AMÁLIA CINTHIA MENESES DO RÊGO

BIODISTRIBUIÇÃO DO PERTECNETATO DE SÓDIO APÓS DESVIO GÁSTRICO EM Y DE *ROUX* (TÉCNICA DE CAPELLA).

NATAL – RN

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DEDICATÓRIA

A meu esposo, Irami Filho, meu grande amor;

A meu filho João, fruto do nosso amor.

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SUMÁRIO

RESUMO

O desvio gástrico em Y de *Roux* é a técnica cirúrgica mais utilizada no tratamento da obesidade mórbida. Esta operação reduz o volume do estômago e o comprimento do intestino delgado, gerando alterações estruturais e metabólicas que podem influenciar no resultado de exames cintilográficos de pacientes operados. Com o objetivo de avaliar a biodistribuição pós-operatória do pertecnetato de sódio ($Na^{99m}Tc$) em órgãos de ratos *Wistar* submetidos à técnica do *bypass* (desvio) gástrico em Y de *Roux* (BGYR), foram utilizados 12 ratos distribuídos aleatoriamente em grupo tratado (n=6), submetido à cirurgia do BGYR e o grupo controle (C; n=6). No 15º dia de pós-operatório foi administrado 0,1 mL via plexo orbital de $Na^{99m}Tc$ aos animais dos dois grupos, com atividade radioativa média de 0,66MBq. Após 30 minutos, os ratos foram mortos e retirados fragmentos de tireóide, coração, pulmão, fígado, estômago, rim e fêmur. As amostras foram lavadas com solução salina 0,9%, pesadas e submetidas ao Contador Gama 1470, *Wizard*^{TM} *Perkin-Elmer-Finlândia* para determinação do percentual de atividade radioativa total por grama (%ATI/g) de cada órgão. Empregou-se o teste t de *Student* para análise estatística, considerando-se significantes as diferenças das médias quando $p<0,05$. Redução significante na média de %ATI/g foi observada no fígado, estômago e fêmur dos animais submetidos à cirurgia de BGYR comparada ao grupo controle ($p<0,05$). Nos demais órgãos não houve diferença estatisticamente significativa entre os grupos. Em conclusão, a cirurgia BGYR em ratos modificou a biodistribuição do $Na^{99m}Tc$ em alguns órgãos, podendo ter implicações clínicas na interpretação de exames cintilográficos. Este estudo

teve um caráter multidisciplinar com a participação de pesquisadores das áreas de Cirurgia Experimental, Farmácia, Radiobiologia, Medicina Nuclear e Estatística.

Palavras chave: Biodisponibilidade biológica. Cirurgia bariátrica. Pertecnetato Tc 99m de Sódio. Radiofármacos. Derivação Gástrica. Anastomose em Y de Roux.

1 INTRODUÇÃO

A obesidade é um problema de saúde pública mundial. Caracteriza-se como obeso mórbido o paciente com Índice de Massa Corpórea (IMC = peso/altura²) maior que 40 ou que está 100 kg acima do peso ideal[1].

Considerada uma síndrome metabólica, a obesidade mórbida está associada ao diabetes *mellitus*, hipertensão arterial, acidente vascular cerebral, doenças articulares, apnéia do sono, asma, entre outras comorbidades que reduzem a expectativa de vida e consomem altos custos em tratamento[2].

Mudanças no estilo de vida bem como a terapia medicamentosa utilizados em pacientes obesos mórbidos são ineficazes na manutenção da perda ponderal a longo prazo, devido à não aderência a programas dietéticos, exercícios físicos e efeitos adversos de anorexígenos[3].

A cirurgia bariátrica, nas últimas quatro décadas, tornou-se o tratamento de escolha dessa síndrome metabólica, controlando as comorbidades, reduzindo o peso e a morbi-mortalidade, com melhora na qualidade de vida[4,5].

As operações bariátricas são classificadas em três tipos distintos: restritivo, disabsortivo e misto[1].

As técnicas restritivas criam um pequeno reservatório gástrico com ajuda de um anel de silicone, reduzindo o volume do estômago, promovendo saciedade precoce. São conhecidas como técnicas restritivas a banda gástrica horizontal e vertical, utilizadas desde 1980[1,2]. O desvio intestinal é uma técnica disabsortiva, hoje em desuso, que induz a perda ponderal por interferir na digestão e absorção do alimento[2].

As operações bariátricas mistas combinam os mecanismos anteriormente citados em um único procedimento, por isso são as mais eficientes e, consequentemente, as mais utilizadas[6].

O *bypass* gástrico em Y de *Roux* (BGYR) é a técnica mista de cirurgia bariátrica mais realizada no momento. Nessa estão contidos a redução volumétrica do estômago e o desvio intestinal (*bypass*) que juntos promovem um efetivo emagrecimento[6]. Por esse motivo é utilizada como uma alternativa de re-intervenção nos pacientes que não obtiveram bons resultados com outros procedimentos, restritivos ou disabsortivos isoladamente[7].

Devido ao seu caráter misto, são descritas no pós-operatório alterações hormonais, estruturais e bioquímicas, necessitando controle e reposição nutricional. Isso tem provocado a realização de pesquisas para identificar e quantificar tais mudanças[8-14].

A cintilografia com o emprego de radiofármacos é um método diagnóstico utilizado no estudo de alterações orgânicas[15-18]. Desde a década de 60, o ^{99m}Tecnécio vem sendo empregado na área biomédica devido a sua natureza física, química e econômica como um elemento biodistribuível, sendo o radiofármaco mais utilizado em medicina nuclear[19,20].

Ensaios experimentais evidenciaram alterações na biodistribuição do ^{99m}Tecnécio provocadas por agentes terapêuticos (químio e fitoterápicos) e operações abdominais[21-27]. Contudo, não há na literatura pesquisas que correlacionem sua atividade tecidual no pós-operatório da cirurgia bariátrica, em particular o BGYR.

Eventuais alterações na biodistribuição desse radiofármaco em qualquer dos órgãos em estudo, levam a suspeitar de que exames cintilográficos pós-operatórios

podem produzir resultados falso-positivos ou falso-negativos, obrigando sua repetição e exposição de pacientes a radiações ionizantes indesejáveis.

Deve-se ressaltar o caráter multi e interdisciplinar desta pesquisa, uma vez que contou com a participação ativa e a constante troca de conhecimentos entre profissionais das áreas de Medicina Nuclear, Cirurgia Geral, Farmácia, Biologia e Estatística.

Tratando-se de uma operação mutilante, de importantes repercussões metabólicas, o trabalho principal anexado a esta tese teve como objetivo avaliar a biodistribuição pós-operatória do ^{99m}Tecnécio na forma de pertecnetato de sódio ($Na^{99m}Tc$) em órgãos como tireóide, coração, pulmão, fígado, estômago, rim e fêmur de ratos *Wistar* submetidos à técnica do BGYR.

Adicionalmente, outros dez trabalhos experimentais estão anexados neste volume, publicados em periódicos com indexação internacional, fazendo parte da formação da autora como Doutora em Ciências da Saúde.

2 REVISÃO DA LITERATURA

2.1 Desvio (*bypass*) gástrico em Y de *Roux* (BGYR)

A cirurgia do BGYR foi inicialmente descrita por Mason e Ito em 1966. Griffen modificou o procedimento com uma gastrojeunostomia retrocólica em Y de *Roux*, de modo que as complicações de refluxo biliar fossem significativamente reduzidas. Desde então, tornou-se a operação mais realizada para perda de peso, considerada como o procedimento bariátrico "padrão ouro" há três décadas[7,28]. Seus sucessos na perda ponderal com baixa morbidade e melhor qualidade de vida têm assegurado a sua utilização continuada na era moderna[4].

A operação realizada por videolaparoscopia desde 1994, consiste na criação de uma pequena bolsa gástrica, que pode conter 30-50 mL de sólidos ou líquidos. Tal reservatório é construído imediatamente abaixo da junção gastroesofágica, aplicando-se um grampeador linear horizontal na pequena curvatura e em sentido cefálico ao ângulo de His (Figura). Com a divisão, um remanescente gástrico é criado, dito estômago excluso, que juntamente com o duodeno e o jejuno proximal irão formar o canal biliopancreático[29].

Em seguida, o jejuno proximal e o mesentério são divididos a uma distânicia de 30-40 cm do ligamento de Treitz. O segmento distal jejunal é trazido em direção à nova bolsa gástrica como canal alimentar, onde se realiza uma anastomose com grampeador circular reforçada por suturas interrompidas. O comprimento deste segmento é cuidadosamente medido em 75-150 cm, altura em que o canal biliopancreatico é anastomosado ao canal alimentar, originando o canal comum, contendo apenas 75-150 cm para a digestão e absorção (Figura). O tempo cirúrgico é geralmente de 2-4 horas[29,30].

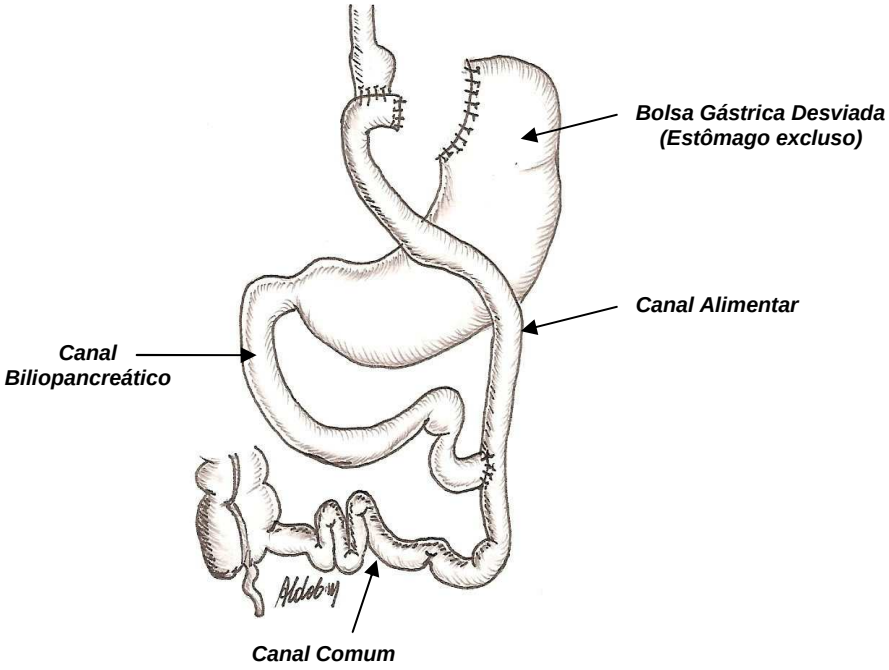

Figura. Medeiros AC. Desvio (bypass) gástrico em *Y de Roux*.

A cirurgia aberta é reservada para cenários difíceis, como nos pacientes superobesos (IMC>50Kg/m^2), formas corporais andróides, história de cirurgia gastrintestinal prévia, e quando os cirurgiões não têm experiência e habilidade técnica com o acesso laparoscópico[6].

Inúmeras variações desta técnica são descritas, porém contendo desfechos em comum: alteração do fluxo biliar, restrição do tamanho do estômago, mudanças na anatomia intestinal e no fluxo de nutrientes, manipulação hepática e vagal intra-operatórias, bem como modulação dos hormônios intestinais e do tecido adiposo[31-33].

A eficácia da técnica BGYR está comprovada em termos de perda de peso e melhora de comorbidades. No entanto, esta intervenção não é isenta de riscos e um seguimento a curto e longo prazo é necessário por várias razões[34]. Em primeiro lugar, há o potencial de complicações cirúrgicas mediatas (vazamentos, fístula, ulceração, hemorragia, obstrução, estenose da anastomose, hérnia interna,

insuficiência hepática) particularmente no caso de pacientes com sintomas digestivos agudos[35-38].

Além disso, é essencial acompanhar as comorbidades e ajustar os tratamentos, em especial medicamentos hipoglicemiantes e antihipertensivos[39].

Por fim, a operação pode ter um forte impacto psicológico e social sobre os pacientes, sendo descritos casos de depressão e encefalopatias, o que requer apoio multidisciplinar[40-42].

Em qualquer caso, a cirurgia resulta em alterações importantes na fisiologia digestiva: refluxo alcalino, estômago excluso, assinergia entre as diferentes secreções (enzimas, ácidos, hormônios) e os nutrientes, resultando em má absorção[43].

Como consequência, a operação pode induzir alterações patológicas no tubo digestivo e demais órgãos: halitose, aftas, desgaste dentário, defeitos de mastigação, esofagites, câncer no estômago excluído, síndrome do intestino curto, hiperplasia pancreática, hipoglicemia, complicações funcionais (vômitos, desordens intestinais, síndrome de *dumping*)[35,44-47].

Os pacientes podem apresentar a médio e longo prazo desnutrição protéico-calórica, hipovitaminoses, hiperparatireoidismo secundário, osteopenia e osteoporose, os quais requerem vigilância e suporte nutricional pós-operatórios[12,48-51].

Com base nos argumentos supracitados, torna-se implícita a necessidade de exames pós-operatórios que auxiliem no diagnóstico de tais alterações. Dentre estes, um dos mais utilizados na medicina nuclear é a cintilografia, com uso de radiofármacos, elementos radiobiocomplexos que podem sofrer alterações na sua biodistribuição, em virtude das modificações estruturais e metabólicas causadas pela

cirurgia bariátrica em tela, levando ao falseamento de resultados, com exposição excessiva dos pacientes operados à radiação.

2.2 Medicina nuclear

O uso de radionuclídeos como traçadores ou radiofármacos têm sido extremamente útil[19,20]. Técnicas inovadoras com radionuclídeos necessitam constantemente de um contato permanente com os avanços nesta área. Este conhecimento é utilizado em procedimentos de medicina nuclear, que ajudam profissionais da saúde no diagnóstico e tratamento de infecções, doenças hematológicas, em oncologia e outras condições patológicas[52].

Os procedimentos de medicina nuclear estão principalmente relacionados com o diagnóstico de doenças e uso da cintilografia, tomografia por emissão de fóton único (SPECT) bem como a tomografia por emissão de pósitrons (PET) têm sido aplicado em vários países[53,54]. Além disso, a terapia de doenças com radiofármacos assume papel relevante na melhoria e desenvolvimento de diferentes compostos com esse propósito.

O uso dos radionuclídeos depende de ensaios em radiofarmácia e do estabelecimento de modelos experimentais[52].

Como a maioria dos pacientes que precisam de exames de medicina nuclear estão sob terapia com vários medicamentos ou em pós-operatório de grandes intervenções cirúrgicas, torna-se mandatório tentar entender melhor o fenômeno desta interação com radiofármacos e as consequências para a sua biodisponibilidade[15,16,55]. Este conhecimento evita erros diagnósticos e a repetição de exames nucleares, uma vez que como são utilizados com freqüência, o domínio da radiobiologia e o controle de qualidade em radioproteção cuminam em uma dose de radiação desprezível para o paciente aliado a um mínimo impacto ambiental[52,55].

2.3 Cintilografia

A aplicabilidade da cintilografia nas diversas áreas da saúde é variada. Este exame utiliza, dentre outros, o ^{99m}Técnecio na forma de pertecnetato de sódio (Na^{99m}Tc) ou ligado a radiotraçadores, que carreiam de maneira específica o radiobiocomplexo ao local a ser examinado, pois possuem afinidades próprias às diferentes estruturas e moléculas[20].

Estudos clínicos enfatizam sua importância no diagnóstico patológico e avaliação funcional de órgãos como tireóide, coração, pulmão, fígado, ossos e rim[56-66], contribuindo também para localização de focos infecciosos através da marcação de leucócitos e mediadores inflamatórios[67-69].

No âmbito da cirurgia bariátrica, exames cintilográficos são realizados no pós-operatório para avaliar o esvaziamento gástrico, a patência de anastomoses, bem como a ocorrência de fístulas e hemorragia digestiva, complicações precoces ou tardias deste procedimento mutilante[70-75].

Em oncologia, a cintilografia figura como um método de alta sensibilidade e especificidade na pesquisa de mestástases e seguimento de doenças neoplásicas a longo prazo[76-78].

A cintilografia é realizada por meio de gama câmera que capta energia de um determinado radionuclídeo na forma de fóton. Esse pulso luminoso é transformado em imagem de acordo com a distribuição do elemento radioativo no organismo[19]. Para ser utilizado em exame cintilográfico, o radiofármaco tem de emitir baixa radiação, toxicidade tolerável, ser estável e possuir meia vida suficiente para o preparo e realização do exame, sem causar maiores danos ou seqüelas[20].

2.4 ^{99m}Tecnécio

O ^{99m}Tecnécio é um dos radionuclídeos mais utilizados em medicina nuclear assim como em pesquisa básica. Ele preenche muitos dos critérios de um radionuclídeo ideal, possui baixa radiação, uma meia vida de 6h, sofrendo 10% de conversão interna, o que resulta em mínima dose para o paciente[20,52].

A forma química utilizada para leitura em gama câmera é o pertecnetato de sódio (Na^{99m}Tc) que necessita de redução iônica para a marcação de substâncias, células ou órgãos em estudo. Para tal processo a substância mais utilizada é o cloreto estanoso. Apesar disso, o Na^{99m}Tc pode entrar ou sair do meio intracelular livremente, num processo de difusão passiva[79,80].

Estudos experimentais evidenciaram a interferência de químioterápicos e ansiolíticos na biodistribuição do Na^{99m}Tc em células sanguíneas e tecidos orgânicos[22,81-83].

A terapia com extratos obtidos de plantas medicinais pode alterar a marcação das frações solúvel e insolúvel de constituintes sanguíneos e resultar em efeitos inesperados na captação radioativa[23,84,85].

Os dados da literatura demonstram que, apesar de utilizado em exames cintilográficos pós-operatórios, não se conhece a influência que o BGYR pode ter na atividade do Na^{99m}Tc como radiofármaco. Portanto, no presente estudo procurou-se analisar, em modelo animal, a interferência da cirurgia do BGYR na biodistribuição do Na^{99m}Tc.

3 ANEXAÇÃO DE ARTIGOS

3.1 Artigo I

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Biodistribution of sodium pertechnetate after Roux-en-Y gastric bypass (Capella technique) in rats[1].

Biodistribuição do pertecnetato de sódio após desvio gástrico em Y de Roux (Técnica de Capella) em ratos.

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ABSTRACT
Purpose: The biodistribution of sodium pertechnetate, the most used radiopharmaceutical in nuclear medicine, has not been studied in details after bariatric surgery. The objective was to investigate the effect of Roux-en-Y gastric bypass (RYGB) on biodistribution of sodium pertechnetate ($Na^{99m}Tc^-$) in organs and tissues of rats. **Methods:** Twelve rats were randomly distributed into two groups of 6 animals each. The RYGB group rats were submitted to the Roux-en-Y gastric bypass and the control group rats were not operated. After 15 days, all rats were injected with 0.1mL of $Na^{99m}Tc^-$ via orbital plexus with average radioactivity of 0.66 MBq. After 30 minutes, liver, stomach, thyroid, heart, lung, kidney and femur samples were harvested, weighed and percentage of radioactivity per gram (%ATI/g) of each organ was determined by Gamma Counter Wizard Perkin-Elmer. We applied the Student t test for statistical analysis, considering $p<0.05$ as significant. **Results**: Significant reduction in mean %ATI/g was observed in the liver, stomach and femur in the RYGB group animals, compared with the control group rats ($p<0.05$). In other organs no significant difference in %ATI/g was observed between the two groups. **Conclusion**: This work contributes to the knowledge that the bariatric surgery RYGB modifies the pattern of biodistribution of $Na^{99m}Tc^-$.

Key words: Biological availability. Bariatric surgery. Radiopharmaceutical. Pertechnetate. Rats.

RESUMO
Objetivo: Avaliar o efeito da cirurgia de desvio gástrico em Y de Roux (BGYR) na biodistribuição do pertecnetato de sódio ($Na^{99m}Tc^-$) em órgãos e tecidos de ratos. **Métodos:** Doze ratos *Wistar* foram aleatoriamente distribuidos em dois grupos de 6 animais cada. O grupo BGYR foi submetido à técnica cirúrgica do desvio gástrico em Y de Roux e o grupo controle não foi operado. No 15º dia de pós-operatório foi administrado 0,1 ml de $Na^{99m}Tc^-$ via plexo orbital aos animais dos dois grupos, com atividade radioativa média de 0,66MBq. Após 30 minutos os ratos foram mortos e retirados fragmentos de fígado, estômago, tireóide, coração, pulmão, rim e fêmur. As amostras foram lavadas com solução salina 0,9%, pesadas e submetidas ao Contador Gama 1470, Wizard^{TM} Perkin-Elmer para

se determinar o percentual de atividade radiotiva por grama (%ATI/g) de cada órgão. Empregou-se o teste t de *Student* para análise estatística, considerando p<0,05 como significante. **Resultados:** Redução significante na média de %ATI/g foi observada no fígado, estômago e fêmur nos animais submetidos a cirurgia de BGYR comparado com o grupo controle (p<0,05). Nos demais órgãos não houve diferença significante no %ATI/g entre os dois grupos. **Conclusão**: A cirurgia BGYR em ratos modificou a biodistribuição do $Na^{99m}Tc$ em alguns órgãos, podendo ter implicações clínicas na interpretação de exames cintilográficos.

Descritores: Biodisponibilidade. Cirurgia bariátrica. Pertecnetato. Radiofármaco. Ratos.

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INTRODUCTION

Obesity is rapidly increasing in the United States, with the prevalence of class 3 obesity approaching 8% in some populations[1,2]. Overall, in the past decades, the Brazilian population has experienced relatively rapid socioeconomic improvement, resulting in many lifestyle modifications that have promoted increased prevalence of obesity and associated diseases, such as diabetes and dyslipidemias[3]. Current treatment options for morbid obesity include pharmacologic agents, low-calorie diets, behavioral modification, exercise, and surgery. Dietary treatments produce an initial weight loss of less than 15% of the starting weight, and weight reductions generally decay to zero at 5 years[4]. The failure of most current approaches to control morbid obesity has led to the development of surgical procedures of the upper gastrointestinal tract designed to induce weight loss (bariatric surgery)[5].

The goal of bariatric surgery is to reduce caloric intake by either restricting the amount of calories that an individual can take in (restrictive procedure) or reducing the amount of calories absorbed from the gastrointestinal tract (malabsorptive procedure). This can be accomplished in a number of ways. The most commonly described techniques are the Roux-en-Y gastric bypass (restrictive and malabsorptive), laparoscopic adjustable banding (restrictive only), and the bilio-pancreatic diversion with duodenal switch (malabsorptive and restrictive). Roux-en-Y gastric bypass (RYGB), is the predominant approach used in the United States[6]; RYGB also creates a small stomach pouch to restrict food intake, but a portion of the jejunum is attached to the pouch to allow food to bypass the distal stomach, duodenum, and proximal jejunum. Bypassing this segment of the gastrointestinal tract might contribute to the clinical success of RYGB by altering the secretion of hormones that influence glucose regulation and the perception of both hunger and satiety[7,8]. Although the procedures can be successful, they can be associated with a number of complications. Unfortunately, these complications can sometimes be subtle and difficult to diagnose early. Since these patients have limited physiologic reserve, it is imperative that complications are identified early by image procedures and appropriately managed[9].

One of the most used diagnostic methods in identifying a great number of diseases and metabolic disorders is the use of radiopharmaceuticals, radioactive compounds used in diagnostic procedures as sources of radiation or tracers[10]. Examination of gastric emptying, liver function, thyroid function, bone disorders, distant metastasis, etc, are used frequently in patients undergoing bariatric surgery[10]. Radiopharmaceuticals biodistribution may provide important information about its uptake to some target organs, but post-surgery data are scarce[11]. It is relevant to investigate sodium pertechnetate biodistribution after RYGB, because it is highly secreted by gastric mucosa. After this operation, the stomach is mostly bypassed and alterations are expected to occur on biodistribution. Several drugs and surgery may interfere with the biological behavior of radiopharmaceuticals used in scintigraphic examinations and sodium pertechnetate is used in more than 80% of scintigraphic examinations[12-14]. They may change the biological effects of the sodium pertechnetate and

their uptake to some organs. As a major surgery, RYGB may result in important anatomical and metabolic changing and unpredictable complications. So, scintigraphic studies may be needed in the postoperative period. If the sodium pertechnetate biodistribution is changed in important organs and tissues due to bariatric surgery, scintigraphic examinations can generate false-positive or false negative images, leading to repetition of nuclear medicine procedures with unnecessary radiation exposure for patients.

The aim of this study was to analyze the effects of RYGB on biodistribution of sodium pertechnetate in several organs and tissues of rats.

Methods

The use of laboratory animals followed the Council for International Organization of Medical Sciences Ethical Code for animal experimentation and the Brazilian guidelines for scientific use of animals (Law nº 11.794). The protocol was approved by the Ethical Committee of Research, UFRN. Rats were observed in individual polypropilene cages in room temperature of 24ºC, 45% relative humidity, 12-hour light/dark cycles, and they had free access to their diet and tap water. For preoperative procedures, rats were deprived of food for 16 to 18 hours and were anesthetized with intramuscular injection of 0.1 mL/100g weight, of a solution prepared with 1.0 mL of ketamine (50mg/mL) and 1.0 mL of xilazine (20mg/mL). For postoperative pain control, tenoxicam (Roche, Brazil) 1.5 mg/kg, was given IM to the rats postoperatively, once a day for 3 days.

Operative and laboratory procedures

Twelve Wistar rats (349.3 ±10.7 g) were equally divided into RYGB and control groups. The rat abdomens were shaved and prepared with 70% alcohol. A midline incision was performed, and the stomach and distal esophagus were exposed. The stomach was divided 1 cm down of the esophagus and both gastric ends were oversewn using a running 6-0 polypropilene suture, and the suture lines were embrocated. The jejunum was divided 16 cm below the ligament of Treitz, creating a 16 cm biliary-pancreatic limb. A 4 to 5 mm end-to-side gastrojejunostomy was sewn using interrupted 6-0 polypropilene sutures on the anterior surface of the gastric fundus. The stump of proximal jejunum was closed with a running suture. A 7 to 8 mm side-to-side jejunojejunostomy was sewn 10 cm below the gastrojejunostomy. The procedure lasted approximately 30 minutes and the abdomen was closed in layers, using a running 4-0 nylon suture. Rats drank water and a 10% glucose plus 2% saline solution starting 24 hours after the operation and for the first 3 days. This was followed by a solid diet (Labina-Purina). For the first three postoperative days, rats were hydrated with normal saline solution (20ml) injected subcutaneously to prevent dehydration. A surgical microscope with 10x magnification (DF Vasconcelos, São Paulo, Brazil) was used for the anastomosis. A single intramuscular dose of 75 mg/kg of ceftriaxone sodium (Roche, Brazil) was given as antimicrobial prophylaxis 30 minutes before the surgical procedures. The control group rats (n=6) were not operated.

The animals remained under observation for 15 days after surgery and were then injected with 0.1 ml of sodium pertecnetate via the orbital plexus, corresponding to radioactive activity of 0.66 MBq. Thirty minutes after radiopharmaceutical administration, the animals were killed with an anesthesia overdose (thiopental sodium, intracardiac) and underwent surgery for removal of samples from the liver, kidney, heart, lung, thyroid, stomach and femur. The tissue samples were washed in 0.9% saline, weighed on a precision scale (Mark 160®, Bel equipment, Italy) and their radioactivity was determined in an automatic gamma counter (Wizard 1470, Perkin-Elmer, Finland). The results were shown in counts per minute (CPM), corrected by disintegrations per minute (DPM). The efficiency of the gamma counter was 86%, as specified by the manufacturer. The specific activity of each

sample was calculated by dividing its absolute count in DPM by its weight (DPM/g). The percentage of radioactivity of each sample (% ATI/g) was calculated by dividing its specific activity (DPM/g) by the total radioactivity of each animal. The total activity administered to each animal was calculated from the average of three patterns with the same volume injected.

The data were expressed as mean ± standard deviation. Statistical analysis for comparison between groups was performed by the Student t test, using a significance level of 0.05.

Results

All animals survived for the duration of the study. Table 1 summarizes the descriptive results of the percentage of radioactivity (% ATI/g) in organs of rats from groups RYGB and control. We applied the Student t test for independent samples. The significance level established for the test was 5%.

TABLE 1 - Percentage of radioactivity (% ATI/g) of each organ sample in animals submitted to Roux-en-Y gastric bypass and controls.

Organs	*%ATI/g*		*P*
	RYGB	Control	
Liver	0.33 ± 0.05	0.56 ± 0.08	0.024
Kidney	0.58 ± 0.22	0.41 ± 0.07	0.119
Heart	0.21 ± 0.05	0.27 ± 0.07	0.128
Lung	0.53 ± 0.18	0.37 ± 0.14	0.098
Tyroid	2.91 ± 0.97	3.46 ± 1.67	0.506
Stomach	0.54 ± 0.32	2.99 ± 1.65	0.005
Femur	0.14 ± 0.03	0.22 ± 0.07	0.035

The values appear as mean±standard deviation. RYGB, Roux-en-Y gastric bypass

In the kidney and lung we observed an increased uptake of radioactivity on rats submitted to RYGB, comparing with controls, but the difference was statistically insignificant ($p>0.05$). However, in the RYGB rats a significant decrease in the radioactive uptake (%ATI/g) occurred in the stomach, liver and femur, comparing with controls ($p<0.05$). When comparing the difference between the radioactive uptake of heart and thyroid from RYGB and control rats, there was a tendency for a further decrease in % ATI/g of RYGB animals, although this was not statistically significant ($p>0.05$).

Discussion

Obesity has become an epidemic condition, and in the United States the percentage of obese adults increased from 15.3% in 1995 to 23.9% in 2005. Approximately 4.8% of patients are considered morbidly obese. Worldwide it is estimated that over 300 million people are obese[14]. Obesity results in an increased risk for serious diseases, including diabetes mellitus, cardiovascular disease, hypertension, dyslipidemia, degenerative arthritis, some forms of cancer and respiratory problems, and to generate socio-economic and psicossocial disturbs[15]. Thus, some weight loss therapies, such as diet and pharmaceutical systems complemented by physical exercises have been proposed. However, almost 95% of patients who have morbid obesity (BMI> 40kg/m^2) fail to achieve satisfactory weight loss[16]. Therefore, many efforts

have been implemented to get good results with surgical techniques, and the bariatric operations have obtained good results at a significant weight loss for many years[17]. Despite the good results, bariatric surgery can cause anatomical and metabolic complications[18]. The diagnosis of these disturbs may require imaging examinations such as radiography and scintigraphy.

The technetium-99m is the most widely used radionuclide in nuclear medicine and research studies. It has a short half-life (6h), emits low radiation and small doses are needed for diagnostic procedures[19]. Changes in the biodistribution of sodium pertecnetate in organs and tissues are well identified in several clinical studies. It has been used in vivo and in vitro, in the study of diseases, drugs, chemotherapy and plants that interfere with their distribution[20,21]. However, in the postoperative period of major surgical procedures, there are few studies that focus on the biodistribution of radiopharmaceuticals. To understand and explore the relationship between the RYGB and biodistribution of radiopharmaceuticals in organs and tissues, we used a reproducible and well characterized animal model. Scintigraphic examinations in the postoperative period are used to diagnose bleeding in gastrointestinal tract gastroesophageal reflux and the patency of anastomosis. Diagnosis of metastases by cancer and postoperative changes in the kidneys, liver, lung, heart and other organs are made using scintigraphy with radiotracers and sodium pertecnetate[19]. In this study, the RYGB did not affect the biodistribution of sodium pertecnetate the heart, lung, kidney and thyroid.

The stomach is often examined in the postoperative of bariatric surgery, primarily for diagnosing the presence of fistulas, since the escape from the anastomosis may be early or late complication of this operation. A large case series of 63 patients with leaks after RYGB reports that most were not detected by CT imaging and that most of them required surgery (63%), with morbidity of 53% and mortality of 10%[22]. In case of small fistulas, the rate of escape of contrast will be small, so that leaks may be overlooked by radiography. The morphofunctional radionuclide evaluation provides the efficiency of anastomosis of digestive tract, by showing the emptying time of the gastric remnant, excluding reflux and any radionuclide stagnation. Using scintigraphy, a small amount of diffused sodium pertechnetate is absorbed by the gastric mucosa due to the high affinity for this radiopharmaceutical. It has been suggested that scintigraphy has high accuracy in diagnosis of complications in postoperative bariatric surgery[23]. In this study the uptake of sodium pertechnetate was significantly decreased in the remnant functional stomach post RYGB, meaning that scintigraphic examination of this organ in the postoperative of bariatric surgery must be interpreted considering this finding.

Several different mechanisms may explain the observed changes in bone metabolism after bariatric surgery. Poor absorption of minerals and vitamins, including calcium and vitamin D has been documented[24]. A sustained deficiency of vitamin D in obese patients may result in metabolic and skeletal abnormalities, which may be detected before and after bariatric surgery. The increased turnover and reduced bone mineral density may occur as a physiological adaptation of weight loss and changes in the mechanics of the skeleton or as a result of pathophysiological responses to surgery[25]. All these metabolic changes, especially the increase in bone turnover may explain the decrease in biodistribution of sodium pertecnetate in the femur of the animals submitted to RYGB as compared to the control group. In our study the uptake of sodium pertecnetate by the liver of rats submitted to RYGB was significantly lower than in control rats, meaning that the liver function may be affected in operated animals. This results need to be treated with some caution. In fact, some authors demonstrated significant improvement in the levels of aminotransferases and gamma glutamyl transferase in the postoperative of obese patients submitted to RYGB[26], and others found postoperative values of aminotransferases transiently increased by fivefold to eightfold[27] The rat model for RYBG appears to be a reasonable model and will enable continued research involving metabolic changes, in order to validate our findings on biodistribution of

radiopharmaceuticals. In conclusion, this work contributes to the knowledge that the bariatric surgery RYGB modifies the pattern of biodistribution of sodium pertechnetate.

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3.2 Artigo II

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Biodistribution of samarium-153-EDTMP in rats treated with docetaxel[1]

Biodistribuição de EDTMP-153-samário em ratos tratados com docetaxel

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ABSTRACT

PURPOSE: Many patients with metastatic bone disease have to use radiopharmaceuticals associated with chemotherapy to relieve bone pain. The aim of this study was to assess the influence of docetaxel on the biodistribution of samarium-153-EDTMP in bones and other organs of rats. METHODS: Wistar male rats were randomly allocated into 2 groups of 6 rats each. The DS (docetaxel/samarium) group received docetaxel (15 mg/kg) intraperitoneally in two cycles 11 days apart. The S (samarium/control) group rats were not treated with docetaxel. Nine days after chemotherapy, all the rats were injected with 0.1ml of samarium-153-EDTMP via orbital plexus (25µCi). After 2 hours, the animals were killed and samples of the brain, thyroid, lung, heart, stomach, colon, liver, kidney and both femurs were removed. The percentage radioactivity of each sample (% ATI/g) was determined in an automatic gamma-counter (Wizard-1470, Perkin-Elmer, Finland). RESULTS: On the 9th day after the administration of the 2nd chemotherapy cycle, the rats had a significant weight loss (314.50±22.09g) compared ($p<0.5$) to pre-treatment weight (353.66± 22.8). The % ATI/g in the samples of rats treated with samarium-153-EDTMP had a significant reduction in the right femur, left femur, kidney, liver and lungs of animals treated with docetaxel, compared to the control rats. CONCLUSION: The combination of docetaxel and samarium-153-EDTMP was associated with a lower response rate in the biodistribution of the radiopharmaceutical to targeted tissues. Further investigation into the impact of docetaxel on biodistribution of samarium-153-EDTMP would complement the findings of this study.

Key words: Taxoids. Samarium. Biological Availability. Drug Therapy. Rats.

RESUMO

OBJETIVO: Muitos pacientes com metástases ósseas são tratados com radiofármacos associados com quimioterapia para alívio da dor óssea. O objetivo do trabalho foi estudar a influência do docetaxel na biodistribuição do EDTMP-153-samário nos ossos e outros órgãos de ratos. MÉTODOS: Ratos Wistar foram aleatoriamente alocados em 2 grupos de 6 animais cada. O grupo DS (docetaxel/samário) recebeu docetaxel (15 mg/kg) intraperitoneal em dois ciclos com 11 dias de intervalo. Os ratos do grupo S (samário/controle) não foram tratados com docetaxel. Nove dias após a quimioterapia, todos os animais receberam 0,1ml de EDTMP-153-samário via plexo orbital (25µCi). Após 2 horas, os animais foram mortos e retiradas amostras de cérebro, tireóide, pulmão, coração,

estômago, cólon, fígado, rim e fêmures. O percentual de radioatividade por grama (%ATI/g) de tecido de cada biópsia foi determinado em contador gama automático (Wizard-1470, Perkin-Elmer, Finland).RESULTADOS: No 9º após 2º ciclo de docetaxel os ratos tiveram perda de peso significante, passando de 353,66± 22,8g (controle/pré-tratamento) para 314,50±22,09g (p<0,5). Os % ATI/g nos órgãos dos ratos tratados com EDTMP-153-samário e docataxel tiveram redução significante nos fêmures direito e esquerdo, rim, fígado e pulmão, quando comparados com os não tratados com docetaxel. CONCLUSÃO: A combinação de docetaxel com EDTMP-153-samário foi associada com resposta mais baixa na biodistribuição do radiofármaco em órgãos alvo. Futuras investigações sobre o impacto do docetaxel na biodistribuição do EDTMP-153-samário poderão complementar os achados teste estudo.

Descritores: Taxóides. Samário. Disponibilidade Biológica. Quimioterapia. Ratos.

Introduction

Bone metastases are a frequent complication of cancer, occurring in up 70% of patients suffering from advanced breast or prostate cancer[1]. Bone metastases patients often present with severe bone pain, especially in the advanced stage of disease[1,2]. The major pain mechanism of small metastases appears to be the stimulation of nerve endings in the endosteum by a variety of chemical mediators. Larger bone metastases produce stretching of the periosteum, which leads to pain[2]. The resulting bone pain interferes with the patient's quality of life and requires effective treatment. Unfortunately, various non-radiotherapeutic modalities such as analgesics, hormone therapy, orchidectomy, cytostatic and cytotoxic drugs, bisphosphonates, and surgery are not effective in all cases, especially in the late stage of the disease[1,2]. Systemic radionuclide therapy using boneseeking radiopharmaceuticals is considered a valuable and effective method for treating patients with widespread skeletal metastases and increased bone turnover, especially in patients with bone metastases from prostate and breast cancer[3,4,5].

In the 1980s the radiopharmaceutical samarium-153 ethylenediamine tetramethylene phosphonate (Sm-153-EDTMP) with short physical half-life was developed, and has been considered effective in the treatment of bone pain[4,5]. Docetaxel is one of the taxane class chemotherapies that is being increasingly used to treat various solid tumors, including those of prostate and breast cancer. It acts by Bcl-2 protein inactivation in the metastatic cells, causing the death of tumor cells by apoptosis[6]. In patients with prostate cancer, docetaxel has been suggested in various studies as the first-line choice for treating metastatic hormone-refractory disease. This treatment increases survival, compared to previously accepted chemotherapy schemes[6].

A significant number of patients with metastatic bone disease are often subjected to a series of concomitant treatments, including the use of radioisotopes to relieve bone pain, associated with chemotherapy. Accordingly, the aim of this study was to assess the influence of docetaxel on the biodistribution of Sm-153-EDTMP, especially in the bones of rats, enabling a more thorough knowledge of its applicability.

Methods

We used male rats provided by the Health Science Center vivarium, Federal University of Rio Grande do Norte, Brazil. The animals were randomly allocated into 2 groups of 6 rats each. They were weighed and placed in individual cages with water and food (Labina Purina®) "ad libitum" and acclimatized in the lab for 7 days. The rats were kept under temperature (21ºC), humidity (60 - 70%) and lighting (12 / 12 hour cycle light / dark)

control and they were handled in accordance with International Standards of Care and Use of Laboratory Animals, following the guidelines of the Brazilian College of Animal Experimentation.

The animals of the DS group (docetaxel/samarium) were administered docetaxel (Trixotene®, Novartis, São Paulo, Brazil), intraperitoneally, using a 15 mg/kg dose in two administration cycles 11 days apart. The S group (samarium/control) rats were not treated with docetaxel. The animals remained under observation for 9 days after the 2nd cycle and were then injected with 0.1 mL of Sm-153-EDTMP via the orbital plexus, corresponding to radioactivity of 925 Bq (25µCi). Two hours after radiopharmaceutical administration, the animals were killed with an anesthesia overdose (thiopental sodium, via the intracardiac cannula) and underwent surgery for removal of samples from the brain, thyroid, lung, heart, stomach, colon, liver, kidney and both femurs. The tissue samples were washed in 0.9% saline, weighed on a precision scale (Mark 160®, Bel equipment, Italy) and their radioactivity was determined in an automatic gamma counter (Wizard 1470, Perkin-Elmer, Finland). The results are shown in counts per minute (CPM), corrected by disintegrations per minute (DPM). The efficiency of the gamma counter was 86%, as specified by the manufacturer. The specific activity of each sample was calculated by dividing its absolute count DPM by its weight (DPM/g). The percentage of radioactivity of each sample (% ATI/g) was calculated by dividing its specific activity (DPM/g) by the total radioactivity injected in each animal. The total activity administered to each animal was calculated from the average of three patterns with the same volume injected.

The data were expressed as mean ± standard deviation. Statistical analysis for comparison between groups was performed by the Student and Tukey tests, using a significance level of 0.05.

Results

No animal died during the experiment. On the eleventh day after the 1st chemotherapy cycle with docetaxel, the animals showed weight loss (mean 329.16±29.2 g), but not significantly different from pre-treatment weight (mean 353.66± 22.8). Meanwhile, on the 9th day after administration of the 2nd chemotherapy cycle, there was significant weight loss (314.50±22.09g) compared to pre-treatment weight (Figure 1). We observed no changes in animal behavior or side effects, such as diarrhea and hair loss. During the laparotomy to remove organ samples, few peritoneal adhesions were observed in DS group rats. No macroscopic changes were detected in the abdominal organs.

The analysis of the percentage of total activity of Sm-153-EDTMP injected per gram of tissue (% ATI/g) in the samples analyzed showed greater radiopharmaceutical activity in the liver, kidney, right femur, left femur and thyroid, when compared to other organs, in both the DS group (treated with docetaxel) and control group S (Table 1). In the rats treated with docetaxel, the Sm-153-EDTMP activity was smaller in all the organs studied, when compared to the control group, except in the brain. Using the Student t-test, we showed that this reduction was statistically significant in the right femur, left femur, kidney, liver and lungs of animals treated with docetaxel, compared to the control group rats ($p<0.05$). Consequently, there was no significant reduction in radioactivity biodistribution in brain, thyroid, heart, stomach and colon samples (Table 1).

TABLE 1 - Comparison of percentage of radioactivity/g (% ATI/g) of samples from each organ of rats treated with Sm-153-EDTMP (group S) and with docetaxel + Sm-153-EDTMP (group DS)

Organs	% ATI/g		p
	Group S	Group DS	
Brain	0.01 ± 0.002	0.01 ± 0.005	0.753022
Thyroid	0.40 ± 0.079	0.30 ± 0.066	0.07301
Lung	0.17 ± 0.033	0.13 ± 0.025	0.043126
Heart	0.12 ± 0.019	0.11 ± 0.019	0.300239
Stomach	0.18 ± 0.042	0.14 ± 0.032	0.091458
Colon	0.13 ± 0.041	0.11 ± 0.038	0.484202
Liver	1.48 ± 0.187	0.96 ± 0.267	0.007438
Kidney	1.21 ± 0.160	0.86 ± 0.144	0.007411
Femur (right)	0.73 ± 0.105	0.46 ± 0.093	0.002484
Femur (left)	0.72 ± 0.004	0.50 ± 0.086	0.003599

Mean ± SD, Student t test. S, samarium; DS, docetaxel+samarium.

Discussion

Bone pain is a common symptom of metastatic cancer and may be difficult to control with analgesic medication alone. Systemic agents such as chemotherapy, bisphosphonates and hormonal therapy, as well as external beam radiation in the form of hemibody or focal bone irradiation are also used as palliative measures to control metastatic bone pain[7]. Radiation in the form of radiopharmaceuticals has been investigated for the relief of bone metastasis pain and several systemically administered beta-emitters have been evaluated for the treatment of painful bone metastases, two of which (32-P and 89-Sr) have been studied extensively[7]. Both agents are effective in relieving pain from osteoblastic metastases, but they have properties that limit their usefulness. The very high beta energy of 32-P coupled with a low lesion-to-normal bone ratio has inhibited widespread use of this radiopharmaceutical as a palliative agent for bone pain[7]. The long physical half-life of 89-Sr (50.5 days) results in a slow delivery of the radiation dose. Consequently, the onset of pain relief may not occur for several weeks after administration[8]. The long half-life can also produce a prolonged and variable suppression of hematopoietic elements[8], limiting the availability or efficacy of concurrent myelosuppressive therapies (e.g. external radiation, surgery or chemotherapy) that these patients frequently require. Therefore, we used 153-samarium in this study because of its physical and biological proprieties, ease of use in laboratory, as well as its efficacy in treating bone pain[5]. The physical half-life of 153-Sm is 46.3 h, and its beta particle has a maximum range of 1.7 mm in bone and 3.1 mm in soft tissue. The gamma emission at 103 keV (29% abundance) is suitable energy for imaging using standard scintigraphic cameras, and is low enough result in minimal external exposure levels when administered at therapeutic levels[9]. Although many radiopharmaceuticals have been examined for bone pain palliation, samarium-153 has been the most extensively examined in clinical trials. The advantages of radiopharmaceutical use include the ease of administration, the ability to treat multiple sites of metastatic disease, the improved potential therapeutic ratio due to bone location and the potential of combining with chemotherapy agents and external beam radiation for an enhanced therapeutic effect[4,5]. A subset of trials has suggested improved pain palliation by combining a radiopharmaceutical agent with chemotherapy[4,5,12].

Taxanes (docetaxel and paclitaxel) are currently one of the most extensively studied new chemotherapeutic agents for metastatic breast cancer. Single-agent docetaxel has demonstrated significant survival advantages over other recognized regimens in two trials involving patients with anthracycline-pretreated metastatic breast cancer[10]. Some data suggest that docetaxel is one of the most active agents available for treating these patients[11].

The primary objective of this study was to evaluate if combined chemotherapy interferes with the biodistribution of Sm-153-EDTMP to bone and other organs. It was based on the possibility of using Sm-153-EDTMP combined with docetaxel for the treatment of bone metastasis pain in patients with prostate or breast cancer. Recently, Ricci *et al.*[12] reported that Sm-153-EDTMP is effective in bone pain relief, with minimal toxicity. When administered in combination with chemotherapy (estramustine phosphate or mitoxantrone plus prednisone), it prolonged survival.

Many factors, such as drug therapy, radiation therapy, surgery, in addition to the pathological process, could affect the biodistribution of different radiopharmaceuticals[13-15]. In this work, when Sm-153-EDTMP was administered to rats previously treated with docetaxel, the biodistribution of the radiopharmaceutical to bone, kidney, liver and lung was significantly lower than that of controls. Based on these data, we can infer that an additional dose of Sm-153-EDTMP may be necessary to get the desired pain relief effect when docetaxel is used in combination with this radiopharmaceutical. It was reported that the average duration of bone pain palliation in patients treated with Sm-153-EDTMP alone was about 3-4 months, while it was about 9-10 months in patients who started receiving estramustine phosphate or mitoxantrone plus prednisone after treatment with the bone-seeking radionuclide[12]. However, information about the clinical effect of docetaxel associated to Sm-153-EDTMP is scarce. The results obtained in the present work provide a rationale for further studies aimed at assessing combined chemotherapy and bone-targeted radiotherapy (docetaxel plus Sm-153-EDTMP) as a therapeutic strategy in patients with advanced hormone resistant prostate cancer.

In conclusion, the combination of docetaxel and Sm-153-EDTMP was associated with a lower response rate in the biodistribution of the radiopharmaceutical to targeted tissues. Further investigation into the impact of docetaxel on the biodistribution of Sm-153-EDTMP would complement the findings of this study.

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3.3 Artigo III

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Influence of Splenectomy on the Biodistribution of Technetium-99m Dimercaptosuccinic acid (Tc-99m-DMSA) in Rats.

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ABSTRACT

This study aimed to evaluate if the splenectomy alter biodistribution of Tc99m-DMSA and renal function in Wistar rats. Used the groups: splenectomy (n = 6) and control (n = 6). After15 days postsplenectomy, administration of 0.1ml of Tc99m-DMSA IV (0.48 MBq). Thirty minutes later, biopsies from kidney, heart, lung, thyroid, stomach, bladder, femur, blood. Samples were weighed and the percentage of radioactivity/g (%ATI/g) counted with Wizard Gamma Counter Perkin-Elmer. Serum urea and creatinine, hematocrit, leukocytes and platelets were measured. Statistics by t test, significance p< 0.05. There was a significant reduction in %ATI/g in kidney and blood (p <0.05) of splenectomized animals, a significant increase (p <0.05) of urea (88.8 ± 18.6 mg / dL) and creatinine (0.56 ± 0.08), compared to the controls (51.5±1.6, 0.37±0.02mg/dL, respectively) as well as leukocytosis, increase in platelets and hematocrit reduction. Conclusion: in rats, splenectomy changed the renal function and the uptake of Tc-99m-DMSA.

Key-words: Splenectomy, Biodistribution, Technetium, Rats.

INTRODUCTION

The spleen has a complex physiologic role, as evidenced by its multiple immunologic, filtration, reservoir, and hematopoietic functions. Several immunologic roles are uniquely exercised by the spleen. It is more efficient in removing non-opsonized bacteria, mostly encapsulated organisms, than is the liver. It is the main site of the opsonins, tuftsin and properdin, as well as of immunoglobulin-M antibody synthesis (Brendolan et al., 2007). Splenectomy has been indicated for trauma, tumor and hematological disorders (Petroianu, 2005). In some splenectomized patients severe infection and disseminated intravascular coagulation (DIC) with post-mortem congestion in every organ as well as bleeding may occur. Frequent causes of death are progressive renal failure, pneumococcal sepsis, DIC, hemorrhage, pulmonary and renal congestion (Tajiri et al., 2007). In spite of the evidence of renal complications in the postoperative of splenectomy, this problem has been scarcely studied.

Technetium-99m-dimercaptosuccinic acid (Tc-99m-DMSA) scintigraphy is the standard method used to evaluate permanent renal damage in children with urinary tract infection (Stokland et al., 2007; Piepsz et al., 1999). The use of Tc-99m-DMSA for renal cortical scintigraphy, introduced in the 1970s, has been shown to be sensitive and specific for diagnosing pyelonephritis in children (Majd & Rushton, 1992).

Quantitative single-photon emission computed tomography (SPECT) measurement of Tc-99m-DMSA uptake by the kidneys is a reproducible method that can reliably be used to monitor serial changes in individual renal function. The method provides information concerning the percentage injected dose per cubic centimeter of renal tissue and functional kidney volumes, and can obtain individual kidney uptake, which provides a practical index for evaluating individual renal function (Groshar et al., 1997). The renal uptake of 99mTc-DMSA is dependent on renal blood flow and proximal tubular cell membrane transport function (Taylor, 1982).

When a radiopharmaceutical is administered to a patient, the process of biodistribution begins; this consists of absorption, distribution, metabolism and excretion. The biodistribution and labeling of blood constituents by radiopharmaceuticals can be changed by surgical procedures performed in animal models (Araújo-Filho et al., 2007; Chacon et al., 2007), and by phytotherapic and allopathic drugs (Rebello et al., 2007; Neves et al., 2007; Fonseca et al, 2007; Xavier-Holanda et al., 2006). The biodistribution of radiopharmaceuticals is based on the same pharmacokinetic principles described for therapeutic agents. So, when a patient is using a given drug, or was previously submitted to a surgical procedure, the biodistribution of a radiopharmaceutical injected intravenously to perform scintigraphy might change. It may bypass the target organ and undermine the effectiveness of image diagnostic methods of nuclear medicine (Braga et al., 2000).

Therefore, the present study aimed at investigating whether splenectomy, an operation that may alter immunology, coagulation and hematologic functions, could change the biodistribution of Tc-99m-DMSA to some organs, especially the kidney of rats. An additional objective was to determine if splenectomy could affect the red/white blood cell and platelet count, as well as renal function.

MATERIAL AND METHODS

Male Wistar rats, weighing 295±31g, were supplied by the Center of Experimental Surgery of the Federal University of Rio Grande do Norte, Brazil. The animals were housed under standard conditions, and fed rodent chow and water *ad libitum*. The protocol for this study was approved by the Institutional Animal Care Committee, and all surgical procedures and care administered to the animals were in accordance with Brazilian College of Animal Experimentation guidelines. After 7 days of acclimatization in the laboratory, the rats were randomly allocated to two groups of 6 rats each. The groups were denominated splenectomy (SP) and control (C).

After overnight fast, the rats were anesthetized with intraperitoneal thiobarbital (20 mg/kg weight) and ketamine (50 mg/kg weight) IM. A midline laparotomy was performed after the skin was shaved and sterilized with 70% ethanol. The spleens of SP group rats were removed. The laparotomy wound was closed with 4-0 nylon in layers and individual rats were placed in separate cages. They were allowed water *ad libitum* and rat chow after 24 h postoperatively. The C rats (n=6) were neither anesthetized nor operated on. Hydration was performed with normal 0.9% saline (10 mL/100g weight) injected subcutaneously in the back of the rats for the first two postoperative days. Postoperative pain was treated with tenoxicam (Roche Pharm., Brazil); 10 mg/kg was given IM to the rats once a day for the first three days. Body weight was monitored weekly throughout the entire 15-day experimental period. Activity, mucosa and skin color were observed daily.

On the 15th day all the animals were anesthetized again, and injected with 0.lmL of Tc-99m-DMSA (corresponding to radioactivity of 0.66MBq) in the orbital plexus. After 30 min, blood was collected by cardiac puncture for dosages and the animals were killed by a lethal dose of anesthetic. Samples of the kidney, heart, lung, thyroid, stomach, bladder, femur and blood were

harvested. The samples were washed in 0.9% saline, weighed on a high-precision digital scale (Bel-Mark 160-II Itália®) and subjected to radioactivity detection using a 1470 Wizard^{TM} Gamma Counter - Perkin-Elmer, Finland, with automatic correction of radiation decline. The percentage of radioactivity/g (%ATI/g) of each organ was calculated by dividing the activity per gram of the sample tissues by the total activity administered to each animal.

Serum urea and creatinine were measured with a Konelab 60i Spectrophotometer (assay kit from Weiner, São Paulo, Brazil). Hematological analysis of hematocrit (HCT), white blood cells (WBC) and platelets was performed using an Abbot Cell Dyn 3500 Automatic Analyzer, São Paulo, Brazil. The data were expressed as mean±standard deviation (SD). Group comparison was made using ANOVA and the post-hoc Student test, with a significance level set at $p<0.05$.

RESULTS

Table 1 shows significantly lower kidney and blood uptake levels of Tc-99m-DMSA in animals subjected to splenectomy, than those found in control rats ($p<0.05$). The radioactive uptake predominated in the kidney and bone (femur), the main target tissues for Tc-99m-DMSA. Specifically, the lung demonstrated higher uptake in splenectomized rats than in controls ($p=0.008$). In the other organs (heart, thyroid, stomach, bladder and femur) we did not detect significant differences in Tc-99m-DMSA uptake between splenectomized and control rats ($p>0.05$).

Table 1 - Results of Tc-99m-DMSA biodistribution in each organ, and the p value to investigate the existence of statistically significant differences between splenectomy and control groups.

Organs	*%ATI/g*		P
	Splenectomy	Control	
Kidney	2.10 ± 0.30	3.27 ± 0.81	0.041
Heart	0.13 ± 0.04	0.12 ± 0.02	0.066
Lung	0.23 ± 0.05	0.12 ± 0.06	0.008
Thyroid	0.07 ± 0.02	0.06 ± 0.02	0.357
Stomach	0.08 ± 0.02	0.06 ± 0.02	0.225
Bladder	0.34 ± 0.43	0.42 ± 0.35	0.736
Femur	2.04 ± 0.01	1.93 ± 0.01	0.924
Blood	0.30 ± 0.09	0.53 ± 0.16	0.012

Mean±Standard deviation; %ATI/g, percent of radioactivity per gram.

Postsplenectomy symptoms such as hematuria, pale mucosa and lethargy were observed in 6 animals, but not in control rats. We observed no significant body weight loss in splenectomized rats, compared to controls.

Table 2 – Values of urea, creatinine, platelets, hematocrit (HCT) and white blood cell (WBC) count at the 15th postoperative day following splenectomy, compared to controls.

Dosages	Splenectomy	Control	P
Urea (mg/dL)	88.3 ± 18.5	51.7 ± 1.3	0.01
Creatinine (mg/dL)	0.56 ± 0.08	0.37 ± 0.02	0.0014
Platelets(k/μL)	1252± 248	530 ± 251	0.002
HCT (%)	22.8±4.7	46.8±9.02	0.0002
WBC (k/μL)	15.2±2.9	3.3± 0.8	<0.001

Mean±standard deviation; WBC, white blood cells; p-value from t test for independent samples; HCT, hematocrit.

To examine the effects of prior splenectomy on kidney injury, we assessed the levels of urea and creatinine. (Table 2). In splenectomized rats, urea and creatinine values (88.3 ± 18.5 mg/dL and 0.56 ± 0.08 mg/dL respectively) were significantly ($p<0.05$) higher than in controls (51.7 ± 1.3 mg/dL and 0.37 ± 0.02 mg/dL respectively). Changes in platelet and WBC counts are summarized in Table 2. At postoperative day 15,, the number of platelets and WBC was significantly higher in splenectomized rats than in controls ($p<0.05$). Hematocrit was significantly lower in splenectomized rats (22.8±4.7%) than in controls (46.8±9.02%; $p=0.0002$).

DISCUSSION

Splenectomy is recognized as having a significant immunomodulating effect, best described with regard to postsplenectomy sepsis and graft survival after renal transplant (Shaw & Print, 1989). During the last ten years, non operative management has become the primary method of preserving the spleen. Splenectomy is now required for around 50% of splenic trauma injuries (Velmahos et al., 2000). Sepsis following splenectomy, known as postsplenectomy infection syndrome and associated with a high mortality rate, is the most feared postoperative complication (Malangoni et al., 1984; Chhaikof & McCabe, 1985).

The white blood cell count (WBC) is an integral part of sepsis diagnosis. Early WBC trends alert the physician about the possibility of sepsis and allow prompt therapeutic response. However, postsplenectomy diagnosis of sepsis based on elevated WBC is confounded by the fact that leukocytosis is a physiologic response to splenectomy, similar to the phenomenon of elevated postsplenectomy platelet count (Bessler et al., 2004; Horowitz et al., 1992). Some reports suggest that postsplenectomy WBC in patients with sepsis is greater and more persistent than the WBC in patients without sepsis (Rutherford et al., 1994).

In our study, we sought to determine if splenectomy was able to change the biodistribution of Tc-99m-DMSA to some organs, especially to the kidneys, the preferential uptake target of this radiopharmaceutical. As all animals had hematuria, leukocytosis and a significant platelet increase, we suspect that the splenectomized animals were affected by sepsis associated with disseminated intravascular coagulation. Increased white blood cell count and platelets are frequently the only hematological abnormalities observed in splenectomized patients (Petroianu et al., 2006). The phenomenon of temporary leukocytosis following splenectomy has been well known for many decades as a physiologic response to removal of the spleen. Leukocytosis is also a

prominent finding of postoperative sepsis, a common and much feared complication of splenectomy. Therefore, confusion exists as to whether postsplenectomy leukocytosis should be considered a normal finding or a warning sign mandating treatment (Horowitz et al., 1992). Associated with hematuria, the animals in this study had significant kidney function impairment, due to the increase in urea and serum creatinine. These findings may explain the marked reduction in Tc-99m-DMSA uptake by renal parenchyma, reinforcing the characterization of postsplenectomy renal failure in rats.

Some physiological and pharmacological properties related to the radiobiocomplex sodium pertechnetate ($Na^{99m}TcO_4$) have been reported. After intravenous injection, it is weakly bound to serum proteins (70-80%). The pertechnetate ion ($^{99m}TcO_4^-$) diffuses slowly through the capillary membranes to the interstitial fluids, from where it is cleared by various organs. In the kidneys, $^{99m}TcO_4^-$ is filtered in the glomeruli, but 86% is reabsorbed in the proximal tubes. When associated with the radiotracer DMSA, the uptake will occur mainly in the kidneys. (Owunwanne et al., 1995; Zuckier et al., 2004).

Most investigators encourage using DMSA scintigraphy for the early diagnosis of many diseases, because of its cost-effectiveness and safety. A focal reduction or absence of uptake in one or more areas of the kidney is considered abnormal, indicating renal damage. In positive cases, DMSA scintigraphy is found to be highly sensitive in detecting multiple lesions (Piepsz et al., 1999; Stogianni et al., 2007). Besides leukocytosis and renal impairment evidenced by changes in urea and creatinine, we observed that the splenectomized animals had intense anemia associated with low hematocrit in all operated rats. In fact, they all showed clear signs of pale mucosa and hematuria at the 15th postoperative day. Certainly these hematologic findings contributed to the renal failure and to the low uptake of the radiopharmaceutical in the blood of splenectomized animals.

In conclusion, the results suggest that splenectomy in rats results in important hematological changes as well as impairment in renal function and Tc-99m-DMSA uptake.

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RESUMO

Estudo com objetivo de avaliar se a esplenectomia altera a biodistribuição do Tc99m-DMSA em ratos Wistar e a função renal. Usados 2 grupos: esplenectomia(n=6) e controle(n=6) animais não operados. Após 15 dias, administração de 0,1 ml de Tc99m-DMSA via plexo orbital (0,48 MBq). 30 minutos depois, retiradas biópsias rim, coração, pulmão, tireóide, estômago, bexiga, fêmur, sangue. Após pesadas as amostras, foi determinado o percentual de radioatividade/g (% ATI/g) em cada uma delas, com o *Wizard Gama Counter Perkin-Elmer*®. Dosadas uréia e creatinina sérica, hematócrito, plaquetas e leucócitos. Estatística pelo teste t, significância 0,05. Foi observada redução significante no %ATI/g no rim e sangue ($p<0,05$) dos animais esplenectomizados, aumento significante ($p<0.05$) da uréia (88,8±18,6 mg/dL) e creatinina (0,56±0,08), comparado aos controles (51,5±1,6; 0,37±0,02mg/dL, respectivamente) assim como leucocitose, aumento de plaquetas e redução de hematócrito. Conclusão: em ratos a esplenectomia alterou a captação de Tc99m-DMSA pelo rim, e a função renal.

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3.4 Artigo IV

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Biodistribution of the Radiopharmaceutical Technetium-99m-sodium Phytate in Rats after Splenectomy.

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ABSTRACT

Drugs and surgery can interfere with the biodistribution of radiopharmaceuticals and data about the effect of splenectomy on the metabolism of phytate-Tc-99m are scarce. This study aimed at evaluating the interference of splenectomy on phytate-Tc-99m biodistribution and liver function in rats. The SP group rats (n=6) underwent splenectomy. In group C(control) the animals were not operated on. After 15 days, all rats were injected with 0.1mL of Tc-99m-phytate via orbital plexus (0.66MBq). After 30 minutes, liver samples were harvested, weighed and the percentage of radioactivity per gram (%ATI/g) was determined by a Wizard Perkin-Elme gama counter. The ATI%/g in splenectomized rats (0.99±0.02) was significantly higher than in controls (0.4±0.02), (p=0.034). ALT, AST and HDL were significantly lower in SP rats (p= 0.001) and leukocytosis was observed in SP rats. In conclusion, splenectomy in rats changed the hepatic biodistribution of Tc-99m-phytate and liver enzimatic activity.

Key-words: Splenectomy, Sodium phytate, Biodistribution, Liver

INTRODUCTION

The spleen is the largest lymphoid organ in the body. Its functions are many, but they are generally related to 1 of 4 categories: filtration, immunologic, reservoir, and hematopoietic. Many of the spleen's immunologic functions, therefore, are common to other immunologic organs. For example, the spleen is efficient at removing non-opsonized bacteria, mostly encapsulated organisms (Müftüoğlu et al., 2000). Splenectomy is frequently performed for a multitude of reasons, including trauma and various pathologic processes. Blunt abdominal trauma remains the most common indication for splenectomy, but patients with a variety of hematologic disorders also benefit from this procedure. Loss of the spleen, however, leads to a lifelong higher risk of sepsis and severe infection (Bader-Meunier et al., 2001, Waghorn et al., 1997) and may be associated with an increased rate of thromboembolic complications, enhanced arteriosclerosis, and late coronary heart disease (Schilling, 1997). It has been reported that splenectomy might promote hepatic regeneration, prevent liver fibrosis (Akahoshi et al.,

2002, Murata et al., 2001), reduce serum bilirubin concentration and improve liver function (Shimada et al., 2000, Lin et al., 1999). However, considering the relationship between the spleen and liver physiology, nuclear medicine may have an important role in studying the diagnosis and physiology of the liver after splenectomy.

Since its introduction a number of years ago, Tc-99m-phytate colloid has been used as an imaging agent for the liver and spleen (Huet et al., 1980). Subjective assessment of parameters such as liver dimension, colloid shift, and radiopharmaceutical uptake in the bone marrow have been used for both liver disease diagnosis and evaluation of its progression. Subjective assessment of radiocolloid distribution has been shown to be unreliable, and quantitative techniques have been used to evaluate liver function (Jago et al., 1987). The distribution of radiocolloid uptake in the liver and bone marrow has been shown to correlate well with the severity of chronic liver disease, the severity of histologic fibrosis, prognosis, and hepatic function (Hoefs et al., 1997). Thus, quantitation of liver uptake of 99mTc-phytate colloid provides a practical index of hepatic function, by using planar and ***single photon emission computed tomography*** (SPECT) techniques (Strauss et al., 1984). The ability to quantitate individual organ volumes and radiopharmaceutical concentrations with SPECT (Front et al., 1987, Iosilevsky et al, 1989) stimulates the use of Tc-99^{m}-phytate colloid scintigraphy of the liver as a quantitative test of hepatic function. Radiopharceutical biodistribution may provide important information about its uptake to some target organs but post - surgery data are scarce (Chacon et al., 2007, Araújo-Filho et al., 2007).

This study aimed at evaluating if splenectomy interferes with liver function and the biodistribution of Tc-99^{m}-fytate in the liver of Wistar rats.

MATERIAL AND METHODS

Animals: Male Wistar rats weighing 274±21g were obtained from the Center for Health Sciences vivarium, Universidade Federal do Rio Grande do Norte (UFRN), Brazil. The animals were housed in polypropylene cages and fed standard rat chow and water *ad libitum*. Prior to surgery, the rats were fasted overnight in separate cages. After 7 days of acclimatization, they were allocated to two groups of 6 animals each. The groups were denominated splenectomy (SP) and control (C). The protocol for this study was approved by the Institutional Animal Care Committee, and the research was performed in accordance with Brazilian College of Animal Experimentation guidelines

Surgical procedure for splenectomy: The rats were anesthetized with ketamine 50 mg/kg IM and thiopental (20 mg/kg IP), shaved and placed on an operating board and secured with tape. Midline laparotomy (3cm) was performed after skin sterilization with 70% ethanol. The animals were then covered with a small sterile drape with a 6cm circular opening. In group SP (n=6) the spleen was identified and resected after ligature of the splenic vessels with vicryl 5-0 (Ethicon, São Paulo, Brazil). The peritoneal cavity was irrigated with warm (37°C) normal saline. The laparotomy wound was closed with 4-0 nylon in layers and individual rats were placed in separate cages. They were allowed water *ad libitum* and rat chow 24 h postoperatively. The C rats (n=6) were neither anesthetized nor operated on. Hydration was done with normal saline (10 mL/100g weight) injected

subcutaneously in the back of the rats for the first two postoperative days. Postoperative pain was treated with tenoxicam (Roche Pharm., Brazil); 10 mg/kg was given i.m. to the rats once a day for three days.

Body weight and clinical observation: Body weight was monitored weekly throughout the entire 15-day experimental period. Activity, mucosa and skin color were observed daily.

Radioactivity count: Sodium pertechnetate ($Na^{99m}TcO_4$) was obtained by elution of a $^{99}Mo/^{99m}Tc$ generator (*Instituto de Pesquisas Energéticas e Nucleares, Comissão Nacional de Energia Nuclear*, *Brazil*), and 99mTc-phytate was prepared. On the 15th day all the animals were anesthetized again, and injected with 0.lmL of 99mTc-phytate in the orbital plexus, corresponding to radioactivity of 0.66MBq. After 30 min, blood was collected by cardiac puncture for dosages and the animals were killed by lethal dose of anesthetic. Samples of the liver were harvested. The samples were washed in 0.9% saline, weighed on a high-precision digital scale (Bel-Mark 160-II Itália®) and subjected to radioactivity detection using a 1470 Wizard^{TM} Gamma Counter - Perkin-Elmer, Finland, with automatic correction of radiation decline. The percentage of radioactivity/g (%ATI/g) of each organ was calculated by dividing the activity/g of the hepatic tissue by the total activity administered to each animal.

Laboratory analysis: A blood sample was used for hematological analysis of white blood cells (Abbot Cell Dyn 3500 automatic analyzer) and for the serum measure of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactic desydrogenase activity, we used the Konelab 60i spectrophotometer (assay kit from Weiner, São Paulo, Brazil).

Statistics: The data were expressed as mean±standard deviation (SD). The groups were compared using ANOVA and the post-hoc Student test, considering a significance level of $p<0.05$.

RESULTS

Postsplenectomy symptoms such as hematuria, pale mucosa and lethargy were observed in 3 animals but not in control rats. We observed no significant body weight loss in splenectomized rats, compared to controls. Radioactivity per gram of liver tissue ((%ATI/g) was increased by prior splenectomy in the SP group rats (0.99±0.2), when compared to the control (C) group (0.4±0.2). The difference between the two groups was significant (p=0.034; Table 1). To examine the effects of prior splenectomy on liver function, we assessed the levels of ALT, AST and LDH activity, as a marker of liver injury, at 15 days postsplenectomy (Table 1). In splenectomized rats, ALT, AST and LDH values were markedly reduced, compared with the control group rats (p=0.001; Table 1). In addition, splenectomy resulted in increased white blood cell counts in the SP rats (13.8± 2.7 k/μL), when compared to the C rats (2.5±0.9 k/μL; Table 1).

Table 1 – Results of percentage of radioactivity per gram (%ATI/g) of liver, hepatic enzyme activity and WBC count

Examinations	Splenectomy	Control	p[(1)]
Liver (%ATI/g)	0.99 ± 0.2	0.4 ± 0.2	0.034
ALT (U/L)	114.6 ± 10.9	157.5 ± 13.2	0.001
AST (U/L)	51.6 ± 7.8	62.0 ± 2.5	0.041
LDH (mg/dL)	8.2 ± 2.4	16.1 ± 1.1	0.001
WBC (k/µL)	13.8±2.7	2.5± 0.9	<0.001

Mean±standard deviation; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactic dehydrogenase; p-value from t test for independent samples; WBC, white blood cells.

DISCUSSION

Based on current knowledge, there is no doubt that the spleen is a lymphoid organ. Filtration, immunology, reservoir, and hematopoiesis are such important functions, that partial splenectomy or the preservation of the organ by suture is indicated in cases of traumatic injuries (Resende and Petroianu., 2003). Various complications and beneficial effects of the operation are described in postoperative splenectomy (Cadili and Gara., 2008). Splenectomy might promote hepatic regeneration to a certain extent and prevent liver fibrosis (Murata et al.,2001, Chen et al., 1998).

In this study we demonstrated that in splenectomized rats the biodistribution of 99mTc-phytate to the liver was higher than in controls, suggesting that the operation favored the hepatic uptake of the radiopharmaceutical. This result coincided with the improvement in liver function, confirmed by the better alanine aminotransferase, aspartate aminotransferase and lactic dehydrogenase activities in splenectomized rats, compared with controls. In some studies splenectomy promoted hepatic regeneration (Akahoshi et al., 2002, Murata et al., 2001), prevented liver fibrosis (Chen et al., 1998), reduced serum bilirubin concentration and, consequently, reflected the effect of splenectomy on reducing the burden of hepatocyte bilirubin metabolism, improving liver function (Shimada et al, 2000, Lin et al., 1999).

Tomikawa et al (1996) reported that splenectomy increased hepatocyte growth factor (HGF) activities in plasma, suggesting that the spleen played an inhibitory role in hepatic regeneration. HGF, first identified as the most potent mitogen for primary hepatocytes, not only stimulates hepatic regeneration but also accelerates liver function. Moreover, the fact that a large amount of splenic tissue connects to liver tissue through the portal vein system, suggests the existence of a humoral factor originating in the spleen, which thus inhibits hepatic regeneration and promotes liver fibrosis. In rats submitted do hepatic ischemia/reperfusion, prior splenectomy ameliorated acute multiple organ damage (Jiang et al., 2007). These findings, associated to the favorable serum activity of ALT, AST and LDH in our splenectomized rats, partially explain the high hepatic biodistribution of 99mTc-phytate in the operated animals.

The postsplenectomy diagnosis of sepsis based on elevated white blood cell (WBC) counts is confounded by the fact that leukocytosis is considered a physiologic response to splenectomy (Horowitz et al., 1992). Some reports suggest that postsplenectomy WBC

counts in patients with sepsis are greater and more persistent than the WBC in patients without sepsis (Weng et al.,2005, Rutherford et al., 1994).
In this study we were not able to diagnose sepsis in the splenectomized rats. Nevertheless, all the operated animals had leukocytosis (WBC ranging from 11 to 18 k/μL) in the 15th postoperative day. It has been reported that by the fifth day following post-traumatic splenectomy, the WBC count may help the physician to confirm the development of sepsis and the need for treatment. If the WBC is greater than 15x10^{3}/μL on that day, the physician should seriously consider treatment by empiric antibiotics and further diagnostic follow-up to prevent the untoward sequelae of postsplenectomy sepsis (Toutouzas et al., 2002). In conclusion, splenectomy improved the liver uptake of Tc-99m-phyate in rats, coinciding with changes in hepatic enzymatic activity.

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RESUMO

O radiofármaco fitato-Tc-99m. é usado no diagnóstico através de exames de imagem, na dependência de sua biodistribuição. O objetivo do trabalho foi avaliar efeito da esplenectomia na biodistribuição do fitato-Tc-99m e função hepática em ratos Wistar. Sob anestesia e técnica asséptica, os animais do grupo SP (n=6) foram esplenectomizados. Grupo C(controle; n=6) não operado. Após 15 dias, injeção de 0,1ml de fitato-Tc-99m via plexo orbital (0,66MBq). Após 30 minutos, retiradas biópsias hepáticas para determinação do percentual de radioatividade/grama (% ATI/g), usando-se contador gama WizardPerkin-Elmer®. Realizada dosagem de ALT, AST e HDL, e leucometria. Estatística pelo teste t, significância 0,05. O %ATI/g nos ratos esplenectomizados foi 0,99 ± 0,2 e nos controles 0,40 ± 0,2 (p=0,034). ALT, AST e HDL tiveram dosagens significativamente menores nos esplenectomizados (p=0,01), com leucocitose, comparando com controles. Em conclusão**, e**m ratos a esplenectomia provocou alteração na captação de fitato-Tc-99m pelo fígado, coincidindo com alteração da função hepática.

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3.5 Artigo V

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Efeitos da aplicação tópica do mel de *Melipona subnitida* em feridas infectadas de ratos.

Effects of topical application of the honey of *Melipona subnitida* in infected wounds of rats.

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RESUMO

OBJETIVO: Avaliar efeitos do uso tópico do mel da abelha silvestre *Melipona subnitida* na evolução de feridas infectadas de pele. **MÉTODO:** Ratos Wistar foram distribuídos aleatoriamente em grupos de 6, anestesiados com tiopental sódico 20mg/Kg IP e cetamina 30mg/Kg IM e submetidos a exérese de segmento de 1 cm^2 de pele total do dorso. Os ratos do grupo C (não infectado) foram tratados com solução salina sobre a ferida diariamente e no grupo MEL (não infectado) as feridas foram tratadas com mel uma vez por dia. Nos grupos C/I e MEL/I as feridas foram inoculadas com solução polimicrobiana. Culturas foram feitas 24 horas após. Caracterizada a infecção, as feridas foram tratadas com solução salina e mel, respectivamente. No terceiro dia de tratamento foi feita nova cultura. Após epitelização foi contado o tempo de cicatrização e as feridas foram biopsiadas para histopatologia e dosagem de TNF-α, IL-1β e IL-6 no tecido. **RESULTADOS:** O tempo médio de cicatrização do grupo MEL/I foi menor que nos demais grupos ($P<0,05$). Verificou-se que a densidade de colágeno, leucócitos, fibroblastos e dosagem de citocinas (especialmente TNF) foi maior no grupo infectado e tratado com mel que nos demais grupos. Houve significante redução de bactérias Gram-negativas e positivas nas feridas após o tratamento com mel. **CONCLUSÃO:** O uso tópico de mel de *Melipona subnitida* em feridas infectadas da pele de ratos estimulou a resposta imunológica, reduziu a infecção e o tempo de cicatrização.

Descritores: Mel; Abelha; Himenópteros/classificação; Cicatrização de feridas; Citocinas; Agentes antibacterianos/uso terapêutico; Infecção; rato.

ABSTRACT

BACKGROUND: The current study investigated the antimicrobial, immunological and healing effects of Melipona subnitida honey on infected wounds of rat skin.

METHOD: Wistar rats were anesthetized with sodium thiopental 20mg/Kg IP and ketamine 30 mg/kg IM. We evaluated the effects of honey using rats by generating 1 cm^2 full-thickness skin wounds on the dorsum. The wounds of Group C rats (not infected) were treated daily with topic saline solution (0.9%) and in the Group HONEY (not infected) the wounds were treated with topic honey once a day. In the Groups C/I (infected) and HONEY/I (infected) the wounds were inoculated with polymicrobial solution, and tissue bacterial culture was performed 24 hours later. These wounds were treated with topic saline solution and honey, respectively. In the third day of treatment it was made a new bacterial culture. After epithelialization, wound tissue biopsies were used for cytokines dosage and histology. **RESULTS:** The HONEY/I wounds showed more rapid healing and re-epithelialization than in the other groups, and the difference was significant ($p<0.05$). It was observed that the density of collagen, fibroblasts, macrophages and the expression of TNF-á, IL1-â and Il-6 were higher on the HONEY/I wounds then in the other groups. After the treatment with honey, the amount of Gram-positive and Gram-negative bacteria in the infected wounds decreased significantly. **CONCLUSION:** The results may encourage the use of honey in skin infected wounds because it stimulated cytokine production, reduced the healing time and had antibacterial activity.

Key words: Honey; Bee; Hymenoptera/classification; Wound Healing; Cytokines; Anti-bacterial agents/ therapeutic use; Infection; Rat.

INTRODUÇÃO

O tratamento de feridas infectadas continua sendo um tema de grande importância médica, particularmente na prática cirúrgica, numa época em que o uso indiscriminado de antimicrobianos tem dificultado o tratamento hospitalar dos pacientes, com repercussão no aparecimento cada vez maior de microorganismos resistentes aos antimicrobianos. Diversas terapias alternativas têm sido testadas, dentre as quais o uso tópico de substâncias com elevada osmolaridade[1], sendo o açúcar e seus derivados citados como agentes cicatrizantes e antimicrobianos[2,3]. Nesse sentido, o uso do mel de abelha como elemento terapêutico tem mostrado resultados promissores. Tem sido demonstrada sua atividade como antibacteriano e facilitador da cicatrização no tratamento de feridas[4] e queimaduras, atuando como importante barreira viscosa, impedindo a entrada de substâncias e a perda de fluidos para o meio externo[5,6]. A atividade antibacteriana do mel ocorre devido à sua alta osmolaridade, que o faz agir como bactericida e bacteriostático[7]. Estudos *in vitro* têm observado que o mel é eficaz contra bactérias antibiótico-resistentes como o *Estaphylococcus aureus* e *Pseudomonas aeruginosa*[8-11].

Tendo em vista estas propriedades do mel de abelha, o presente trabalho teve como objetivo avaliar a influência do mel de *Melipona subnitida,* abelha silvestre comum no nordeste Brasileiro, conhecida como Jandaíra, no tratamento de feridas infectadas da pele de ratos.

MÉTODO

Foram usados 24 ratos da raça Wistar, com peso de 221± 18g, provenientes do Biotério do Núcleo de Cirurgia Experimental da Universidade Federal do Rio Grande do Norte. O protocolo foi aprovado pelo Comitê Institucional de Ética em Pesquisa e o manuseio dos animais seguiu as normas e orientações do Colégio Brasileiro de Experimentação Animal (COBEA). Foram selecionados aleatoriamente e alocados em

quatro grupos, com seis ratos cada: Grupo C – ratos com feridas não infectadas, submetidos a tratamento tópico com solução salina 0,9%. Grupo C/I – ratos com feridas infectadas tratadas com solução salina. Grupo MEL – ratos com feridas não infectadas tratadas com mel. Grupo MEL/I – ratos com feridas infectadas tratadas com mel.

Metodologia empregada na confecção das feridas - Os ratos foram anestesiados com tiopental sódico 20 mg/Kg IP e cetamina 30 mg/Kg IM, submetidos à depilação da pele dorsal e antissepsia com álcool 70% (Figura 1A). Posteriormente, foi provocada uma ferida aberta pela retirada de um fragmento de $1cm^2$ de pele total do dorso (Figura 1B). Nos grupos de feridas infectadas C/I e MEL/I as feridas foram inoculadas com duas gotas de solução polimicrobiana de fezes frescas dos próprios animais (Figura 1C). Esta solução foi preparada diluindo-se 1g de fezes em 10 ml de solução salina estéril com posterior tamização com gaze dobrada quatro vezes. As feridas de todos os animais foram cobertas com lâmina de polietileno esterilizada, fixada sobre a lesão com quatro pontos simples de monofilamento de nylon 4-0 (Figura 1D). A dor pós-operatória foi tratada com tenoxicam (Roche Farm., Brasil); 10 mg/Kg foram injetados no subcutâneo uma vez por dia durante três dias.

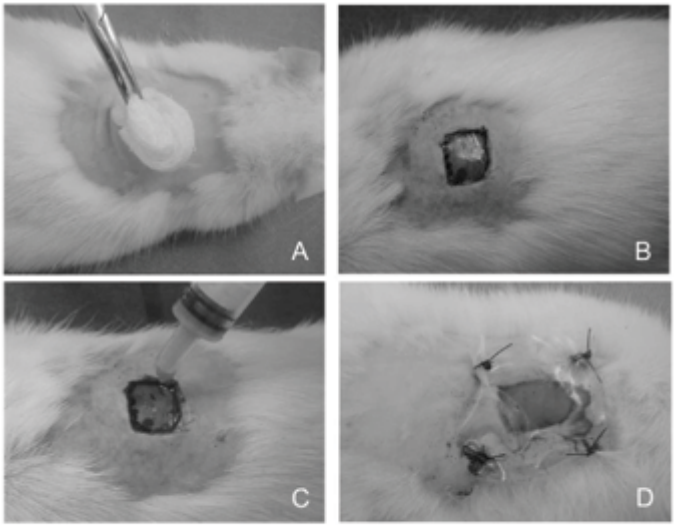

Figura 1 - *1A, antissepsia da pele com álcool a 70%; 1B, Excisão de 1cm² de fragmento de pele do dorso do animal; 1C, inoculação com solução polimicrobiana; 1D, proteção da ferida com lâmina de polietileno fixada com fio de nylon 4-0.*

Tratamento das feridas - Nos animais dos Grupos C (não infectado) e C/I (infectado) as feridas receberam tratamento tópico com uma dose diária de duas gotas de solução salina. Nos Grupos MEL (não infectado) e MEL/I (infectado) as feridas foram tratadas com duas gotas de mel, uma vez por dia.

Análise do mel – Foi utilizado o mel da abelha *Melipona subnitida* adquirido do C. Sertanejo, Natal-RN, Brasil. A análise físico-química do mel foi realizada no Laboratório de Tecnologia de Alimentos do Departamento de Farmácia da UFRN. Todos os parâmetros analisados foram resultantes da média de três repetições. A

umidade das amostras foi determinada por meio de um refratômetro Abbe com escala de refração (ND) 1300-1720 e 0-95%, temperatura de 20ºC, modelo 2 Waj, Biobrix, São Paulo, Brasil. A prova do Hidroximetilfurfural (HMF) foi realizada para se verificar a possibilidade de adulteração por adição de açúcar, estocagem inadequada ou superaquecimento, através da determinação da absorbância da amostra, utilizando espectrofotômetro digital, com faixa de 200-1000 nm (monofeixe), banda de passagem de 5 nm (fixa), modelo SP-220, Bioespectro, São Paulo, Brasil. O teor de cinzas das amostras foi determinado em forno mufla Splabor, São Paulo, Brasil, a 600ºC por cinco horas, com temperatura máxima de trabalho permitida 1.100ºC. Foi utilizado o pHmetro digital de bancada modelo pH21, Hanna Instruments, São Paulo, Brasil, para a medida do pH e a acidez total foi dosada pelo método da titulação simples. Sólidos insolúveis em água foram determinados com uso de estufa 515-Fanem, São Paulo, Brasil, a 135ºC, após lavagem do mel em água a 80ºC para eliminação de açúcares. Todos esses parâmetros foram comparados com os estabelecidos pelo Regulamento Técnico de Identidade e Qualidade do mel do Ministério da Agricultura, Brasil, 2000[12]. Análise microbiológica do mel foi feita utilizando-se os meios de cultura agar sangue e agar McConkey.

Procedimentos utilizados para avaliação das feridas - Vinte e quatro horas após a inoculação, foi feita coleta de secreção de todas as feridas com swab estéril para cultura microbiológica, utilizando os meios agar sangue e agar McConkey para identificação de microrganismos Gram positivos e Gram negativos respectivamente. No terceiro dia de tratamento foi feita nova cultura para se verificar a ação do mel sobre os microrganismos identificados na primeira cultura.

Foi considerado tempo de cicatrização o intervalo entre a confecção da ferida e a manhã em que as feridas apresentavam-se completamente epitelizadas. Após epitelização as feridas foram biopsiadas, utilizando-se metade dos materiais para dosagem de citocinas teciduais e outra metade para histopatologia. Os materiais foram triturados e o homogeneizado foi utilizado para dosagem das citocinas fator de necrose tumoral alfa (TNF-α), interleucina-1 beta (IL-1β) e interleucina 6 (IL-6), utilizando-se *Kit Preprotec, USA*, e técnica ELISA. Um segmento de cada ferida foi fixado em formalina 10% e incluído em parafina. Cortes de 5 µm foram corados com hematoxilina e eosina e analisados através de microscopia óptica por patologista experiente, sem conhecimento prévio dos respectivos grupos. A análise quantitativa foi feita quanto ao montante de leucócitos, fibroblastos e fibras colágenas, utilizando um sistema digitalizador e analisador de imagens. A área total dos campos microscópicos foi observada em microscópio óptico (Olympus BX50), cuja imagem foi capturada por câmera *Samsung* de alta resolução e digitalizada através do *Software Image Pro-plus*, (*Media Cybernetics- LP, USA*). Cada campo digitalizado foi quantificado em unidades *pixel* com coordenadas definidas, sendo avaliados cinco campos microscópicos aleatórios por lâmina. Após selecionada a resolução desejada, as imagens foram armazenadas para quantificação da densidade da reação inflamatória.

Estatística – A avaliação estatística foi realizada por meio da análise de variância (ANOVA), seguida pelo teste de Bonferroni, considerando-se diferenças significantes quando $p<0,05$.

RESULTADOS

A análise físico-química do mel utilizado demonstrou que todos os parâmetros estavam de acordo com o estabelecido pelas normas do Ministério da Agricultura, Brasil, podendo ser considerado de boa qualidade (Tabela 1). O exame de cultura revelou que o mel não apresentava contaminação bacteriana.

Tabela 1 – *Parâmetros da análise físico-química do mel de Melipona subnitida (jandaíra), comparados com os estabelecidos pela legislação do Ministério da Agricultura-Brasil.*

Amostras	Umidade (%)	HMF (mg/Kg)	pH	Acidez (meq/kg)	Cinzas (%)	Sólidos Insolúveis (%)
Mel de jandaíra	18,06	23,90	3,85	41,66	0,01	0,01
Referência: Ministério Agricultura	Máximo 20	Máximo 40	3,3 a 4,6	Máximo 50	Máximo 0,6	Máximo 0,1

HMF, hidroximetilfurfural.

Não houve diferença significante no tempo de cicatrização das feridas dos animais dos Grupos C, C/I e MEL ($p>0,05$) (Tabela 2). Entretanto, quando feridas infectadas foram tratadas com mel (Grupo MEL/I), observou-se diminuição significante no tempo de cicatrização, quando comparado com os demais grupos. (Figura 2)

Tabela 2 - *Comparação do tempo de cicatrização entre os grupos estudados.*

Grupos	Dias
C	13,5 ± 0,86
C/I	14,0 ± 2,5
MEL	14,0 ± 2,6
MEL/I	11,3 ± 2,2 *

* *P<0,05 comparado com C, C/I e MEL.*

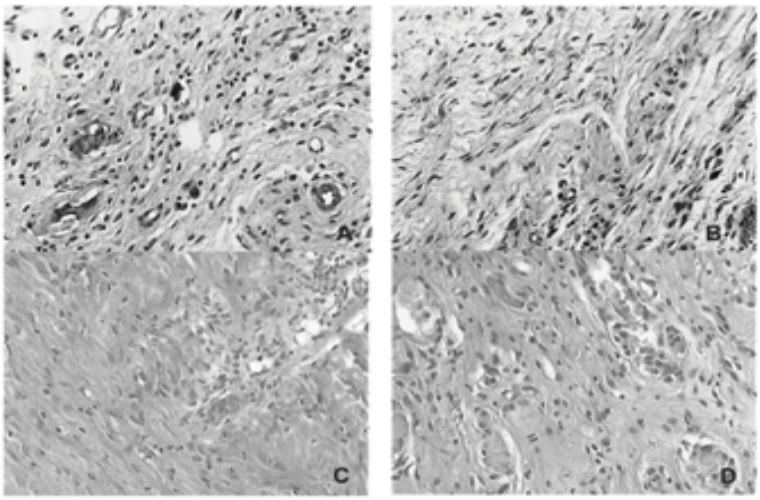

Figura 2 – *Estudo histológico da cicatrização das feridas. A: densidade do colágeno, leucócitos e fibroblastos em lâmina de animal do grupo controle. B: a densidade dos elementos estudados mostra-se maior no grupo MEL do que no grupo controle. C: aspecto histológico de ferida infectada tratada com solução salina (grupo C/I). D: alta densidade de leucócitos, colágeno/fibroblastos em animal do grupo MEL/I. (HE, 100x).*

O tratamento de feridas infectadas com mel (Grupo MEL/I) resultou em reação cicatricial com densidade de colágeno, leucócitos e fibroblastos significativamente maior do que nos demais grupos. Igualmente, a presença do mel em feridas não infectadas fez surgir colágeno, leucócitos e fibroblastos com densidade maior do que no Grupo controle (C), caracterizando diferença significante ($p<0,05$) (Tabela 3).

Tabela 3 - *Densidade do colágeno e fibroblastos na pele de ratos tratados ou não tratados com mel de abelha jandaíra.*

	Observação até a Epitelização das Feridas			
Animal	**Grupo C**	**Grupo C/I**	**Grupo MEL**	**Grupo MEL/I**
1	433,87	228,90	772,12	890,84
2	386,40	350,23	653,28	1137,30
3	543,22	280,78	681,40	1256,89
4	456,66	321,67	802,43	1103,65
5	397,12	255,88	744,27	950,12
6	427,80	402,90	651,89	933,50
Média±dp	440,8 ± 56,2	306,7 ± 64,3	717,56± 64,25	1045,38± 142,8*

*****Diferença significante pelo teste t de Bonferroni, comparado com os grupos C, C/I, MEL ($p<0,05$).*

A presença do mel de abelha em feridas não infectadas (Grupo MEL) estimulou a produção das citocinas TNF-α e IL-1β, uma vez que, comparando-se os valores com os observados nas feridas dos ratos controles, as diferenças foram estatisticamente significantes ($p<0,05$). Quanto à IL-6, não foi detectado aumento significante na dosagem tecidual no grupo MEL, quando comparado ao controle (Tabela 4). Nos tecidos cicatriciais das feridas infectadas tratadas com mel (Grupo MEL/I) foi observado um aumento significante ($p<0,05$) da expressão das citocinas TNFα, IL-1β e IL-6, em comparação com as feridas infectadas tratadas com solução salina (Grupo C/I) (Tabela 4).

Tabela 4 - *Dosagem de citocinas de acordo com os grupos estudados.*

Grupos	**TNF-α**	**IL-1β**	**IL-6**
C	77 ± 8,2	53 ± 9,6	48 ± 7,1
MEL	98 ± 4,7*	84 ± 8,3*	53 ± 8,9
C/I	115 ± 12	91 ± 11	84 ± 6,6
MEL/I	175 ± 18,4**	154 ± 17**	122 ±10,2**

*****$p<0,05$ comparado com C. ******$p<0,05$ comparado com C, MEL, C/I.*

Na secreção das feridas limpas dos animais dos Grupos C e MEL não ocorreu crescimento de microrganismos nos meios de cultura. Nas feridas infectadas tratadas com solução salina (Grupo C/I) foi observado crescimento de bactérias Gram-positivas

(*Staphylococcus sp*) e Gram-negativas (*Proteus mirabilis, Klebsiella sp, Acinetobacter sp, Escherichia coli e Pseudomonas sp*) na primeira cultura, 24 horas após a inoculação das feridas. Na segunda cultura de secreção colhida no terceiro dia de observação as mesmas bactérias estavam presentes nos meios de cultura. Nos animais cujas feridas infectadas foram tratadas com mel (MEL/I) observou-se que em 100% dos casos o tratamento foi eficaz na eliminação de microrganismos Gram-negativos como *Pseudomonas sp, Klebsiella sp, Acinetobacter sp e Escherichia coli*. O mel de *Melipona subnitida* inibiu com menor eficácia, porém de modo significante, o crescimento de *Proteus mirabilis* e de *Staphylococcus sp.* Esse efeito ocorreu em 50% dos espécimes em que esses microrganismos estiveram presentes nas feridas infectadas, por ocasião da primeira cultura (Tabela 5).

Tabela 5 - *Microrganismos isolados nas feridas infectadas tratadas com mel (grupo MEL/I) na 1ª cultura 24 h após inoculação das feridas e no 3º dia de tratamento com mel.*

Rato nº	1ª cultura (24 horas após inoculação das feridas)	2ª Cultura (3º dia de tratamento com mel,) das feridas infectadas
01	*Staphylococcus sp* Acinetobacter sp *Proteus mirabilis*	*Proteus mirabilis*
02	*Staphylococccus sp* Klebsiella sp; Acinetobacter; *Proteus mirabilis*	*Staphylococcus sp*
03	*Staphylococus sp; Pseudomonas sp;* *Acinetobacter; Proteus mirabilis*	*Proteus mirabilis*
04	*Staplylococcus sp;* *Pseudomonas sp; Proteus mirabilis*	*Proteus mirabilis*
05	*Staphylococcus sp; Pseudomonas sp;* *Acinetobacter; Proteus mirabilis*	*Staphylococcus sp*
06	*Staplylococcus sp;* *Pseudomonas sp; Escherichia coli*	*Staphylococcus sp*

DISCUSSÃO

Nos últimos cinco anos tem aumentado o interesse pelo uso do mel de abelhas no tratamento de feridas, mas ainda há pouca evidência científica para dar suporte ao seu uso tópico em feridas e queimaduras[13]. No presente trabalho, foram estudados os efeitos em feridas infectadas, do mel de *Melipona subnitida*, uma abelha silvestre sem ferrão, conhecida como Jandaíra, comum na região do semi-árido do Nordeste do Brasil. Seu mel tem sido utilizado empiricamente na medicina popular para tratamento de doenças respiratórias, doenças de pele e tecidos moles, e não há na literatura relatos a respeito do seu uso para tratamento de feridas limpas e infectadas. A análise microbiológica e físico-química do mel de *Melipona subnitida* usado na pesquisa revelou que se encontrava de acordo com as normas de qualidade estabelecidas pelo Ministério da Agricultura-Brasil, adequado para uso no presente trabalho. Para uso do mel em humanos deve ser assegurada boa procedência e segurança do ponto de vista físico-químico e microbiológico, aliado ao fato de ter boa relação custo-efetivo[14].

O mel de abelhas em geral é composto por cerca de 40% de frutose, 20% de água, aminoácidos, vitaminas (ácido nicotínico, piridoxina e tiamina), enzimas (diastase, invertase, catalase e glicose-oxidase), peróxido de hidrogênio, e minerais

(potássio, ferro, magnésio, fósforo, cobre, zinco e cálcio)[15,16]. Em nosso estudo, a avaliação microbiológica nas feridas infectadas dos animais dos Grupos MEL/I e C/I mostrou atividade antimicrobiana do mel da abelha *Melipona subnitida* contra bactérias Gram-negativas, incluindo seu efeito anti-*Pseudomonas*. Os resultados desse estudo também mostraram claramente que o mel tem potencial para ser usado como antimicrobiano para prevenir e controlar infecções por Gram-positivos (*Staphylococcus*). Estudo *in vitro* realizado por French *et al*[17] demonstrou esse potencial e outros trabalhos têm apontado eficácia do mel contra cocos Gram-positivos e *Pseudomonas* considerados multiresistentes[8-10]. A atividade antibacteriana do mel está relacionada com algumas propriedades: é uma solução supersaturada com potente atividade osmótica, tem o pH entre 3,2 e 4,5 e esta acidez é suficiente para inibir o crescimento de muitos microrganismos. A propósito, o pH do mel de *Melipona subnitida* foi caracterizado como sendo 3,85. O peróxido de hidrogênio produzido pela glicose-oxidade é certamente o componente antibacteriano mais importante do mel e vários outros fatores fitoquímicos e imunoquímicos estão sendo avaliados[7,16]. Estudo comparativo entre mel e açúcar demonstrou que o mel é mais eficaz contra infecção de feridas[18].

No presente estudo a análise histopatológica das lesões infectadas e tratadas com mel revelou significante aumento da presença de leucócitos, notadamente macrófagos, fibroblastos e colágeno nos tecidos examinados. Macrófagos são células importantes para a regulação da cicatrização de feridas, de modo que substâncias tópicas que ativam macrófagos podem gerar estímulo pró-inflamatório, proliferação celular e progressão do processo cicatricial[19].

Estabelecidos os efeitos do mel de abelhas em feridas limpas e infectadas, resta o esclarecimento a respeito dos mecanismos responsáveis pela aceleração do processo de cicatrização e atividade antimicrobiana. Estudos têm mostrado que o efeito do mel na cicatrização de feridas pode estar relacionado ao aumento na liberação de TNF-α[20]. No presente trabalho conseguimos detectar que a maior expressão de citocinas ocorreu com o TNF-α, seguido das interleuninas IL_1β e IL-6 nos tecidos de feridas infectadas e tratadas com mel. Este achado pode em parte explicar a aceleração da cicatrização dessas feridas quando comparado com aquelas não tratadas com mel. De fato, após estudo realizado por Tonks *et al* [21] em que utilizaram mel de abelha e mel artificial para elucidar a liberação das citocinas TNF-α, IL–1β e IL-6 por monócitos, foi sugerido que os efeitos regulatórios do mel sobre a cicatrização das feridas estão relacionados a outros componentes além dos açúcares presentes no mel, alguns fatores ainda desconhecidos, que induzem a liberação de citocinas. Seu estudo demonstrou que a atividade dos monócitos, intimamente envolvidos na cicatrização das feridas, é modulada pelo mel. Entretanto, os mecanismos pelos quais o mel afeta a liberação de agentes antiinflamatórios, pró-inflamatórios e fatores do crescimento são desconhecidos, podendo atuar em monócitos, macrófagos, células endoteliais e fibroblastos.

Em conclusão, nossos achados sugerem que parte da eficácia do mel de *Melipona subnitida* em melhorar a cicatrização e tratar a infecção em feridas pode ser devida a um incremento na defesa imunológica orgânica e tecidual, além de sua atividade antimicrobiana.

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3.6 Artigo VI

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Biodistribution of the radiopharmaceutical sodium pertechnetate after biliopancreatic bypass with a duodenal switch

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ABSTRACT

Study with the purpose to examine the effects of duodenal switch (DS), regularly performed in morbidly obese patients, on biodistribution of sodium pertechnetate in several organs of rats. There was no early or late mortality in either rats groups. The values of percent radioactivity per gram of tissue (%ATI/g), showed no significant difference in liver, stomach, small bowel, duodenum, kidney, heart, bladder, bone and brain, when compared the DS rats with sham and controls rats. A postoperative significant increase ($p<0.05$) in mean %ATI/g levels was observed in spleen, pancreas and muscle in group DS rats, as compared to group S and C rats. In the lung there was an increase and in thyroid a decrease in mean %ATI/g of DS rats, when compared to sham rats ($p<0.05$). In conclusion, the biliopancreatic diversion with duodenal switch in rats modified the biodistribution of sodium pertechnetate in thyroid, lung, pancreas, spleen and muscle.

Key words: Bariatric surgery, duodenal switch, sodium pertechnetate, biodistribution

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INTRODUCTION

Worldwide, it is estimated that more than 300 million people are obese (Haslam & James, 2005). Obesity, particularly abdominal obesity, is associated with increased risks of hypertension, diabetes, hyperlipidemia, sleep apnea, coronary heart disease, and stroke (Li et al., 2005). The accumulating evidence identifying obesity-related mortality and comorbidities is an important factor that has led to increased numbers of patients seeking treatment through bariatric surgery. This is a surgical procedure that reduces caloric intake by modifying the anatomy of the gastrointestinal tract and provides effective treatment for many patients with morbid obesity. Bariatric operations are classified as either restrictive or malabsorptive. Restrictive procedures limit intake by creating a small gastric reservoir with a narrow outlet to delay emptying. Malabsorptive procedures bypass varying portions of the small intestine where nutrient absorption occurs (DeMaria & Jamal., 2005). The biliopancreatic diversion with duodenal switch

(DS) is a hybrid operation involving both components of weight loss surgery. In the DS, a lateral gastrectomy provides a restricted gastric volume, while excess fat absorption is limited by shortening the functioning length of the intestine. This involves diversion of the biliopancreatic secretions by partitioning the bowel into two limbs – an alimentary channel, and the biliopancreatic (afferent) limb. These two limbs of small bowel are reconnected to form the common channel (Hess, 1998; Marceau et al., 1998). DS produces a sustained weight loss, with low side effects and without any increase in the perioperative morbidity and mortality rate, comparing to other bariatric operations (Biron et al., 2004; Rabkin., 2004; Anthone et al., 2003).

There are numerous complications that may arise following any of the bariatric surgical procedures that require understanding and delineation of the specific anatomy of the operation performed. These complications may include nutrient deficiencies or gastrointestinal pathology. Anastomotic leak and stricture commonly occurs (Schauer et al., 2000; DeMaria et al., 2002). The most frequently reported complication of gastric band placement is prolapse of stomach superiorly through the band producing obstruction at the band (O'Brien et al., 1999). Radiographs and scintigraphies may show an air fluid level in the gastric pouch, malposition, angulation of the band bands, problems with gastric emptying, and gastric obstruction (O'Brien et al., 2005).

The radiopharmaceuticals are frequently used in diagnostic procedures (Bingener-Casey et al., 2002, Carter & Kotlyarov, 2005). Examinations of gastric emptying, patency of anastomoses, enterogastric reflux, hepatic and thyroid diseases, osteoporosis and metastasis, frequently are carried after bariatric surgery (Kitabaiashi et al., 2002; Fonseca et al., 2000; Badiali et al., 2001; Obradovic et al., 2000). Several works have studied the relationship between chemotherapy, phytotherapy and other drugs with the biodistribution of sodium pertechnetate (Braga et al., 2000; Oliveira et al., 2002; Gomes et al., 1998; Ripoll-Hamer et al, 1995; Simões et al., 1997; Feliciano et al., 2002, Abreu et al., 2006., Santos et al., 1995). With regard to potential consequences of bariatric surgery in the biodistribution of radiopharmaceuticals, little or no study was published until now.

As DS is a restrictive and malabsorptive operation, of raised anatomical and metabolic repercussion, postoperative evaluation of patients through scintigraphy can be necessary. If the biodistribution of sodium pertechnetate to organs and tissues is modified as a result of bariatric surgery, scintigraphic examinations can be false-positive or false-negative, resulting in repetition of examinations with unnecessary exposition of patient to ionizing radiations. The purpose of this study was to examine the effects of DS, similar to that performed in morbidly obese patients, on biodistribution of sodium pertechnetate in several organs and tissues of rats.

MATERIAL AND METHODS

Male Wistar rats (12 weeks of age and weighing 328g ± 33g) were obtained from Center of Experimental Surgery-UFRN, Brazil. Animals were housed in polypropilene cages for 1 week to acclimatize them to the study laboratory: 12-h light/dark cycle, room temperature of 25°C, and 50% relative humidity. Rats were allowed free access to water and standard rat chow (Labina, Purina®). The study was approved by the Institutional Animal Care Committee of the University Hospital-

UFRN, Brazil, and the international guidelines for the care and use of laboratory animals were followed throughout the study.

Rats were randomly divided into three groups: duodenal switch group (DS), control group (C), and sham-operated group (S). After 12 h of food deprivation, rats were anesthetized with a ketamine and xylazine mixture (200 mg: 5 mg, 0.1 ml/100 g, IM), the abdomen was shaved and prepared, and the operations were performed with aseptic technique. All the surgical procedures were performed by the same investigator, a well trained and experienced surgeon in animal surgery and three previous series of experiments were performed in sequence to develop the DS model. A single intramuscular dose of 75 mg/kg of ceftriaxone sodium (Roche, SP, Brazil) was given as antimicrobial prophylaxis 30 minutes before the surgical procedures.

In the DS rats (n=7) the surgery was performed via an upper 3 cm midline incision. A sleeve 75% longitudinal gastrectomy was performed leaving a tabularized stomach. (Figure. 1). The duodenum was divided about 1 cm beyond the pylorus. The stump of duodenum was closed with running sutures. The small bowel was divided at its midpoint, and the distal end (alimentary limb) was anastomosed to the proximal duodenum. The proximal end of the divided small bowel, now the distal end of the iliopancreatic limb, was anastomosed to the ileum 5 cm from the ileocecal valve to create a 10cm common channel. (Figure 1). The anastomosis were hand sewn using interrupted polypropilene 6-0 sutures (Prolene® - Ethicon), using a surgical microscope (DFV- M900, São Paulo, Brazil). The hydration was done with normal saline (10ml) injected subcutaneously into the back of the rats for the first 2 postoperative days. Postoperatively pain was treated with tenoxicam (Roche Pharm., Brazil); 0.5 mg/kg was given to the rats once a day for 3 days.

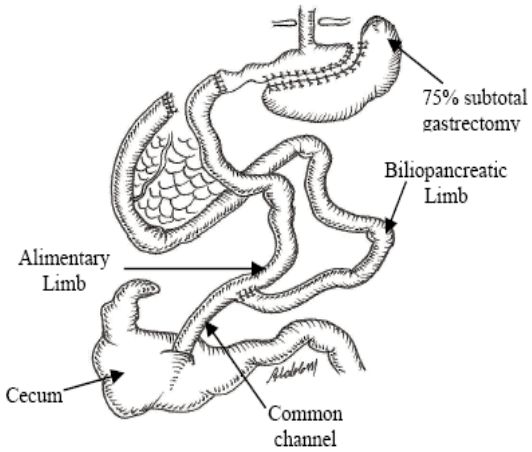

Figure 1 - The biliopancreatic diversion with duodenal switch operation

Rats were allowed to drink and eat 24 h after surgery. Liquid diet (Nestogeno, Nestlé, Brazil, 1 cal/g) was provided for the first 2 days, followed by ground Purina Labina chow. The sham rats (n=7) were submited to a laparotomy and soft malipulation of

stomach, duodenum and small bowel. The control rats (n=7) were not operated. Body weight, using a digital scale (Filizola®, São Paulo, Brazil), and operative complications were evaluated daily for 10 days.

On the 10th day all the animals were anaesthetized again, and injected with 0.l mL of $Na^{99m}TcO_4$ in the orbital plexus, corresponding to activity of 0.66 MBq. After 30 minutes, the animals were killed by lethal dose of anesthetic. Samples of the liver, spleen, stomach, small intestine, duodenum, pancreas, kidney, heart, lung, thyroid, bladder, muscle, bone (femur) and brain were harvested. The samples were washed in 0.9% NaCl, weighed on a high-precision digital scale (Bel-Mark 160-II ItáliaÒ) and subjected to radioactivity detection using a 1470 Wizard^{TM} Gamma Counter- Perkin-Elmer, with automatic correction of radiation decline. The percentage of activity/g (%ATI/g) of each organ was calculated by dividing the activity/g of the tissue by the total activity administered to each animal.

Data are expressed as mean ± standard deviation. Statistical analysis was performed using one-way analysis of variance and the Tukey test as appropriate. $p<0.05$ were considered to be statistically significant.

RESULTS

There was no early or late mortality in either rat groups. The values of all sodium pertechnetate biodistributions, expressed as percent radioactivity per gram of tissue (%ATI/g), are shown in Table 1. There was no significant difference in %ATI/g in liver, stomach, small bowel, duodenum, kidney, heart, bladder, bone and brain, when compared the DS rats with S and C rats. A postoperative significant ($p<0.05$) increase in mean %ATI/g levels was observed in spleen, pancreas and muscle in DS rats, as compared to S and C rats. Looking at each group separately, in the lung there was a significant ($p>0.05$) increase and in thyroid a decrease in mean %ATI/g of DS rats, when compared to sham rats.

Preoperative and postoperative data of weight loss of DS rats are summarized in Figure 2. Before treatments, there were no significant differences between the groups in terms of weight. The operative time for the sham operation was equivalent to that of DS. Postoperatively in group DS there was a significant decrease in body weight during the 10 days of observation attributed to the effects of operation ($p<0.05$). Body weight in the C and S groups gradually increased by day 10, when the rats were euthanized (Figure 2). So, the differences in the mean weight of DS rats at the end of the 10 days were significant, when compared to C and sham rats ($p<0.05$).

Body weight decreased significantly ($p<0.05$) in the duodenal switch group when compared with the sham and control groups. No difference was observed between sham and control rats.

In day 1 (operative day) no difference was observed among all the rat weights, meaning that the groups were uniform.

DISCUSSION

Obesity has become an epidemic condition and in the United States, the percentage of adults who are obese increased from 15.3% in 1995 to 23.9% in 2005. Approximately 4.8% are considered to be extremely or morbidly obese. Worldwide, it is estimated that more than 300 million people are obese (Ogden et al., 2006; Haslam & James, 2005).

Obesity results in a major risk for serious diseases, including diabetes mellitus, cardiovascular disease, hypertension, dyslipidemia, degenerative arthritis, certain forms of cancer and respiratory problems and may result in socioeconomic and psychosocial impairment (Ali et al., 2006). Therefore many weight-lowering therapies, such as dietary and pharmaceutical regimens completed with physical exercise, have been proposed. However, almost 95% of morbidly obese (BMI $\geq$40 kg/m^2) patients fail to achieve acceptable long-term weight loss with any form of non-operative treatment Ali et al., 2006). Hence, great efforts have been made to achieve better results using surgery. The bariatric operations have proved very successful and cost effective in achieving marked and maintained weight loss. In spite of good results, bariatric surgery may cause anatomic and metabolic complications (DeMaria et al., 2002). The diagnostic of these side effects may require image exams such as radiography and scintigraphy.

To understand and explore the relationship between bariatric surgery and biodistribution of radiopharmaceuticals to organs and tissues, a well-characterized and reproducible animal model was used. To achieve this purpose, this is a report of the experience with a biliopancreatic diversion with duodenal switch rat model.

Technetium-99m (99mTc) is the most used radioisotope in nuclear medicine as well as in basic research. It has a low mean life (6 h), low radiation and a low doses is needed for diagnostic procedures (Braga et al., 2006). It has been used in vivo and in vitro under the form of sodium pertechnetate, in the study of diseases, drugs, chemotherapics and phytoterpics that interfere in its biodistribution (Oliveira et al, 2002; Amorim et al., 2003). Red blood cells and leukocytes labeled with 99mTc have been used in the study of drugs, and in the evaluation of the mononuclear system (Palestro et al., 2006).

Postoperative scintigraphic exams are accomplished to diagnose digestive bleeding (Bingener-Casey et al., 2002), gastroesofageal reflux (Adachi et al., 1999)**,** as well as anastomoses patency (Blachar, 2004)**.** Diagnosis of cancer metastasis (Leen., 1999)**,** and postoperative chances in kidney, liver, lung, heart and other organs are done using scintigraphy with radiotracers and pertechnetate (Aktas et al., 2005; Chalela, 1999).

In the present work the DS did not affect the biodistribution of sodium pertechnetate in liver, stomach, small bowel, duodenum, kidney, heart, bladder, bone and brain. The stomach is commonly examined in the postoperative of bariatric surgery to diagnose mainly leaks, because leakage from the reservoir or the connecting tube is a late complication of bariatric surgery. In small leaks the escape rate of a radio-contrast agent may be low, and hence these leaks may be overlooked on radiography. By contrast, using scintigraphy, the slowly diffusing pertechnetate is re-absorbed by

peritoneal blood vessels and subsequently absorbed into the gastric mucosa, because of high gastric affinity for pertechnetate. This fact may explain because, in this study, biodistribution of pertechnetate was not affected in stomach. It has been suggested a higher accuracy of scintigraphy as compared with radiography in the assessment of leakage in bariatric surgery. (Van DenBossche et al., 2002). The liver, small bowel, duodenum, kidney, heart, bladder, bone and brain have a few affinity for pertechnetate and we did not find alteration in biodistribution of this radiopharmaceutical in them. So, if a scintigraphy is to be done to study anyone after bariatric surgery, false results certainly are not expected.

The biodistribution of pertechnetate showed elevated in spleen and this organ may be the target of future scintigraphies. Splenectomy during exploratory laparotomy after bariatric surgery significantly increases morbidity and mortality. Peters et al, (1990) related that six of 200 patients having primary or revisional vertical banded gastroplasty for morbid obesity or failure of previous bariatric surgery had splenic injury. Eventual scintigraphy in these patients should be interpreted with caution.

Obese patients often complain of dyspnea despite not having demonstrable lung disease It has been hypothesized that increased chest wall mass along with increased abdominal size imparts a restrictive ventilatory defect, which then imposes an increased work of breathing (Sahebjani, 1996). The bariatric procedures are performed in morbidly obese patients who tend to have reduced chest wall compliance, reduced lung volume, less functional residual capacity, and increased physiologic intrapulmonary shunt during mechanical ventilation (Damia et al., 1988). Therefore, morbidly obese patients may be at risk for intraoperative and postoperative complications, which may be diagnosed by scintigraphy. Together, pulmonary emboli, anastomotic leaks, and respiratory failure account for 80 percent of all deaths in the first 30 days following bariatric surgery (Virji & Murr, 2006). As in this study the biodistribution of pertechnetate was higher in lungs of operated rats then in controls, the interpretation of eventual lung exams (Giordano et al., 1997) in patients has to be with caution.

In the past five years, several confirmed cases of pancreatic disorders occurred in persons who had undergone bariatric surgery (Service et al., 2005). Some patients presented with repeated episodes of symptoms of profound postprandial neuroglycopenia associated with endogenous hyperinsulinemic hypoglycemia and demonstration of diffuse beta-cell hypertrophy and hyperplasia in resected pancreatic tissue. A plausible explanation, with broader implications, is that bariatric surgery results in long-term stimulation of beta-cell growth and activity by gut hormones (glucagon-like peptide 1) that are perturbed as a result of the altered gastrointestinal transit. These metabolic disorders may explain the high radioactivity observed in pancreas of DS operated rats (D'Alessio & Vahl, 2004). Moreover, at least in rodents, GLP-1 triggers beta-cell neogenesis and proliferation while inhibiting apoptosis (Brubaker & Drucker, 2004).

In this study the %ATI/g in thyroid was decreased in DS rats compared to sham rats. Paradoxically, a great percentage of obese patients have hypothyroidism, that is improved after bariatric surgery (Raftopoulos et al., 2004). The reduction in the %ATI/g in thyroid can be related to the postoperative energy deficiency, knowing that half of the small bowel is defunccionalyzed and the stomach is highly reduced. Steps et al had attributed this phenomenon to a reduction of the transport of sodium

pertechnetate to the thyroid gland, as happen with iodine in malnourished patients (Passos et al., 2000; Passos et al., 2002). Further studies are necessary to explain these findings.

Bariatric surgery has become the treatment of choice for morbid obesity and it greatly changes the body composition for years following surgery. The lean body mass (muscle) is significantly reduced in the postoperative period (Tanner et al., 2002). In this study we found a paradoxical relationship between weight loss, muscle loss, and increased muscle %ATI/g. So that, questions remain with regard to the physiological mechanisms and pathophysiological consequences of duodenal switch, and biodistribution of radiopharmaceuticals is one of them. It has been related that the polyneuropathy and miopathy after bariatric surgery are resultant of the deficiency of B12 vitamin, tiamin and vitamin D respectively (Koffman et al., 2006)**.** The highly significant muscular uptake of sodium pertechnetate in DS rats may be a consequence of muscle inflammation for vitamin D deficit (Plotnikoff & Quigley, 2003).

In conclusion, the data of this study permits the conclusion that the biliopancreatic diversion with duodenal switch in rats modified the biodistribution of sodium pertechnetate in tireóide, lung, pancreas, spleen and muscle.

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3.7 Artigo VII

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Biodistribution of the radiophamarceutical sodium pertechnetate ($Na^{99m}TcO_4$) after massive small bowel resection in rats[1]

Biodistribuição do radiofármaco pertecnetato de sódio ($Na^{99m}TcO_4$) em ratos submetidos a ressecção extensa de intestino delgado

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ABSTRACT

PURPOSE: To evaluate the biodistribution of sodium pertecnetate ($Na^{99m}TcO_4$) in organs and tissues, the morphometry of remnant intestinal mucosa and ponderal evolution in rats subjected to massive resection of the small intestine. **METHODS:** Twenty-one Wistar rats were randomly divided into three groups of 7 animals each. The short bowel (SB) group was subjected to massive resection of the small intestine; the control group (C) rats were not operated on, and soft intestinal handling was performed in sham rats. The animals were weighed weekly. On the 30th postoperative day, 0.l mL of $Na^{99m}TcO_4$, with mean activity of 0.66 MBq was injected intravenously into the orbital plexus. After 30 minutes, the rats were killed with an overdose of anesthetic, and fragments of the liver, spleen, pancreas, stomach, duodenum, small intestine, thyroid, lung, heart, kidney, bladder, muscle, femur and brain were harvested. The biopsies were washed with 0.9% NaCl.,The radioactivity was counted using Gama Counter Wizard™ 1470, PerkinElmer. The percentage of radioactivity per gram of tissue (%ATI/g) was calculated. Biopsies of the remaining jejunum were analysed by HE staining to obtain mucosal thickness. Analysis of variance (ANOVA) and the Tukey test for multiple comparisons were used, considering $p<0.05$ as significant. **RESULTS:** There were no significant differences in %ATI/g of the $Na^{99m}TcO_4$ in the organs of the groups studied ($p>0.05$). An increase in the weight of the SB rats was observed after the second postoperative week. The jejunal mucosal thickness of the SB rats was significantly greater than that of C and sham rats ($p<0.05$). **CONCLUSION:** In rats with experimentally-produced short bowel syndrome, an adaptive response by the intestinal mucosa reduced weight loss. The biodistribution of $Na^{99m}TcO_4$ was not affected by massive intestinal resection, suggesting that short bowel syndrome is not the cause of misleading interpretation, if an examination using this radiopharmaceutical is indicated.

Key words: Short Bowel Syndrome. Sodium Pertechnetate Tc 99m. Pharmacokinetics. Rat.

RESUMO

OBJETIVO: Avaliar em modelo animal com ressecção extensa do intestino delgado a biodistribuição de pertecnetato de sódio ($Na^{99m}TcO_4$) em órgãos e tecidos, a evolução ponderal e a morfometria da mucosa do intestino delgado remanescente. **MÉTODOS:** Vinte e um ratos *Wistar* foram aleatoriamente divididos em três grupos de sete animais cada. O grupo intestino curto (IC) foi submetido a ressecção extensa do intestino delgado, o grupo controle (C) não foi operado e o grupo *sham* foi submetido a leve manipulação cirúrgica das alças intestinais.Todos foram pesados semanalmente. No 30º dia pós-operatório foi administrado 0,l mL de $Na^{99m}TcO_4$ aos animais dos três grupos, IV no plexo orbital, com atividade radioativa média de 0,66MBq. Após 30 minutos os ratos foram mortos e retirados fragmentos do fígado, baço, pâncreas, estomago, duodeno, intestino delgado, tireóide, pulmão, coração, rim, bexiga, músculo, fêmur, e cérebro. As amostras foram lavadas com solução de NaCl 0,9%.A radioatividade foi contada pelo Contador Gama 1470, Wizard^{TM} Perkin-Elmer e calculado o percentual de atividade radioativa por grama (%ATI/g) de cada órgão. Biópsias do jejuno foram submetidas a análise da espessura da mucosa (coloração HE). Utilizou-se avaliação estatística paramétrica (ANOVA) e teste de Tukey, considerando $p<0,05$ como significante. **RESULTADOS:** Não houve diferenças significantes da %ATI/g nos órgãos dos grupos estudados ($p>0,05$). Verificou-se acentuada redução inicial de peso, em seguida um aumento do peso dos animais tratados a partir da segunda semana de observação e aumento da espessura da mucosa jejunal do grupo IC, comparado com os demais. **CONCLUSÃO:** Em ratos com síndrome do intestino curto, uma adaptação na espessura da mucosa contribuiu para reversão na perda de peso inicial e para que a biodistribuição do $Na^{99m}TcO_4$ não fosse afetada pela ressecção extensa do intestino, sugerindo que o intestino curto não é causa de interpretações duvidosas, quando exame cintilográfico com este radiofármaco estiver indicado.

Descritores: Síndrome do Intestino Curto. Pertecnetato Tc99m de Sódio. Farmacocinética. Ratos.

Introduction

Radioisotopes are used in nuclear medicine for diagnostic and therapeutic purposes. The labelling capacity of these isotopes for the plasmatic proteins is well known, and their bioavailability and pharmacokinetics can be modified by drugs and diseases[1,2]. Among the most useful artificial radioisotopes, technetium-99m, in the chemical form of sodium pertechnetate ($Na^{99m}Tc0_4$), is the most important. Its use in nuclear medicine is due to the emission of gamma energy of 140 KeV, enabling it to take advantage of the scintillation detectors and obtain enhanced image quality; this is known as scintigraphy[3]. It has a short life, is low cost, and is obtained from a molybdenum/technetium generator ($^{99}Mo/^{99m}Tc$).[3] It is easily distributed through the vascular fluid, intersticial space with uptake by the salivary glands, thyroid, stomach, intestines and other organs[3]. It is rapidly eliminated by the urine and when incorporated to specific substances, produces organ images of different densities and functions[4]. Experimental studies carried out with the labelling of red blood cells with ^{99m}Tc identified important biological effects, in addtion to alterations in the labelling process[5,7]. Gomes et al carried out a study with mitomicin-C that described alterations in the labelling of red blood cells[6]. The literature reports that several natural drugs reduce the efficiency of labelling red blood cells with ^{99m}Tc[7]. An experimental study with Vincristin, used in oncology protocols, showed an interaction of this drug with ^{99m}Tc in several organs[8]. However, there are no reports of research studying the

biodistribution of radiopharmaceuticals after surgical procedures. In the present study, we used an experimental model of massive resection of the small intestine, characterizing the short bowel syndrome, which results in unsuitable water and nutrient absorption, causing malnutrition[9,10]. In spite of the short bowel syndrome, the intestine can be adapted through physiologic, cellular and moleculares mechanisms[9]. In some patients, dilation and lengthening of the remnant small intestine occur as a phenomenon of functional adaptation. Surgical techniques have been reported that attempt to lengthen this intestinal segment. Such procedures are complex and frequently ineffective, and call for assessments of their efficacy[11]. Recently, new therapeutic methods, such as isolated small intestine transplantation or combined with liver transplantation, have been an alternative for cases of hepatic failure due to total parenteral nutrition in the treatment of short bowel syndrome[12]. Given the antiabsorptive effect of the operation, with great repercussions on the metabolism, radioisotope images may be necessary in the postoperative, in order to control the series of pathological conditions resulting from short bowel syndrome. Scintigraphy can be used in the postoperative of intestinal resections to assess the morphology and metabolism of several organs. Under these conditions, it becomes relevant to study the biodistritubion of $Na^{99m}Tc0_4$ in specific organs and tissues. Therefore, the present paper aims to study, in an animal model of massive resection of the small intestine, the biodistribution of $Na^{99m}TcO_4$ in several organs by means of radiation counting in organs and tissues in the postoperative period. We also evaluated the ponderal evolution of the animals after the operation, as well as the mucosal morphometry of the remnant small intestine.

Methods

Twenty-one Wistar rats, age around 180 days, weighing 265g±31g were used. They were supplied by the animal colony of the Nucleus for Experimental Surgery of the Federal University of the Rio Grande do Norte, Brazil. All the animals were weighed and observed in individual cages with water and food (Labina ® Purina) *ad libitum* and acclimated in the laboratory for 7 days. They were maintained under temperature control (21ºC), air humidity (60 – 70 %) and lighting (12/12 hours light/dark cycle) and handled in accordance with the Animal Experimentation Code of Ethics (Council for International Organizations of Medical Sciences) and the rules of the Brazilian College of Animal Experimentation. They were randomly divided into three groups: the experimental group rats, denominated short bowel, (SB, n=7) were subjected to massive resection of the small intestine (90%); control group rats were not operated on (C, n=7) and the third group underwent a simulated operation, called sham (n=7). Rats were fasted overnight before surgery, and anesthetized with sodium pentobarbital (20mg/kg intraperitoneal) and ketamine (20mg/kg intramuscular); they were operated on under sterile conditions. A 3 cm midline laparotomy was performed and intestinal transections were done 5 cm above the ileocecal junction and 5 cm from the duodenojejunal transition. With the aid of a surgical microscope (DF Vasconcelos, São Paulo, Brazil), interrupted sutures of 6-0 prolene (Ethicon®, Brazil) were used for bowel anastomosis. The animals typically have a small bowel length of 100 cm, and accordingly, residual length was 5 cm of jejunum and 5 cm of ileum (10 cm), corresponding to 90% of resection. After surgery, the abdomen was closed with interrupted sutures of 4-0 nylon suture (Ethicon®). The animals were allowed water immediately after surgery and food on the second postoperative day. The sham rats were subjected to a 3 cm medium laparotomy and mild manipulation of the small

bowel. The rats were weighed weekly with a digital scale (Filizola® São Paulo, Brazil) and observed for 30 days. On the 30th day all the animals were anaesthetized again, and injected with 0.lmL of $Na^{99m}TcO_4$ in the venous orbital plexus, corresponding to radioactive activity of 0.66MBq. After 30 minutes, the animals were killed by lethal dose of anesthetic. Samples of the liver, spleen, pancreas, stomach, duodenum, small intestine, thyroid, lung, heart, kidney, bladder, muscle, femur and brain were harvested. The samples were washed in 0.9% NaCl , weighed on a high-precision digital scale (Bel-Mark 160-II ItáliaÒ) and subjected to radioactivity detection using a 1470 Wizard^{TM} Gamma Counter- Perkin-Elmer, with automatic correction of radiation decline. The percentage of radioactive activity/g (%ATI/g) of each organ was calculated by dividing the activity/g of the tissue by the total activity administered to each animal. Samples with 2cm of jejunum were harvested 2 cm below the anastomosis. After washed in 0.9% saline, the excised tissues were fixed in 10 % buffered formalin for 48 h, dehydrated and embedded in paraffin. Sections cut at 5mm thickness were stained with hematoxilyn and eosin and morphology was assessed by an observer, who was unaware of the tissue origin. For the morphometric study of intestinal mucosa, Media Cybernetics – LP, USA, Image Pro-Plus software was used with an Olimpus BX-50 microscope fitted with a digital (Samsung®) video camera. The video camera transferred the image from the microscope to the computer screen. The measurement of the mucosal thickness was delimited with a computer mouse from the apex of the villus to the *muscularis mucosae* and was expressed in microns (mm). The analysis was made under 40x magnification using specimens in which the *villi* and the crypts were perpendicular to the *muscularis mucosae*. For the analysis of the different data related to postsurgical weight loss, to the measurements of total mucosal thickness, and to the biodistribution of sodium pertechnetate of the different groups, parametric variance (ANOVA) was used. For the multiple comparisons, the Tukey test was used. A significance level of 5% ($p<0.05$) was established.

Results

All the animals survived the surgical procedures. Table 1 shows the results of the differences in %ATI/g among groups SB, C and sham. We observed an increase in the biodistribution of $Na^{99m}TcO_4$ in the thyroid and duodenum of SB rats, when compared to control rats. However, since the standard deviation was high, there was no significant difference ($p>0.05$). In the stomach, an apparent tendency for reduced biodistribution of the %ATI/g occurred in the SB rats, compared to sham rats, but without statistical significance ($p>0.05$). In several organs the percentages of radioactive activity (%ATI/g) had very similar values among the groups, without significant differences (Table 1). The weight of SB rats decreased during the first and second weeks of survival and, after that, their weight gradually increased until the 30th postoperative day, when it was nearly the same as the C and SHAM rats, which continually increased their weight over time (Figure 1). The differences in the mean weight of SB rats at the end of the second week were significant, when compared to C and SHAM rats ($p<0.05$). The presence of an increase in intestinal mucosa thickness was detected in all IC rats, when compared to C and SHAM rats until the end of the observation period, as seen in Table 2 ($p<0.05$) and Figure 2.

TABLE 1 – Biodistribution of $Na^{99m}TcO_4$ in the organs of the respective groups

Organs	*%ATI g*			ANOVA (1)
	SB	C	Sham	
Liver	0.35 ± 0.089	0.36 ± 0.079	0.39 ± 0.113	0.794400
Spleen	0.22 ± 0.090	0.18 ± 0.031	0.19 ± 0.044	0.565470
Estomach	2.58 ± 0.730	2.72 ± 0.614	4.11 ± 1.793	0.116180
Small bowel	0.28 ± 0.107	0.20 ± 0.052	0.28 ± 0.130	0.690700
Duodenum	1.73 ± 1.814	0.41 ± 0.062	1.13 ± 1.719	0.378723
Pancreas	0.16 ± 0.063	0.14 ± 0.055	0.18 ± 0.125	0.811183
Kidney	0.41 ± 0.086	0.42 ± 0.082	0.36 ± 0.187	0.738872
Heart	0.17 ± 0.075	0.27 ± 0.057	0.17 ± 0.084	0.076831
Lung	0.35 ± 0.105	0.38 ± 0.125	0.31 ± 0.058	0.581337
Thyroid	5.35 ± 1.979	3.71 ± 1.256	3.80 ± 1.058	0.187603
Bladder	0.39 ± 0.114	0.33 ± 0.109	0.27 ± 0.139	0.309546
Muscle	0.07 ± 0.028	0.06 ± 0.019	0.05 ± 0.035	0.570391
Femur	0.16 ± 0.055	0.14 ± 0.036	0.15 ± 0.050	0.760950
Brain	0.02 ± 0.013	0.01 ± 0.003	0.03 ± 0.027	0.482193

Mean ± Standard deviation

(1) P- from analysis of variance (ANOVA).

The results of the test did not show statistically significant differences ($p>0.05$), for all the variables. %ATI/g, percent radioactivity per gram of tissue.

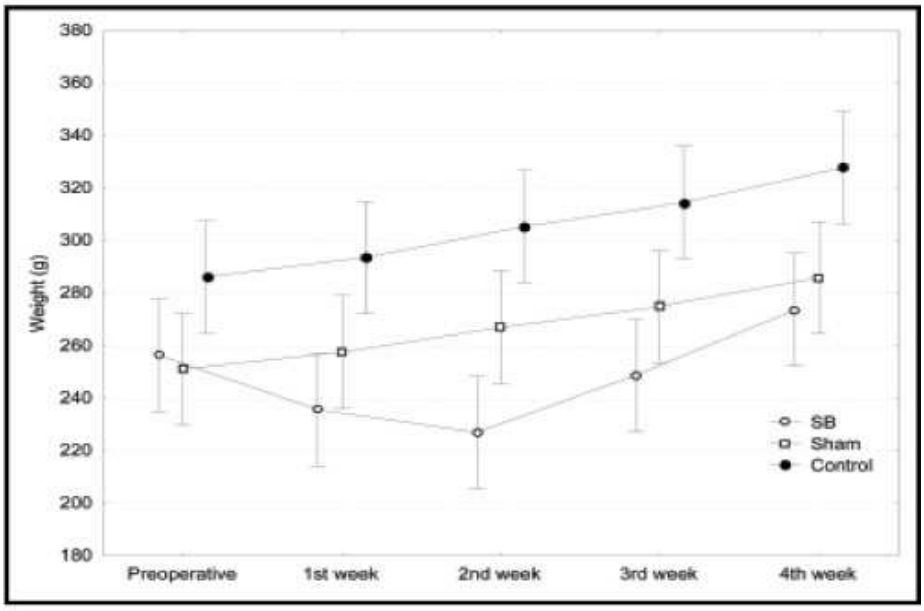

FIGURE 1 – Mean weight of rats in each group and postoperative period; SB, short bowel.

TABLE 2 – Jejunal mucosa thickness of rats and their respective groups.

Variable	Groups			ANOVA[1]
	SB	C	Sham	
Mucosal thickness in μm[2]	334.34 ± 25.9[a]	194.40 ± 39.0[b]	194.22 ± 33.2[b]	0.0000

Values expressed in Mean ± Standard deviation
(1) p-value of analysis of variance (ANOVA).
(2) Groups identified with the same letter differ significantly at a level of 5% (Tukey test).
SB = Short bowel; C = control.

FIGURE 2 – Small intestine morphology and mucosal cell proliferation on day 30 in short bowel (A) control (B) and sham (C) animals. Massive intestinal resection (A) induced significant increases in total mucosal thickness (arrows), when compared to control and sham animals (B, C) (see Table 2; $p<0.05$). HE, 40x

Discussion

The alterations in the biodistribution of $Na^{99m}TcO_4$ in organs and tissues are very well identified in several studies that used no experimental surgical models [1,,6,,8,13,14]. However, in the postoperative of major surgical procedures, there are no reports concerning the biodistribution of radiopharmaceuticals. The present experimental model of the short bowel syndrome in rats submitted to massive resection of the small intestine was used to determine the biodistribution profile of $Na^{99m}TcO4$ in several organs and tissues. Intestinal failure is characterized by malnutrition and/or dehydration as a result of the inadequate digestion and absorption of nutrients. The most common cause of intestinal failure is short bowel syndrome, which occurs when the functional small bowel mass is reduced below the level necessary for adequate nutrient and water absorption. This condition frequently results from a massive resection of the small bowel. Following resection, the intestine is capable of adaptating in response to enteral nutrients as well as other trophic stimulation. Rodents are commonly used in well-characterized models to assess the process of intestinal adaptation[16]. Following small bowel resection in the rat, the remnant intestinal mucosa undergoes compensatory alterations in an attempt to restore normal absorptive capacity. Morphologic and functional changes include increases in mucosal length, enterocyte proliferation, as well as increased electrolyte, glucose and amino acid uptake[16,17]. In humans, the alterations of intestinal absorption due to massive resection of the small intestine usually cause significant weight loss[15]. However, in rodents, there is a rapid adaptation of the intestinal mucous membrane, which minimizes weight loss[16]. These mechanisms of intestinal adaptation take place at physiologic, cellular and molecular levels and they do not correspond to what occurs in the human intestine [17]. Nutrients, electrolytes, hormones, cytokines and other elements take part in the process, which

involves mainly the intestinal mucous membrane. The process begins with apoptosis and continues with an increase in epithelial cells, vilosities and mucosal crypts, and a consequent remodeling of their architecture. Functionally, this allows for increased substance transport through the intestinal mucosa[17]. In the present study a significant decrease was observed in the weights of rats submitted to massive intestinal resection, in the immediate postoperative period, and weight recovery beginning at the end of the second week. These data coincide with a classic study on the subject, where morphological and functional adaptations of the jejunum were observed between the first and second postoperative weeks[18]. This phenomenon was also shown in the morphometry of the jejunal mucous membrane of the animals subjected to massive resection of the present study. Therefore, the mucous membrane hyperplasia observed in the jejunal mucosa of the SB rats of the present experiment, likely contributed to the rapid weight recovery of the animals, starting from the second postoperative week. Welters et al (2002) verified that intestinal function recovery begins with the hyperplasia of the intestinal mucosa and that absorptive function depends on the maturity of the enterocytes, a fundamental factor for nutrient metabolism[19]. The precocious postoperative recovery of the animals, represented by weight and morphology of the intestinal mucosa recovery, and the healthy behavior of the SB rats, comparable to the controls, certainly contributed to the absence of significant clinical alterations and malnutrition. Consequently, there was no negative effect on the biodistribution of $Na^{99m}TcO_4$ in the vital organs. In an experimental study using malnutrition-inducing diets, Passos et al[20] (2000) showed that malnutrition affected the biodistribution of $Na^{99m}TcO_4$ in different organs such as the thyroid, brain, stomach and heart. In their study, the intestine was not surgically manipulated. Studies in animals have been investigating substances that regulate the absorptive function of the intestine[21]. These mechanisms are mediated by multiple factors, including enteral or parenteral nutrition, hormones and growth factors[22]. Recently, studies on the use of the human growth hormone (GH), the epidermal growth factor (EGF) and the glucagons-like peptide-2 (GLP-2), produced in the L-cells of the small intestine, have confirmed them as agents that increase intestinal adaptation after massive resection[23]. The study suggests that, whereas GLP-2 is important in controlling adaptation, there are spatial or regional systems in place that use varying pathways. The significant increase in nutrient-stimulated GLP-2 secretion suggests that GLP-2 is involved not only in the initiation, but also in maintaining the ongoing adaptive process. The increases in mucosal proliferation that are temporally associated with a maintained GLP-2 release, suggest that GLP-2 is important in initiating and maintaining the small intestines adaptive response to resection[24]. Curtis et al (1984) studied rats submitted to massive resection of the small intestine using marker $^{51m}Cr^{13m}C$ and protein, and observed the animals for one week. They concluded that the rats had no alteration in absorption and digestion time when compared to the treated group and the control; this demonstrated the fast physiological adaptation of the animals[25]. A growing number of tissue factors are being investigated for having great potential in promoting intestinal adaptation in animals and humans with short bowel syndrome, in the hope of obtaining effective therapies for the syndrome in the future[23,26]. In summary, massive intestinal resection in the current study did not interfere significantly with the biodistribution of the radiopharmaceutical $Na^{99m}TcO_4$ in the organs studied. Certainly the mucosal hyperplasia of the remnant intestine was a preponderant factor for the quick weight loss reversal of the animals, and consequent preservation of their healthy metabolism. The present study does not allow us to comment on the mechanisms by which intestinal resection results in the stimulation of trophic effects and mucosal adaptation, allowing

normal biodistribution of $Na^{99m}TcO_4$ in rats. Identifying factors that may enhance the process of intestinal adaptation is an exciting area of research with important potential clinical applications. This area will require further studies.

Conclusion

In rats with experimentally-induced short bowel syndrome, an adaptive response by the intestinal mucosa reduced weight loss. The biodistribution of $Na^{99m}TcO_4$ was not affected by massive intestinal resection, suggesting that short bowel syndrome could not be the cause of misleading image interpretation when an examination with this radiopharmaceutical is indicated.

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3.8 Artigo VIII

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Simvastatin improves the healing of infected skin wounds of rats[1]

A sinvastatina melhora a cicatrização de feridas infectadas da pele de ratos

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ABSTRACT

PURPOSE: This study explores the potential of the simvastatin to ameliorate inflammation and infection in open infected skin wounds of rats. **METHODS:** Fourteen *Wistar* rats weighing 285±12g were used. The study was done in a group whose open infected skin wounds were treated with topical application of sinvastatina microemulsion (SIM, n=7) and a second group with wounds treated with saline 0.9 % (SAL, n=7). A bacteriological exam of the wounds fluid for gram positive and gram negative bacteria, the tecidual expression of TNFá and IL-1â by imunohistochemical technique, and histological analysis by HE stain were performed. **RESULTS:** The expression of TNFa could be clearly demonstrated in lower degree in skin wounds treated with simvastatin (668.6 ± 74.7 ìm^2) than in saline (2120.0 ± 327.1 ìm^2). In comparison, wound tissue from SIM group displayed leukocyte infiltration significantly lower than that observed in SAL group ($p<0.05$). Culture results of the samples taken from wound fluid on fourth post treatment day revealed wound infection in only one rat of group simvastatin (SIM), where *Proteus mirabilis*, *Escherchia coli* and *Enterobacter sp* were isolated. In the rats whose wounds were treated with saline (SAL), polymicrobial infection with more than 100,000 CFU/g was detected in all the wounds. **CONCLUSION:** In addition to its antiinflammatory properties, the protective effects of simvastatin in infected open skin wounds is able to reduce infection and probably has antibacterial action. The potential to treat these wounds with statins to ameliorate inflammation and infection is promising.

Key Words: Statin. Inflammation. Wound Healing. Wistar rat. Skin. Cytokine.

RESUMO

OBJETIVO: O presente estudo avaliou o potencial da sinvastatina para atenuar a inflamação e a infecção em feridas abertas infectadas de pele de ratos. **MÉTODOS:** Foram utilizados 14 ratos *Wistar* pesando 285±12g. O estudo foi realizado com um grupo de animais cujas feridas abertas infectadas foram tratadas com aplicação tópica de sinvastatina microemulsão (SIM,

n=7) e um segundo grupo com feridas tratadas com solução salina 0,9% (SAL n=7). Foi realizado exame bacteriológico do fluido das feridas para detecção de bactérias gram positivas e negativas, a expressão tecidual de TNFá e IL-1â por imunohistoquímica e análise histológica pela coloração H-E. **RESULTADOS:** A expressão do TNFa pode ser claramente demonstrada em menor grau nas feridas de pele tratadas com sinvastatina (668.6 ± 74.7 ìm^2) do que no grupo salina (2120.0 ± 327.1 ìm^2). Em comparação, os tecidos das feridas do grupo SIM mostrou infiltração leucocitária significantemente menor do que a observada no grupo SAL ($p<0,05$). O resultado das culturas realizadas no fluido das feridas no 4º dia de tratamento revelou infecção em apenas um rato do grupo sinvastatina (SIM), onde *Proteus mirabilis, Escherchia coli* e *Enterobacter sp* foram isolados. Nos ratos cujas feridas foram tratadas com solução salina (SAL), infecção polimicrobiana com mais de 100,000 UFC/g foi detectada em todas as feridas. **CONCLUSÃO:** Além de suas propriedades antiinflamatórias, o efeito protetor da sinvastatina em feridas abertas e infectadas de pele é capaz de reduzir a infecção e provavelmente tem ação antibacteriana. O potencial da droga para atenuar inflamação e infecção de feridas é promissor.

Descritores: Estatinas. Inflamação. Cicatrização de Feridas. Rato Wistar. Pele. Citocinas.

Introduction

The healing of open skin wounds involves the epithelium and underlying stroma. Processes such as angiogenesis, activation, migration, and proliferation of fibroblasts, myofibroblasts and endothelial cells; formation of granulation tissue; and wound contraction are needed to close these defects[1,2]. Some wounds are also frequently inflamed and, in general, stromal involvement and inflammation greatly increase the risk of subsequent complications[3,4] The repair process begins immediately after injury by the release of various growth factors, cytokines, and low-molecular weight compounds. Disruption of blood vessels also leads to the formation of the blood clot, which is composed of cross-linked fibrin, and of extracellular matrix proteins such as fibronectin, tenascin, and thrombospondin[5] . Wound infection develops in 2% to 5% of patients undergoing surgical procedures each year in most hospitals world-wide and continues being considered one of the most important problems in surgical wards nowadays. It is one of the main factors that alter the physiologic evolution of the wound healing[6,7] The bacteria inhibit the angiogenesis, secrete plasminogen activators, and proteolitic enzymes that may affect the extracelular matrix, blocking the wound contraction[8] Several substances have been used to treat infected skin wounds, like honey, sugar, antibiotics, phytotherapics[9,10,11,12,13] Statins are a class of compounds that competitively inhibit the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the first committed step in cholesterol biosynthesis. Increasingly, the pleiotropic properties of statins are being described. In endothelial cells, all of these effects seem to result from the inhibition of cholesterol's precursor mevalonic acid, which is critical to the isoprenylation of a diverse family of proteins[14,15]. Simvastatin, a HMG-CoA reductase inhibitor, have been shown to exhibit important immunomodulatory effects independent of lipid lowering[16]. These pleiotropic effects have been demonstrated to include anti-inflammatory actions[17]. improvement of endothelial and microvascular function, modulation of endothelial nitric oxide synthase (eNOS)[18], ischemia/reperfusion[19] and sepsis[20] . However, statins have not been used to treat skin infected wounds. We thus approached the question of whether topical treatment with simvastatin might improve the healing of skin infected wounds in a rat model.

Methods

The experimental protocol was approved by the Research Ethics Committee (Animal Research Ethics Division) of the University Hospital-UFRN, Brazil. Animals were handled in accordance with the Guide for the Care and Use of Laboratory Animals, Brazilian College of Animal Experimentation.

Animals

Wistar rats (*Rattus norvegicus albinus, Rodentia mammalia*) weighing 282???g were used. They were housed in polypropilene cages and maintained under controlled temperature conditions on a 12h light-dark cycle and allowed *ad libitum* access to commercially available rat chow (Labina, Purina®) and water.

Experimental design and procedures

The animals were randomly separated in two groups of seven rats each. After 12 hours of fasting, the rats were anesthetized by intramuscular injection of 30mg/Kg of ketamine and thiopental 20mg/Kg intraperitoneal. After dorsal skin depilation and anti-sepsis with 1% povidona, the surgical procedures were performed under aseptic conditions. An open skin squared wound ($1cm^2$), was done in the dorsal skin of all the rats. Immediately after the surgical procedure, the wounds were contaminated with topical application of 0.5mL of multibacterial solution prepared with 1g of rats fresh feces and 10mL of saline. In the following day, the infected wounds of the simvastatin group (SIM) (n=7) were topically treated with 0.2 ml of simvastatin microemulsion (10mg/mL) once a day. The wounds of group saline (SAL) (n=7) rats were treated with 0.2 ml of saline solution daily. When the wounds were totally epithelialized, the healing time was recorded and the resection of the scar was performed under anesthesia. The healed tissues were used for histopathological study and immunohistochemical dosage of tumor necrosis factor-a (TNF-a) and interleukine-1 (IL-1b).

Immunohistochemical staining of TNF-a *and IL-1*b.

Immunohistochemical staining for TNF-a and IL-1b was performed on tissue samples obtained from the healing skin. These samples were fixed in 4% paraformaldehyde, processed in routine technique, cut into 5mm-thick frozen sections, and dried at room temperature. Absolute methanol containing 3% hydrogen peroxide was added to block endogenous peroxidase. After three washings with a phosphate-buffered solution (PBS) for 5 min each, these sections were treated with 1% polyoxyethylene-10-octylphenyl ether in PBS for 20 min at room temperature. After washing in the same way, these were reacted with 100 ml of biotinylated anti-rat TNF-a monoclonal antibody (Pharmingen, San Diego, CA) or biotinylated anti-rat IL-1b monoclonal antibody (Pharmingen), diluted in 9 mL of PBS and 1 ml of whole goat serum at room temperature in a moist chamber for 2 h. After washing, the preparations were incubated, with two drops each of avidin solution and biotinylated peroxidase solution in 4.5 ml of PBS and 0.5 ml of 5% skim milk for 2 h at 37°C. After PBS rinsing, diaminobenzidine and nickel were applied for 8 min to achieve permanent color change. Six views were selected randomly for each section and observed under a light microscope (x100). The mean number of reactive cells in the six views was regarded as the data for each sample. Sequential images of microscopic sections were

photographed within 72 hours after immunostaining, by a digital camera (Sony, Tokyo, Japan) mounted on a light microscope (Olympus B-50, Tokyo, Japan) at a magnification of 100x, and saved in jpg file format. Images were then analyzed in ImagePro-Plus software (Media Cybernetics, LP, USA). Briefly, the entire area colored by cytokines was marked, and the total marked area was calculated. The area stained by the antibody of interest was identified and calculated by using the software color algorithm. The integrated optical areas stained by anti-bodys were then recorded. The score index was calculated for each of the antibodies and it was averaged.

Histopathology

The biopsies of skin wounds were processed following the routine and stained with hematoxilin and eosin (HE) for histological analysis of the inflammatory reaction, using the optical microscope Olympus B-50, Japan, Tokyo. The quantification of cells, fibers and elements of the inflammatory reaction was performed by the Image Pro-Plus Média Cybernetics software, LP, USA.

Bacteriological examination

At the 4^{th} postoperative day, exsudato was collected from the wounds for microbiology and for quantitation of bacterial population. The materials were processed and cultured on selective MacConkey's agar, blood agar and salt manitol agar. The agar plates were incubated at 37 ºC and examined for growth after 24, 48 and 72 hours. Any growth in the plates of bacteria of the same biotype as cultured in the wounds was considered positive and expressed as colony-forming units per gram of tissue (CFU/g). All procedures were performed under laminar air flux.

Statistical analysis

Data are presented as the mean±standard deviation. Results were analyzed with ANOVA and Student t test. Statistical significance was assumed at $p< 0.05$.

Results

*Tumor necrosis factor alpha (TNF*a*)*

The expression of TNFa could be clearly demonstrated in lower degree in skin wounds treated with simvastatin (668.6 ± 74.7 ìm^2) than in saline (2120.0 ± 327.1 ìm^2) treated wounds, as can be shown in figure 1 and table 1. So, a distinct decrease of tissue reactivity occurred when the simvastatin microemulsion was applied to the infected wounds.

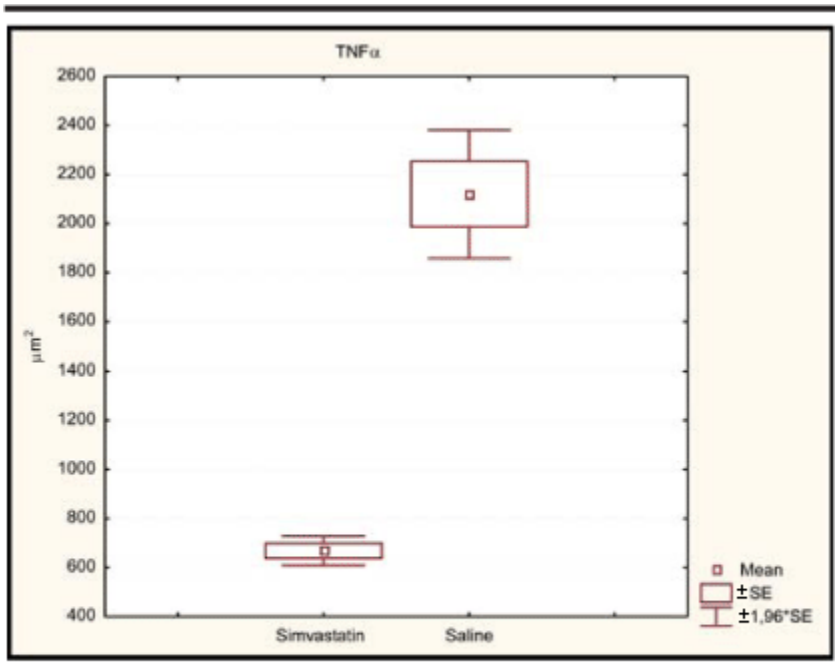

FIGURE 1 - Mean areas corresponding to the expression of TNFα Significant difference comparing the groups simvastatin and saline ($p<0.05$).

TABLE 1 - Mean and statistical analysis of optical density related to the expression of cytokines in tissues of skin infected wounds treated with simvastatin and saline (µm2).

Groups / Cytokines	Simvastatin (SIM)*	Saline (SAL)*	p-value
TNFα	668.6 ± 74.7	2120.0 ± 327.1	0.00001
IL-1β	467.6 ± 55.2	691.6 ± 67.4	0.00001

* Mean ± Standard deviation
1 – Difference statistically significant comparing the groups SIM/ SAL (Student t test).
Image Pro-plus software Media Cybernetics was used.

Interleukin-1b

IL-1b expression was significantly more enhanced in the saline (SAL) group than in the Simvastatin (SIM) group after total epithelialization of the skin wounds. A clearly decreased immunohistochemical stainability could be noticed in the SIM group, whose data are expressed in figure 2 and table 1.

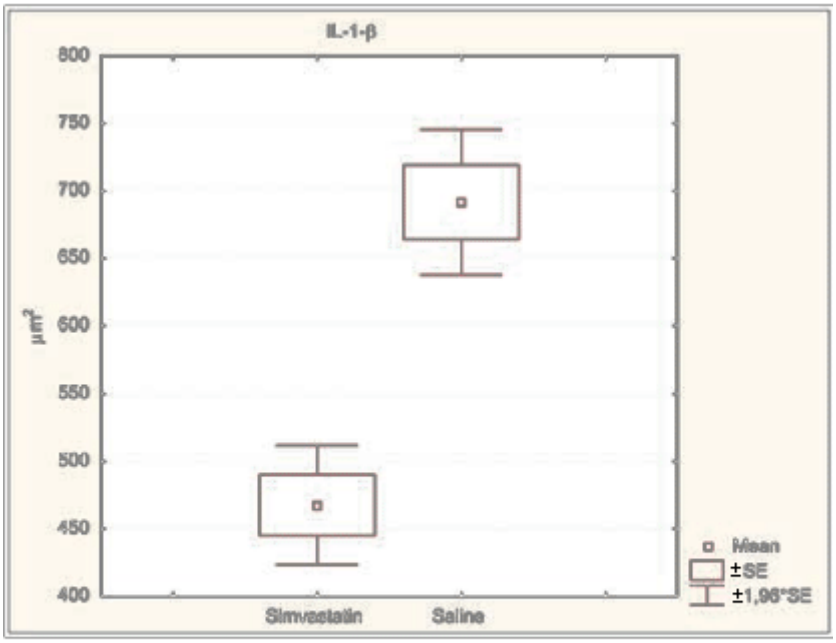

FIGURE 2 - Mean areas corresponding to the expression of IL-1β Significant difference comparing the groups simvastatin and saline ($p<0.05$).

Histopathology

Contamination of skin wounds with multibacterial fecal solution caused intense inflammatory reaction in tissues of group SAL rats, with edema and marked leukocyte infiltration consistent with acute injury (Figure 3). In comparison, wound tissue from SIM group displayed leukocyte infiltration significantly lower than that observed in SAL group. ($p<0.05$). These pathologic changes were reduced by the administration of simvastatin topically in the infected wounds (Table 2) The histological slides (figures 3 and 4) suggest differences in neutrophil accumulation between the SAL and SIM groups.

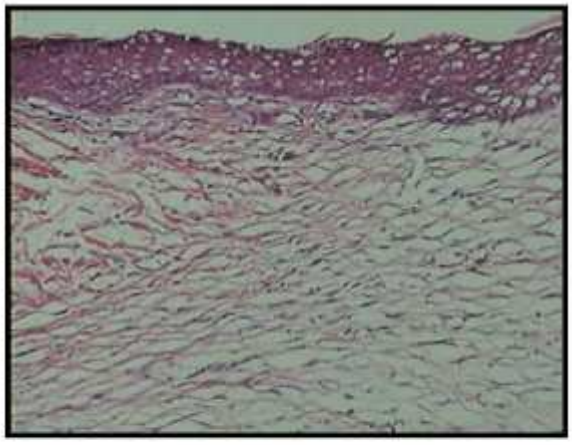

FIGURE 3 - Histological section of wound tissue taken from a SAL group rat, demonstrating significant neutrophil infiltration, giant cells and folicules. HE, 100x.

TABLE 2 - Histological grading based in the optical density related related to the inflammatory reaction in tissues of skin infected wounds treated with simvastatin and saline (μm^2)

Groups / Inflammation	Simvastatin (SIM)*	Saline (SAL)*	p-value
Inflammatory reaction	844.7 ± 65.2	3416.1 ± 233.4	0.00001

* Mean ± Standard deviation
1 - Difference statistically significant comparing the groups SIM/ SAL (Student t test).
Image Pro-plus software Media Cybernetics was used.

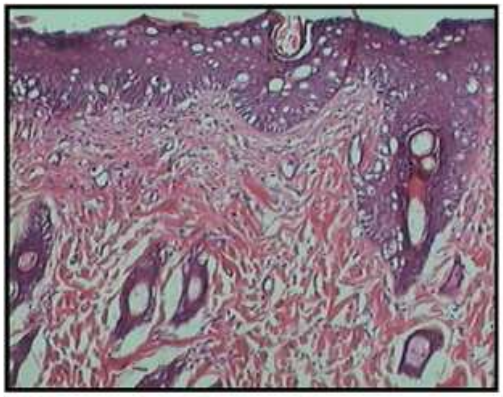

FIGURE 4 - Histological section of wound tissue taken from SIM group rat, demonstrating low neutrophil infiltration and good epithelial regeneration. HE, 100x.

Bacteriological examination

Culture results of the samples taken from wound fluid on fourth post treatment day revealed wound infection in only the rat number 4 of group simvastatin (SIM), where *Proteus mirabilis, Escherchia coli* and *Enterobacter sp* were isolated. In the rats whose wounds were treated with saline (SAL), polymicrobial infection with more than 100,000 CFU/g was detected in all the wounds (Table 3). The most frequently isolated microorganisms can be observed in table 3.

Discussion

Cutaneous wound healing is a complex process involving blood clotting, inflammation, new tissue formation, and finally tissue remodeling[21.] It is well described at the histological level and many experimental and clinical studies have demonstrated varied, but in most cases beneficial, effects of exogenous substances[11,12,13] on the healing process. However, the roles played by these exogenous treatments have remained largely unclear. HMG-CoA reductase inhibitors (statins) are used clinically for lowering hypercholesterolemia because of their inhibitory effect on hepatic biosynthesis of cholesterol at the mevalonate step[22.] These statins such as simvastatin

have been shown to exhibit important immunomodulatory effects independent of lipid lowering[23] These pleiotropic effects have been demonstrated to include anti-inflammatory actions[24]. The present study is one of the first to demonstrate that HMG-CoA reductase inhibitors significantly improve healing of infected skin wounds in an experimental model in rats. The improvement in inflammatory reaction and in cytokines expression corroborates some results in the literature that clearly demonstrate that simvastatin is a potent and effective endothelium-protective agent that reduces leukocyte–endothelial cell interactions independently of its well-known lipid-lowering effects. This effect has been found to be at least partially mediated via downregulation of P-selectin expression on the microvascular endothelium. Thus, HMG-CoA reductase inhibitors like simvastatin have important anti-inflammatory effects besides their well-known lipid-lowering action[25,26]. In the present study we demonstrated that the topical application of simvastatin microemulsion attenuated the inflammatory reaction in wound healing of infected tissues, but to date the mechanism is not clear. Prueffer et al[27]**.**demonstrated a protective effect of simvastatin under conditions of acute inflammation induced by an exotoxin within the microcirculation. In particular, they provide strong evidence that simvastatin is able to attenuate enhanced leukocyte-endothelium interaction after *S aureus* toxin administration. Pore-forming *S aureus* toxin is known to provoke inflammatory acctivation[28,29,30]. These evidences may explain hour results with the topical use of simvastatin in the healing of infected wounds. Statins affect the production of many acute phase reactants, including CRP, which is produced in the liver under stimulation by cytokines (IL-1 and IL-6). In nonatherosclerotic huCRP transgenic mice, statins decreased basal and IL-1*b*-induced plasma huCRP levels independently of cholesterol lowering and of an effect on IL-6 production[31] . In fact, in this work simvastatin was able to induce a marked decrease in TNFa anf IL-1b in healing tissues, as demonstrated by immunohystochemical analysis. A probable antibacterial effect was also observed, and the exact mechanism to explain this action is to be described. In an other study the pretreatment was found to decrease cytokine-stimulated transcription factor activation and iNOS expression in the endothelium, stating that simvastatin affect cytokines with several ways[32].

Conclusion

In addition to its antiinflammatory properties, the protective effects of simvastatin in infected open skin wounds is able to reduce infection and probably has antibacterial action. The potential to treat these wounds with statins to ameliorate inflammation and infection is promising.

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3.9 Artigo IX

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Prevention of bacterial translocation using b-(1-3)-D-glucan in small bowel ischemia and reperfusion in rats[1]

Prevenção de translocação bacteriana com b-(1-3)-D-glucana em isquemia e reperfusão intestinal em ratos

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ABSTRACT

PURPOSE: To investigate the role of b-(1-3)-D-glucan on ^{99m}Tc labelled *Escherichia coli* translocation and cytokines secretion in rats submitted to small bowel ischemia/reperfusion injury. **METHODS:**Five groups (n=10 each) of Wistar rats were subjected to control(C), sham(S), group IR subjected to 45 min of bowel ischemia/60 min of reperfusion(I/R), and group I/R+glucan subjected to 45 min of bowel ischemia/60 min of reperfusion(I/R) and injected with 2mg/Kg intramuscular. Translocation of labelled bacteria to mesenteric lymph nodes, liver, spleen, lung and serum was determined using radioactivity/count and colony forming units/g(CFU/g). Serum TNFa, IL-1b, IL-6, IL-10 were measured by ELISA. **RESULTS:** CFU/g and radioactivity/count were higher in I/R than in I/R+glucan rats. In C, S and S+glucan groups, bacteria and radioactivity/count were rarely detected. The I/R+glucan rats had enhancement of IL-10 and suppressed production of serum TNFa, IL-1b and, IL-6, compared to I/R untreated animals. **CONCLUSION:** The b-(1-3)-D-glucan modulated the production of pro-inflammatory and anti-inflammatory cytokines during bowel ischemia/reperfusion, and attenuated translocation of labelled bacteria.

Key words: Bacterial translocation. Glucan. Intestine. Ischemia. Reperfusion. Prophylaxis.

RESUMO

OBJETIVO: Investigar o papel da b-(1-3)-D-glucana na translocação de *Escherichia coli* marcada com ^{99m}Tc e na secreção de citocinas em ratos submetidos a isquemia e

reperfusão intestinal. **MÉTODOS:** Cinco grupos (n=10 cada) de ratos Wistar foram denominados controle (C), *sham* (S), grupo IR submetido a 45 minutos de isquemia do intestino delgado e 60 minutos de reperfusão(I/R), grupo I/R+glucana com 45 minutos de isquemia e 60 minutos de reperfusão(I/R) e tratados com glucana 2mg/Kg intramuscular. Translocação de *Escherichia coli* marcada com ^{99m}Tc, para Linfonodos mesentéricos, fígado, baço, pulmão e soro foi avaliada usando contagem de radioatividade e de unidades formadoras de colônias/g (UFC/g) Dosagem sérica de TNFa, IL-1b, IL-6, IL-10 foi realizada pelo método ELISA. **RESULTADOS:** CFU/g e contagem de radioatividade foi significantemente maior nos ratos do grupo I/R do que no grupo I/R+glucana. Nos grupos C, S e S+glucana bactérias e contagem radioativa foram raramente detectadas. Os ratos do grupo I/R+glucana tiveram aumento de IL-10 sérica e significante redução da expressão de TNFa, IL-1b e IL-6, quando comparados com os animais não tratados do grupo I/R.**CONCLUSÃO:** A b-(1-3)-D-glucana modulou a produção de citocinas pró-inflamatórias e anti-inflamatórias durante a isquemia/reperfusão intestinal e contribuiu para reduzir a translocação de bactérias marcadas.

Descritores: Translocação bacteriana. Glucana. Intestino. Isquemia. Reperfusão. Profilaxia.

Introduction

Maintenance of bacteria and their products in the intestine is done by both mucin and a layer of epithelial cells, the intestinal barrier that is essential for health and survival. These gut cells are in constant division, metabolizing rapidly and forming an impermeable barrier to harmful intestinal contents. Because they are metabolically active, they are also susceptible to oxygen deprivation with subsequent ischemic damage to enterocytes and their supporting structures[1]. This insult results in epithelial cell damage, decreased absorptive function, and the loss of basement membrane integrity leading to translocation of bacteria[2]. Bacterial translocation (BT) was originally defined and described by Berg and Garlington[3] as the passage of viable bacteria through the intestinal mucosa into the mesenteric lymph nodes (MLN) and to other tissues and organs. It has been suggested that gut ischemia/reperfusion induces disruption of the intestinal mucosal barrier, allowing translocation of bacteria and endotoxin from within the bowel into the blood, an event that may initiate a systemic inflammatory response and the secretion and activation of inflammatory mediators, including cytokines[4]. Although it has been difficult to show BT in clinical cases, patients suffering from hemorrhagic shock or post-surgical syndrome are quite susceptible to endotoxemia and multiple organ failure[5]. b-(1-3)-glucan purified from fungi have been shown to have broad anti-infective activities[6]. It have been shown to bind to receptors on leukocytes and stimulate some immune responses, such as cytokine release[7], and generation of nitric oxide[8]. Soluble b-glucan has also been shown to enhance the clearance of bacteria from the blood, and reduce mortality in rat sepsis models[9]. The present experiment was designed to analyze the effect of soluble b-(1-3)-glucan in rats submitted to bowel ischemia, with and without reperfusion, on translocation of ^{99m}Tc labelled bacteria from the intestinal mucosa to MLN, liver, spleen, lung and serum. Additionally, the levels of serum cytokines were studied and correlated with BT and b-(1-3)-glucan administration.

Methods

Radiolabelling of bacteria

Escherichia coli were labelled with ^{99m}Tc, as follows. Briefly, a sample (0.1 mL) of *E. coli* ATCC-10536 culture, grown overnight in soybean casein medium, was incubated in 10mL of the same medium, under aeration, for 4 hour at 37ºC. After that, different amounts of stannous chloride were added to 2 mL of the medium to reach final concentrations of 40, 130, 290, 400 and 580 mM, respectively. The samples were then incubated at 37ºC for 10, 20, 40 and 60 min. After incubation, 37.0 MBq of ^{99m}Tc were added to each preparation and kept at 37ºC for 10 min. The tubes were then centrifuged at 3000x g for 25 min, washed and resuspended with normal saline. After three washes with saline, the ^{99m}Tc *E. coli* were incubated at 37ºC for 36h. Aliquots (100 mL) of supernatant and resuspended precipitate in saline were withdrawn for determination of radioactivity. This procedure was repeated three times. In order to evaluate the bacterial viability, aliquots were taken from the last suspension, spread into a solid culture medium and incubated at 37ºC for 24 h. The effect of the procedure on the bacterial viability was assessed by comparing the colony-forming units per mL (CFU/mL) of labelled and unlabelled *E. coli*.

Animals

Male Wistar rats weighing 285±14g were maintained under conditions with controlled temperature, on a 12h light-dark cycle and fed *ad libitum* with commercially available rat chow and water.They were randomly divided into four groups (n=10 each), and named, respectively: C group, for non-operated rats, which were the controls, S group, for sham-operated, I/R for rats submitted to 45 minutes of intestinal ischemia and 60 minutes reperfusion, and I/R+glucan for those ischemia/reperfusion group treated with glucan (2mg/Kg) intramuscular. All the animals were gavaged with ^{99m}Tc *E. coli*, two hours before the operative procedures. After fasting overnight, the animals were anesthetized with intramuscular ketamine (50mg/kg) and xilazine (7mg/kg). In the I/R and I/R+glucan groups, the superior mesenteric artery (SMA) was occluded with a microvascular clamp. The laparotomy incision was then closed, to be opened 45 minutes later for removal of the clamp. Reperfusion was confirmed by the return of pulsation to the mesenteric arcade. The incision was again closed and the animals were killed with overdose of anesthetic 60 minutes later.

Glucan administration

For each experiment, soluble b-(1-3)-D-glucan (ImunoglucanÒ) was administered intramuscularly to 10 rats of I/R+glucana group, at a dose of 2mg/Kg of body weight.

Measurement of radioactivity, bacterial counting and cytokines

At the end of the procedures, under aseptic conditions, a midline laparotomy was performed and blood was collected from the portal vein for culture, counting and cytokines assays. One mL of serum was aliquoted for radioactivity counting. One gram

of MLN complex, spleen, liver and lung were removed for counting and culture, if 1g of tissue was available; otherwise, the entire organ was weighed. Tissues were homogenized and solubilized. Aliquots of 0.2mL were processed and were then counted in a PerkinElmer - Wizard TM Gama Counter. Other portions (0,2mL) were cultured on selective MacConkey's agar and blood agar for detection of gram-negative and gram-positive bacteria, respectively. The plates were examined after 24 and 48 hours of incubation at 37ºC. Portal blood samples were used for measurement of tumor necrosis factor-alfa (TNFa), interleukin-1b (IL-1b), interleukin-6 (IL-6), and interleukin-10 (IL-10) assayed using ELISA. Sensitivity of detection was 30 pg/ml for all cytokines. Procedures involving animals and their care were conducted in conformity with the *Guide for the Care and Use of Laboratory Animals*, US National Research Council, 1996. The data analysis were performed using the BioEstat 2.0 program. The results were tabulated and compared by ANOVA using post hoc analysis with Newman-Keuls test. P<=0.05 was considered statistically significant.

Results

All animals survived the experimental protocol. The bacterial viability test showed that the number of colony forming units (CFU) of the *E. coli* under radiolabelling procedure was the same as that grown in absence of ^{99m}Tc (data not shown). When the C and S groups were compared with I/R, and I/R+glucan groups, a significant variation on the labelled bacteria migration to different organs was found. As shown in Table 1, the concentration of radio labelled *E. coli* was the greatest in the MLN, lung, and liver in ischemia/reperfusion (IR) rats. So, the MLN, spleen, liver, lung and serum from I/R rats had significantly higher levels of radioactivity than did the organs from the I/R+glucan ($p<0.01$). The level of positive cultures with CFU was significantly higher in I/R rats than in I/R+glucan group (Table 2). The C group was the only one where the organs and serum were free of any bacterial colony. In the S group the bacteria were rarely detected. As observed with the mean count of radioactivity, bacteria were less detected in the spleen than in the other organs studied (Tables 1,2). The most common bacteria cultured from the organs and serum were *E. coli* and *Enterococcus*. TNF-a, IL-1b, IL-6, and IL-10 were not detected in the serum of the C group, while there concentrations in the serum of S operated rats were 41,7±9,4pg/ml, 34±11pg/mL, 144±17pg/mL and, 94±21pg/mL respectively. Significant increase in serum level of TNF-a (753.7±91pg/ml), IL-1b (588.7±100pg/ml), IL-6 (422.1±56pg/ml) and, IL-10 (311±52pg/mL) was observed in I/R group, when compared with C and S rats ($p<0,01$). The I/R+glucan rats had the serum levels of TNF-a (98±23pg/mL), IL-1b (122±19pg/ml) and, IL-6 (110±31pg/mL) significantly lower than that observed in the I/R rats ($p<0,01$). Nevertheless, an inverse result was observed in the IL-10. There was a significant increase ($p<0,01$) in the level of IL-10 in the I/R+glucan group when compared to the I/R (Table 3).

Discussion

The gut has been suggested to be a port of entry for bacteria after intestinal mucosal injury and endotoxin challenge[10]. The translocation process involves the initial attachment of the bacteria to the gut wall, which by itself can elicit production of cytokines and initiate the subsequent inflammatory response. Once intact microbes penetrate the mucosa, they may be transported to distant organs of even the systemic circulation[11]. As shown in the present study, bowel ischemia and reperfusion promoted

bacteria translocation. In addition, when compared to the control and sham, this phenomenon was significantly higher for MLN, spleen, liver, lungs and, serum in all other groups. Redan et al[12] speculate that the route of BT is through lymphatics into the right side of the heart and then to the lung. The pulmonary vascular bed would then represents the first capillary system in which the translocated bacteria encounter circulating phagocyte cells. In fact, a great amount of colony-forming units of bacteria were found in the lung. The hypoxia, followed by change in intestinal barrier function, generates a vicious cycle of increased permeability, leading to toxic mediators release, and resulting in a further increase in gut permeability, facilitating the BT[13]. However, no significant difference in radioactivity and CFU were found when they were compared the S group, where the intestines were gently manipulated, and in the C group. In this study, increased serum levels of TNF-a, IL-1b, IL-6 and IL-10 reflected the ischemia/reperfusion injury, as demonstrated by other in vivo trials[14,15]. It has been suggested that IL-6 produced by intraepithelial lymphocytes is responsible for the loss of intestinal barrier function following hemorrhage, and the extent of loss can be correlated with plasma levels of this cytokine[16]. In the rats treated with soluble b-(1-3)-D-glucan it was observed a significantly different cytokine response, which was characterized by decreased production of TNF-a, IL-1b, and IL-6, suggesting that immunomodulation with soluble glucan might act to depress the inflammatory cytokine response. The decrease in secretion of these pro-inflammatory cytokines coincided with the increase in IL-10 expression and could, at least in part, be explained by the action of this cytokine known to have anti-inflammatory activity. In fact, IL-10 has been shown to inhibit lipopolissacharide-induced monocyte tissue factor expression in whole blood [17] and to decrease TNF-a production in human monocytes[18]. In a model of murine *E. coli* sepsis, TNF-a and IL-1 levels in soluble glucan-treated mice were significantly lower than in untreated control animals[19]. The levels of radioactivity and colony forming units of bacteria on MLN, spleen, liver, lungs and, serum were lower I/R+glucan rats than the I/R ones, meaning that the use of soluble glucan resulted in an overall decrease in bacterial translocation.

Conclusion

Based on the present data, we conclude that stimulation of the reticuloendothelial system by soluble b-(1-3)-D-glucan modulated the production of pro-inflammatory and anti-inflammatory cytokines during intestinal ischemia/reperfusion, and attenuated the translocation of ^{99m}Tc labelled bacteria.

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3.10 Artigo X

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Effects of simvastatin in abdominal sepsis in rats[1]

Efeitos da sinvastatina na sepse abdominal em ratos

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ABSTRACT

PURPOSE: Statins are widely recognized as hypolipemic drugs, but some studies have observed anti-inflammatory and immunomodulatory effects, known as pleiotropic. The aims of this work was to study possible anti-inflammatory effects of simvastatin in abdominal sepsis. Serum pro-inflammatory cytokines and leukocytes count were determined in an experimental model of abdominal sepsis, using cecal ligation and puncture (CLP) in rats. **METHODS:** Twenty eigth Wistar rats weighing 285±12g were randomly divided in: CLP/Sinvastatin rats (n=7), treated with 10 mg/Kg of oral simvastatin 18 and 2 hs berofe CLP; CLP/Saline group rats (n=7), treated with oral saline; group Sham/Simvastatin (n=7), treated with simvastatin, and group Sham/Saline (n=7), treated with saline. Serum TNF-a, IL-1b and IL-6 by ELISA and total leukocytes, neutrophils, lymphocytes, and eosinophils were determined 24 hs after CLP. ANOVA and Tukey test were used considering significant $p<0.05$. **RESULTS:** It was demonstrated that serum TNF-a, IL-1b and IL-6 were respectively 364,8±42pg/mL; 46,3±18pg/mL and 28,4±13pg/mL in CLP/Sinvastatin rats, significantly lower ($p<0.05$) than in group CLP/Saline (778,5±86pg/ml; 176,9±46pg/ml; 133,6±21 pg/ml, respectively). The same results were observed in total leukocytes and neutrophils counts. **CONCLUSION:** These results clearly demonstrate that simvastatin is an effective agent that reduces cytokines levels and leukocyte count in sepsis, independently of its well-known lipid-lowering effects. Thus, HMG-CoA reductase inhibitors like simvastatin have important anti-inflammatory effects in abdominal sepsis in rats.

Key words: Statin. Inflammation. Abdominal sepsis. Wistar rat. Cytokine. Leukocyte.

RESUMO

OBJETIVO: As estatinas são agentes reconhecidamente hipolipemiantes. Vários estudos têm revelado que eles têm ações pleiotrópicas, como antiinflamatória e imunomoduladora. Tentando-se entender o papel antiinflamatório da sinvastatina na sepse, foram analisados os níveis de citocinas pró-inflamatórias e contagem de leucócitos em modelo de sepse abdominal por ligadura e punção do ceco (LPC) em ratos**. MÉTODOS:** Foram utilizados 28 ratos *Wistar*

pesando 285±12g, assim divididos: grupo sepse (n=14), submetidos a LPC e grupo *sham* (n=14), submetidos a laparotomia e manipulação suave do ceco. No grupo LPC/sinvastatina (n=7) os ratos receberam 10mg/kg de sinvastatina via oral 18 e 2 horas antes da LPC e no grupo LPC/salina (n=7) os ratos receberam injeção oral de solução salina 0,9 %. Os animais dos grupos *sham/*sinvastatina (n=7) e sham/salina (n=7) receberam o mesmo tratamento. Dosagem de TNF-a, IL-1b e IL-6 por ELISA e contagem de leucócitos totais, neutrófilos, linfócitos e eosinófilos foram realizadas em todos os animais. Análise estatística foi feita pelo ANOVA e teste de Tukey, com significância p<0,05. **RESULTADOS:** Ficou demonstrado que as dosagens de TNF-a, IL-1b e IL-6 atingiram valores de 364,8±42pg/ml; 46,3±18pg/ml e 28,4±13pg/ml no grupo submetido à sepse e tratados com sinvastatina, significantemente mais baixos do que no grupo sepse não tratados (778,5±86pg/ml; 176,9±46pg/ml; 133,6±21 pg/ml, respectivamente). O mesmo ocorreu na contagem de leucócitos totais e neutrófilos. **CONCLUSÃO:** A sinvastatina mostrou ação anti-inflamatória em ratos *Wistar,* diminuiu níveis de citocinas e leucócitos, sugerindo uso potencial na prevenção ou atenuação dos efeitos da sepse abdominal.

Descritores: Estatina. Inflamação. Sepse abdominal. Rato Wistar. Citocinas. Leucócitos.

Introduction

Statins are powerful hypolipemic drugs with pleiotropic effects and have been shown to improve survival in the primary and secondary prevention of atherosclerosis in numerous large randomized clinical trials[1,2]. By inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase and the mevalonate pathway to cholesterol, statins reduce not only cholesterol but also the production of several of its nonsteroidal isoprenoid precursor intermediates[3], which are necessary to membrane anchor proteins critical to the binding of signaling proteins involved in various cell functions. Several cellular and animal models demonstrate the pleiotropic activity of statins, including antiinflammatory and antioxidative properties, immunomodulatory effects, improvement in endothelial function, reduction in blood thrombogenicity, and increased nitric oxide (NO) bioavailability. Some or all of these effects may account for a substantial potential impact of statins on the complex pro and anti-inflammatory sequence of events occurring during sepsis. Extensive research has been invested in the last 2 decades and sepsis remains the leading cause of death among patients treated in intensive care units, with mortality rates ranging between 30% and 70%[4,5]. Sepsis is generally viewed as a disease aggravated by the inappropriate and inefficient immune response encountered in the affected individual. Corticosteroids[6,7], activated protein C[8], tumor necrosis factor (TNF) antagonists[9], interleukin-1 receptor antagonists[10], anti-endotoxin antibodies[11], and ibuprofen[12] have all been evaluated in a clinical setting, with improved outcome demonstrated recently for activated protein C. HMG-CoA reductase inhibitors (statins) such as simvastatin have been shown to exhibit important immunomodulatory effects independent of lipid lowering[13]. These pleiotropic effects have been demonstrated to include anti-inflammatory actions[14], improvement of endothelial and microvascular function, and modulation of endothelial nitric oxide synthase (eNOS)[15]. However, statins have thus far not been used to treat severe inflammatory states such as sepsis. Knowing that infection is an important risk factor to operated people and that statins have anti-inflammatory and antioxidant properties, we hypothesized that simvastatin pretreatment would be protective against abdominal sepsis in rats.

Methods

The experimental protocol was approved by the Research Ethics Committee of the University Hospital-UFRN, Brazil. Animals were handled in accordance with the Guide for the Care and Use of Laboratory Animals, US National Research Council, 1996.

Animals

Wistar rats weighing 285??6g were used. Rats were housed in polypropilene cages and maintained under controlled temperature conditions on a 12h light-dark cycle and allowed *ad libitum* access to commercially available rat chow (Labina, PurinaÒ) and water.

Experimental design

A total of 28 Wistar rats were randomly distributed into the following four groups: In the sepsis group (n=14), a half of the (CLP/Sinvastatin) rats (n=7) received 10 mg/Kg of simvastatin microemulsion via gavage, 18 and 2 hours before cecal ligation and puncture (CLP). The remaining (CLP/Saline group) rats (n=7) were treated with oral injection of saline 18 and 2 hs before CLP. In the group sham, 7 rats were treated with simvastatin (Sham/Simvastatin group) and 7 with saline (Sham/Saline group) as sepsis group.

Surgical models

Animals were fasted 12 hr before the experiment and anesthetized with intramuscular injection of 0.1 mL/100g weight, of a solution prepared with 1.0 mL of ketamine (50mg/mL) and 1.0 mL of xilazine (20mg/mL). They breathed spontaneously throughout the procedures. After shaving, the abdominal skin was disinfected with 70% alcohol. All procedures were performed under sterile conditions. Midline laparotomy (3 cm) and gentle manipulation of cecum was performed in the sham group. In the sepsis group the cecum was exposed, ligated with silk 2-0, one cm distally to the ileocecal valve to avoid intestinal obstruction. Four punctures were performed with a 22-gauge needle, squeezed gently to force out a small amount of feces, and then it was returned to the abdominal cavity. The abdominal incision was closed with 4-0 nylon sutures. All animals were observed for 24 hours, weighed again and anesthetized with ketamine intramuscular (50 mg/kg). Thorax was opened, blood was collected by cardiac puncture for cytokine assay and leukocyte count.

Experimental design

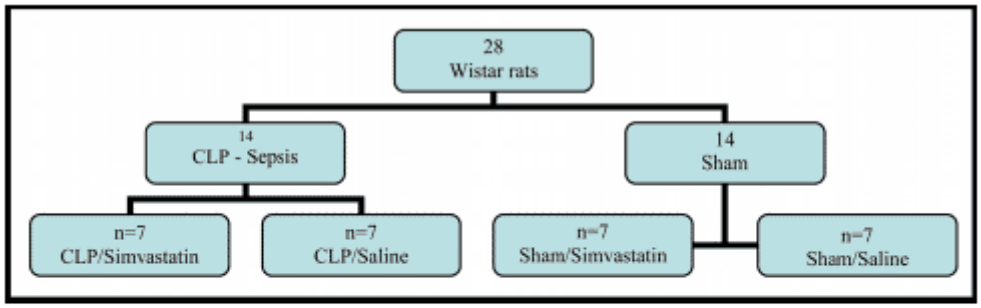

FIGURE 1 - Experimental design: 14 rats were divided into group CLP/sepsis treated with simvastatin (CLP/Simvastatin n=7) and with saline (CLP/ saline n=7). In group sham (n=14), rats were treated with simvastatin (Sham/Simvastatin n=7) and with saline (Sham/Saline n=7).

Cytokine assays

Blood samples were used for measurement of tumor necrosis factor-alfa (TNFa), interleukin-1b (IL-1b) and interleukin-6 (IL-6) , determined using enzyme-linked immunoassay kits (all from PeproTech, Rocky Hill, NJ, USA), according to the manufacturer's recommended protocols. The fluorescence was measured by a Bio-Tec Instruments EL 808 ultra microplate reader, using KC4-V3.0 analysis software. Sensitivity of detection was 20 pg/ml for cytokines.

Leukocyte count

Whole blood was collected by cardiac puncture for leukocyte cell counts using a commercially available automated cell counter (Abbott Cell-Dyn 3500R- CD 3500 5L, USA).

Statistical analysis

Data are reported as mean± SEM. Statistical analyses were conducted with commercially available software SPSS 14.0.1 for Windows. Values of p were reported in cases in which tests were performed. A value of $p<0.05$ was considered significant. ANOVA with post hoc Tukey's test was used to compare the groups.

Results

All the animals survived to experiments. The results were tabulated and exhibited as mean±SD. Leukocyte counts obtained at 24 hours after CLP confirmed significant lowering of WBC and neutrophils in simvastatin treated (CLP/simvastatin) rats of the sepsis group, when compared with the untreated (CLP/saline) rats ($p<0.05$), as can be seen on Table 1. To address possible changes in WBC, neutrophils, limphocytes and eosinophils, secondary to the sham operation, we studied the cells count. No difference was observed comparing the simvastatin (Sham/Simvastatim) treated and saline (Sham/Saline) treated rats ($p>0.05$). To investigate the effects of sepsis and simvastatin treatment on cytokines, serum was isolated from all groups of rats (CLP/saline, CLP-simvastatin, sham/saline, and sham/simvastatin) and subjected to

ELISA assay. The levels of TNFa, IL-1b and IL-6 from CLP/simvastatin treated rats were significantly decreased compared with that of CLP/saline rats (Table 2). Cytokines from CLP animals, treated or untreated with simvastatin, displayed an increased levels compared with the sham operated rats ($p>0.05$), as observed in Table 2. No difference was detected among the values of cytokines (pg/mL) from sham rats treated with simvastatin and sham-operated rats treated with saline. ($p>0.05$). This observation indicates that the simvastatin has interference with the expression of cytokines in septic animals, but not in the absence of sepsis.

Discussion

The 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase inhibitor class of drugs (statins) was introduced into clinical practice in the 1980s. They have become the most widely used drugs for lowering plasma cholesterol. Patients with coronary artery disease, highrisk elderly patients, and those having major surgery, benefit from statin therapy[16-19]. Some works have been presenting several effects (anti-inflammatory, antitrombotic, immunomodulator, etc) of the statins, those denominated together as pleiotropic effects, that do not depend on the reductions in the cholesterol levels[20-23,25]. Enlarging the classic use of the statins, the challenge in subject is to evidence other actions of these molecules seen that, many pleiotropic effects have been told, as well as anti-inflammatory properties, action in the endotelial function and benefits in the hemostasia[23-25,]. In the best attempt to understand the anti-inflammatory effects of the simvastatin in the sepsis, rats were previously treated with this drug and submitted to the model of abdominal sepsis by CLP. The levels of pro-inflammatory cytokines and counting of total leukocytes, neutrophils, lymphocytes and eosinphils were analyzed, considering that they are factors that participate actively of the inflammatory process. The experimental model is one of the main means to study sepsis of abdominal origin. The study of the sepsis in experimental models can be driven with administration lipopolissacárides (LPS) intravascular, bacterial peritonitis induced by introduction of feces or bacteria in the peritonial cavity, opening of an intestinal segment or cecal ligation and puncture[26,27]. The CLP model was adopted in this work by presenting some advantages, as it is easy reproducible, simple, it is not necessary the standardization of an inocule. This is the model that better approaches the human sepsis. The sepsis is polymicrobial and simulates the perforated appendicitis or diverticulitis[26]. It is believed that this experimental model is an appropriate study method to evaluate and to control the septic phenomena from its installation to the moment of failure of the organs and systems in different times in this process[28]. The experimental design of this study was elaborated in a such way that the evidences of the anti-inflammatory effect of the sinvastatin in the abdominal sepse were evaluated in currently used biological models. After the statistical treatment of the results, a discerning analysis of these data resulted in some interesting observations. Except for the groups without infection (group sham), the total leukocytes count indicated an accumulation of these cells as a consequence of the trauma and ischemia on the tissues. The use of simvastatin in the infected rats inhibited the accumulation of the neutrophils, but not in the absence of sepsis. On the other hand, it was observed that in the septic groups, the simvastatin didn't promote significant alteration in the lymphocytes and eosinophils counts. In relation to the cytokines dosages, it was observed that the simvastatin didn't result in a significant change in the levels of TNFa, IL-1b and IL-6 in the sham rats. The abdominal sepsis served to demonstrate a significant anti-inflammatory effect of simvastatin. This fact can be corroborated by the significant

reduction of the levels of these cytokines in the infected animals, when simvastatin was administered. These data suggest an important relationship between the statin and the cells of the immune system in the validity of the mechanisms of repair of the traumatic damage, as well as during the activation of the monocytes. Therefore, it was demonstrated in the present work that the serum TNFa, IL-1b, IL-6, total leukocytes and neutrophils had statistically significant reduction ($p < 0,05$) in the groups submitted to the sepsis and treated with simvastatin, compared with those non treated rats. These data corroborate with the work of Villa et al[29], where the levels of TNF-a, IL-1b and IL-6 became altered in the same model of CLP polymicrobial abdominal sepsis. Koo et al[30] demonstrated in CPL model that the expression of genes for these cytokines happen during the abdominal sepse, not only in the intestinal site, but also in other organs. The results obtained in the present work are also in agreement with those visualized by Merx et al[31]. They demonstrated that the simvastatin, injected 20 hours after CLP in the same concentration used in the present study (10 mg/mL), increased the time of survival, as well as it preserved the heart and hemodinamic functions of the studied rats. In this same work it was demonstrated *in vitro* that the monocytes adhesion was increased in the group sepsis, when compared with the group sham. The adhesion decreased when these cells were incubated with simvastatin. The increasing adhesion is an important factor in the physiopathology of the sepsis. The benefit of the anti-inflammatory action of the statins was also analyzed by Merx et al[31,32], who studied the effect of the atrovastatin, pravastatin, simvastatin and fluvastatin in the survival in a CLP model in murines. The authors demonstrated that the treatment after 6 hours of the induction of the sepse increased the time of survival of the animals, except the fluvastatin, that didn't alter the survival. In the present study we did not find difference in survival between the groups, because no mortality occurred. The host reaction to the peritoneal sepsis involves antibodies production, complement activation, cellular immunity and bacterial destruction by polymorphonuclear leukocytes and macrophages[33-35]. The mechanism of the anti-inflammatory action of simvastin is not completely elucidated. However, some hypothesis exist to explain its action. The bacterial toxins are recognized by a variety of receptors in the monocyte surface, macrophages and granulocytes. The cytokines (TNF, IL-1 and IL-6) increase the expression of adhesion molecules (selectines and iCAMs) recruiting neutrófilos for the infection site[35]. The IL-1 produces several effects similar to the exogen TNF, as fever, anorexia and hypotension. It also produces increase in the leukocyte adhesion, bone reabsorption, inhibition of the lipoprotein-lipase and the synthesis of collagen[36]. Great efforts have been used in the attempt of elucidating the action of the statins in the sepsis, because there great therapeutic potential[37,38]. Few clinical studies have been published recently to support the hypothesis of the action of therapy with simvastatin in sepse. Almog et al[39] performed a prospective observacional cohort study to determine the impact of pre-treatment with statins in the occurrence of severe sepse in infected patients. Of the 361 patients with bacterial infection, 82 (23%) had received statins at least 4 weeks before admission. The mortality tax was low and it didn't differ significantly among the 2 groups (3.7% vs 8.6%, P=0.21). Severe sepsis developed in the 2.4% and 19%, of the patients respectively, in the group with statin and without statin. In other retrospective revision of 388 patient with bacteremia, Liappis et al[40] described a significant reduction in the patients' mortality when they received statins in the period of the admission, compared with those without this therapy.

Conclusion

The data of the present study suggest that simvastatin has potential to attenuate or to prevent the effects of the abdominal sepsis in rats subjected to cecal ligation and punture, represented by the reduction of the levels of serum cytokines, total leukocytes and neutrophils.

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3.11 Artigo XI

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Influence of laparoscopy and laparotomy on gasometry, leukocytes and cytokines in a rat abdominal sepsis model[1]

Influência da laparoscopia e laparotomia na gasometria, leucócitos e citocinas em modelo de sepse abdominal em ratos.

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ABSTRACT

PURPOSE: Laparoscopic surgery is associated with reduced surgical trauma, and less acute phase response, as compared with open surgery. Cytokines are important regulators of the biological response to surgical and anesthetic stress. The aim of this study was to determine if CO_2 pneumoperitoneum would change cytokine expression, gas parameters and leukocyte count in septic rats.**METHODS:** Wistar rats were randomly assigned to five groups: control (anesthesia only), laparotomy, CO_2 pneumoperitoneum, cecum ligation and puncture by laparotomy, and laparoscopic cecum ligation and puncture. After 30 min of the procedures, arterial blood samples were obtained to determine leukocytes subpopulations by hemocytometer. TNFa, IL-1b, IL-6 were determined in intraperitoneal fluid (by ELISA). Gas parameters were measured on arterial blood, intraperitoneal and subperitoneal exsudates. **RESULTS:** Peritoneal TNFa, IL-1b and IL-6 concentrations were lower in pneumoperitoneum rats than in all other groups ($p<0.05$). TNFa, IL-1b and IL-6 expression was lower in the laparoscopic than in laparotomic sepsis ($p<0.05$). Rats from laparoscopic cecum ligation and puncture group developed significant hypercarbic acidosis in blood and subperitoneal fluid when compared to open procedure group. Total white blood cells and lymphocytes were significantly lower in laparoscopic cecum ligation and puncture rats than in the laparotomic ($p<0.01$). Nevertheless, the laparotomic cecum ligation rats had a significant increase in blood neutrophils and eosinophils when compared with controls ($p<0.05$). **CONCLUSIONS:** This study demonstrates that the CO_2 pneumoperitoneum reduced the inflammatory response in an animal model of peritonitis with respect to intraperitoneal cytokines, white blood cell count and clinical correlates of sepsis. The pneumoperitoneum produced hypercarbic acidosis in septic animals.

Key words: Pneumoperitoneum. Carbon Dioxide. Sepsis. Acidosis. Leucocytes.

RESUMO

OBJETIVO: A cirurgia laparoscópica está associada com trauma reduzido e baixa resposta na fase aguda do trauma, quando comparada com a cirurgia aberta. As citocinas e o balanço ácido-base são fatores importantes da resposta biológica ao trauma cirúrgico-anestésico. O objetivo deste estudo foi determinar se o pneumoperitôneo com CO_2 altera a expressão das citocinas, a gasometria e a contagem diferencial de leucócitos em ratos com sepse abdominal. **MÉTODOS:** Ratos Wistar foram aleatoriamente distribuídos em 5 grupos: controle (somente anestesia), laparotomia, pneumoperitôneo com CO_2, ligadura e punção do ceco por laparotomia, ligadura e punção do ceco por laparoscopia. Após 30 minutos dos procedimentos, sangue arterial foi colhido para leucometria diferencial em hemocitômetro. TNFa, IL-1b e IL-6 foram dosadas no líquido intraperitoneal (por ELISA). Os parâmetros gasosos foram medidos no sangue arterial e nos exsudatos intraperitoneal e subperitoneal. **RESULTADOS:** Os valores de TNFa, IL-1b e IL-6 foram significantemente menores nos ratos submetidos ao pneumoperitôneo do que em todos os outros grupos ($p<0.05$). Expressão de TNFa, IL-1b e IL-6 foi menor no grupo sepse induzida por laparoscopia do que por laparotomia ($p<0.05$). Os ratos submetidos a ligadura e punção do ceco via laparoscópica desenvolveram acidose hipercárbica no sangue arterial e exsudato subperitoneal, mais intensa do que no grupo sepse laparotômica. Leucopenia e linfopenia foram mais acentuadas no grupo sepse laparoscópica ($p<0.01$). Entretanto, os animais submetidos a sepse laparotômica desenvolveram significante aumento de neutrófilos e eosinófilos quando comparados com os controles ($p<0.05$). **CONCLUSÕES:** Este estudo demonstrou que o pneumoperitôneo com CO_2 contribuiu para reduzir a resposta inflamatória em ratos submetidos a modelo de sepse abdominal, no que diz respeito à expressão de citocinas intraperitoneais e leucometria diferencial. O pneumoperitôneo também contribuiu para instalação de acidose hipercárbica nos ratos sépticos.

Descritores: Pneumoperitônio. Dióxido de Carbono. Sepse. Acidose. Leucócitos.

Introduction

Operative laparoscopy brought a new dimension to surgical practice, and many experimental and clinical studies have demonstrated feasibility, safety, cost-benefit, and pathophysiologic occurrences. The intraabdominal insufflation of carbon dioxide (CO2) is the most widely used technique for the creation of a pneumoperitoneum. Insufflation under a continuous monitoring of intraabdominal pressure throughout the surgical procedure provides adequate exposure of the operating field. As alternatives, different gases (e.g., helium, argon, nitrous oxide, air) may be used, but they have not been adopted clinically[1,2,3,4,5,6]. The actual knowledge concerning pneumoperitoneum are sometimes inconsistent about the physiologic consequences induced by insufflation of the peritoneal cavity with CO_2. Results from animal models about the effects of a pneumoperitoneum in certain pathologic conditions are often alarming[7]. However, the clinical impact of these changes is unknown, since the majority of patients who undergo laparoscopic procedures do not exhibit any adverse clinical effects either in the short or the long-term course. Laparoscopíc surgery is applied increasingly to abdominal diseases complicated by diffuse or localized peritonitis such as appendicitis,

perforated peptic ulcers and diverticulitis[8,9]. A specific paper has reported the use of laparoscopy in diverticulitis complicated by localized peritonitis with intra-abdominal abscess formation[10], and diagnostic laparoscopy is being advocated in diffuse peritonitis after blunt abdominal trauma[11]. However, a theoretical concern with the use of laparoscopic techniques in clinical cases complicated by intra-abdominal infection and peritonitis, is that carbon dioxide pneumoperitoneum may increase the risk of bacteraemia and sepsis by increasing intra-abdominal pressure. Some studies have demonstrated immunossuppressive effects of carbon dioxide on neutrophil and macrophage function. In one study, CO_2 blocked superoxide release from activated polimorphonuclear leukocytes and significantly reduced the secretion of IL-1 from human peritoneal macrophages[12]. Whereas these effects might be considered beneficial from the standpoint of inflammation following elective surgery, experimental evidence suggests that the CO_2 induced immunossuppression might be deleterious in the setting of infection[13]. This may have an adverse effect on clinical outcome when compared with open procedures. Although some evidences, few data exist regarding the effect of pneumoperitoneum and increased intra-abdominal pressure on sepsis and physiological outcome. The aims of the current study were to investigate the influence of laparoscopic procedures, in particular CO2 insufflation, on the response to sepsis in an animal model—cecal ligation and puncture (CLP) in the rat. Clinical evolution, gasometry, pro-inflammatory cytokines and leukocytes were analyzed.

Methods

Male Wistar rats (Animal Colony from Nucleus of Experimental Surgery, Federal University of Rio Grande do Norte, Brazil), 12 to 13 weeks old, were housed in cages where standard chow and water were available ad libitum. The rats were acclimatized to the laboratory environment for 5 days on arrival and then fasted for 12 hours before any procedures. Anesthesia was obtained using pentobarbital 20 mg/Kg intraperitoneal and ketamine 50 mg/Kg intramuscular. All surgical procedures were performed under aseptic conditions. The animals were allowed to breathe spontaneously for the duration of the experiment. The group C (control) rats were subjected to anesthesia only (n=6). In the LAP (laparotomy) group (n=7) the following procedures were performed: after anesthesia and antisepsis with povidone, a 5cm laparotomy kept the peritoneal cavity exposed to the room air during 30 minutes and the abdominal wall was sutured with nylon 4-0. The PNP rats (n=7) were subjected to CO_2 pneumoperitoneum using a Veress needle under 3 mmHg for 30 minutes. On the CLP/LAP (n=6) the CLP was performed after laparotomy. A cecum ligation and puncture (CLP) by laparoscopy under pneumoperitoneum were performed on the CLP/PNP rats (n=6).

Cecal ligation and puncture - Pneumoperitoneum was achieved by introducing a Veress needle into the peritoneal cavity and insufflating (Endomed insufflator) the abdomen with 3 mmHg CO_2. Laparoscopic procedures were performed using 3-mm instruments (Henke-Sass, Wolf^{TM}) introduced into the abdomen. Cecal ligation and puncture (CLP) consisted of dissection of the cecum, ligation midway between the ileocecal valve and the terminal cecum using a 3-0 chromic catgut tie, and 8-punctures of the isolated cecum with a hollow 25-gauge needle introduced through the abdominal wall. Laparotomy, for the open CLP group, consisted of a 5-cm midline abdominal incision. The duration of the total procedure, and therefore the duration of anesthesia, pneumoperitoneum, and laparotomy, was standardized to 30 minutes for all groups.

Postoperatively, animals were resuscitated with a subcutaneous injection of lactated Ringer's (30 mL/kg) and were again housed in cages where water was available ad libitum. The experimental protocol was approved by the Research Ethics Committee of the Federal University of Rio Grande do Norte, Brazil, and adhered to the Guide for the Care and Use of Laboratory Animals, US National Research Council, 1996.

Gasometry and cytokines dosage - After the surgical procedures, 5mL of buffered saline were injected in peritoneal cavity and the abdomen was softly massaged for 1 minute. Thirty minutes later, whole blood was collected by cardiac puncture and liquid exsudate was collected from peritoneal cavity and from subperitoneal space, using heparinized capilar tube, for determination of pH, pCO_2 and pO_2. An automatic AVL (Roche®) equipment was used. TNFa, IL-1b and IL-6 were determined in the intraperitoneal exsudate, by enzyme-linked immunosorbent assay, using cytokine-kits from PeproTec (Rocky Hill, NJ,USA).

Leukometry - Animals were evaluated 24 hours postoperatively for clinical signs of CLP-induced sepsis (i.e., dark halo around the eyes, piloerection and lethargy). Whole blood was collected by cardiac puncture for leukocyte cell counts and the rats were then killed via anesthetic overdose. The determination of leukocyte cell counts was preformed using a commercially available automated cell counter (Abbott Cell-Dyn 3500R- CD 3500 5L, USA).Data were expressed as mean±standard deviation. Statistical significance was established using the one way analysis of variance ANOVA followed by Newman-Keuls test. Probabilities less than 0.05 were considered significant.

Results

Cytokines release in peritoneal exsudate - Rats in the control, LAP and PNP groups exhibited normal activity and had no piloerection during the 24 hours after interventions. In contrast, all rats that underwent CLP exhibited decreased activity and significant piloerection. The cytokine levels on LAP rats were higher than PNP ones; however, the difference between these groups was not significant ($p<0.05$). Both surgical procedures, PNP/CLP and LAP/CLP, induced higher TNFa, IL-1b and IL-6 in the peritoneal fluid than were found in the control group. In contrast, the peritoneal fluid TNFa, IL-1b and IL-6 levels in the pneumoperitoneum (PNP) group were significantly lower than in the other groups ($p<0.05$). The PNP/CLP rats had a significantly lower elevation of TNFa, IL-1b and IL-6 expression in the peritoneal fluid than LAP/CLP rats ($p<0.05$) (Figures 1, 2 and 3).

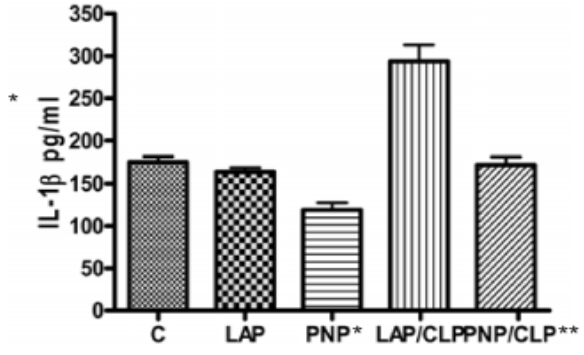

FIGURE 1 - No statistical difference was observed between groups C and LAP. When comparing IL-1β expression in groups LAP/CLP and PNP/CLP**, the difference was significant. ($p < 0.05$)

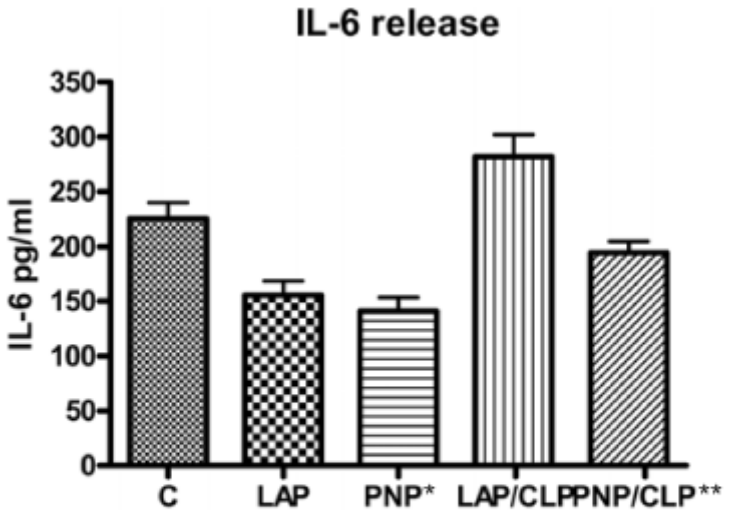

FIGURE 2 - The laparoscopic sepsis group (PNP/CLP) expressed IL-6 significantly lower than laparotomic group ($p < 0.05$). *$p < 0,05$ vs C, PNP/CLP, LAP/CLP

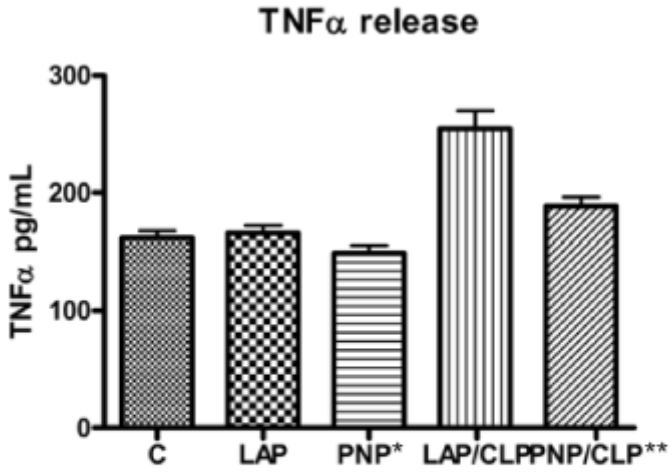

FIGURE 3 - TNF-α levels were significantly lower in laparoscopic sepsis rats (PNP/CLP) than when sepsis was induced by laparotomy (LAP/CLP) ($p < 0.05$)

Gasometry - Arterial blood gas parameters (pH, pO2) in rats from the control group (C) remained significantly higher than in rats from LAP, PNP and PNP/CLP groups ($p<0.01$). Rats from PNP/CLP group developed significant hypercarbic acidosis with mean pH of 7.18±0.05 and pCO_2 60.7±10.2 when compared to LAP/CLP group. The LAP/CLP rats acidosis was not hypercarbic (Table 1). The pCO_2 was significantly higher on PNP/CLP rats than controls ($p<0.01$). Significantly reduced pCO_2 was observed following LAP/CLP, compared to LAP, PNP and PNP/CLP ($p<0.01$). Intraperitoneal exsudate gas analysis revealed acidosis in the PNP group, and the difference was significant when compared with C and LAP groups ($p<0.01$). However, the septic rats subjected to CO_2 pneumoperitoneum (PNP/CLP) developed a profound intraperitoneal acidosis, as a consequence of pCO_2 significantly higher ($p<0.01$) than in all other groups (Table 3). In contrast, the pO_2 was significantly lower than in C and LAP/CLP groups ($p<0.05$). While subperitoneal exsudate pH (6.5±0.05) following laparoscopic CLP using CO_2 (PNP/CLP) was significantly lower ($p<0.01$) than in C, LAP and LAP/CLP rats, the difference did not reach statistical significance when compared with the acidotic pH (6.7±0.08) of PNP group (Table 3). The CO_2 pneumoperitoneum produced a significant increase in pCO_2 in the subperitoneal exsudate, when compared with all the other groups ($p<0.01$).

TABLE 1 – Arterial blood gas parameters

GROUP	pH	pCO2	pO2
C	7,36 ± 0,08*	43,4 ± 4.7§	66,5 ± 29
LAP	7.23 ± 0.03	50.1 ± 9.2	62.4 ± 19
PNP	7.22 ± 0.07	48.6 ± 9.6	61.2 ± 15
PNP/CLP	7.18 ± 0,05**	60.7 ± 10.2	68,7 ± 18
LAP/CLP	7,26 ± 0,04	35.45 ± 7.9†	49.4 ± 19

*P<0,01 vs LAP, PNP, PNP/CLP; **p<0.01 vs LAP/CLP; § p<0,05 vs PNP/CLP; † p<0.05 vs LAP, PNP, PNP/CLP

TABLE 2 – Intraperitoneal exsudate gas parameters

GROUP	pH	pCO2	pO2
C	7,31±0,16	33,6±7,6	113,3±23
LAP	7.30±0.1	39.7±10	95±31
PNP	7.16±0.16**	47±9.7	102±15
PNP/CLP	6,8±0,25*	86,5±41,3*	82,3±15§
LAP/CLP	7,27±0,11	48,3±9,4	122,3±19

*P<0,01 vs C, LAP, PNP, LAP/CLP; **p<0.01 vs C, LAP; § p<0,05 vs C, PNP/CLP

TABLE 3 – Subperitoneal exsudate gas parameters

GROUP	pH	pCO2	pO2
C	7.16±0.2	21±8	146±12
LAP	7.0±0.2	18±9.6	161±20
PNP	6.7±0.08	15±4.4	130±19
PNP/CLP	6.5±0.05*	46±3.8*	122±8.9*
LAP/CLP	7.16±0.1	24±9.8**	165±10.8**

*p<0,01 vs C, LAP, LAP/CLP; **p<0,01 vs PNP/CLP

Leukocytes - White blood cell count in pneumoperitoneum and laparoscopic CLP was similar to those of controls (Table 4). Significantly reduced white cell counts were observed following laparoscopic CLP compared to open CLP ($p<0.01$). No significant difference in blood neutrophil and lymphocyte count was found among control, LAP, PNP and PNP/CLP rats. Nevertheless, the LAP/CLP rats had a significant increase in blood neutrophils when compared with controls ($p<0.05$), as can be observed in Table 4.

The PNP/CLP and LAP/CLP procedures produced a significant reduction in lymphocyte count, compared with controls ($p<0.05$). Additionally, the reduction in lymphocytes was significantly higher in open CLP (LAP/CLP) rats than in PNP/CLP ($p<0.05$). There was a significant reduction in eosinophil count in PNP, LAP/CLP and PNP/CLP, compared with controls ($p<0.05$). The decrease in LAP/CLP eosinophil was greater than in PNP/CLP rats.

Discussion

Because laparoscopic surgery is increasingly used for treating peritonitis and other septic states, a theoretic concern is related to the hypothesis that CO_2 pneumoperitoneum may increase bacteremia with adverse effects for the patient. Some studies have reported technical feasibility of laparoscopic appendectomy, perforated peptic ulcer, perforated diverticulitis and other septic surgical situations[14,15,16]. They are small studies with low numbers of included patients, that can not give strong evidence about improved safety of laparoscopic surgery regarding septicemia. Study in rats showed that pneumoperitoneum causes intestinal ischemia with oxygen free radical production and bacterial translocation, related to mechanical pressure of CO_2[17]. Other studies focused on whether a pneumoperitoneum amplifies the extent and severity of peritonitis or of bacteremia in various animal models[18,19,20,21]. Findings from these investigations are controversial. Whereas some authors reported no increase in bacteremia, intraperitoneal abscess formation, or correlates of sepsis, others reported increased bacterial translocation and severity of peritonitis and sepsis. Laparoscopic surgical technique requires the maintenance of a continuous positive intraperitoneal pressure in patients (approximately 10-15 mmHg) for visualization and manipulation of the viscera. In cases of peritonitis, viable bacteria and bacterial byproducts (including endotoxin) exist free in the peritoneal cavity. Positive intraperitoneal pressure may

increase bacteraemia and endotoxaemia, and thus may worsen clinical sepsis. As experience with laparoscopic surgery increases, its use in more debilitated and critically ill patients is being reported[22]. These patients often have sepsis and suffer from diffuse peritonitis of unclear aetiology, usually the result of a perforated viscus. It has not been clear whether a laparoscopic approach worsens the septic state or whether minimally invasive surgery is beneficial in these critically ill patients. *Experimental model* - Several animal models of peritoneal sepsis have been described varying in many respects, including the specific animal utilized and the method of sepsis[20,23,24,25]. The model of creating sepsis used in this study was adapted from the work of Hanly et al[26]. The convenience of the model of laparoscopic CLP in rats, used in the present experiment, is two-fold. First, the use of a septic animal model magnifies the stress induced by a surgical procedure to more clearly delineate the modifying effects of laparoscopy on the inflammatory response. Second, the combined stressors of bacterial contamination of the peritoneal cavity and bowel ischemia present following laparoscopic CLP provide an environment analogous to clinical situations in which laparoscopy is used to aid in the diagnosis and treatment of patients with peritonitis. The model used in this study presupposes that CLP caused sepsis and that the injury caused by CLP was equivalent between groups. We established the presence or absence of sepsis in rats by evaluating each rat for the presence or absence of periorbital dark halo, the presence or absence of piloerection and normal or decreased activity. All 12 rats that were subjected to CLP were identified as having clinical sepsis, and all 20 rats considered control, or that had received other procedures, were identified as not having sepsis.

Cytokines - In the present study we observed a significant decrease of TNFá, IL-1â and IL-6 in rats subjected to CO_2 pneumoperitoneum with and without sepsis, and these findings coincided with acidosis in arterial blood, in intraperitoneal and subperitoneal exsudates. These data are in agreement with other investigators, who showed inhibition of human peritoneal macrophage cytokine production when these cells were incubated in an acidic extracellular environment, lowered the intracellular pH and attenuated cytokine release[27]. Similarly, Carozzi et al.[28] showed decreased spontaneous release of IL-1, IL-6, IL-8, and TNF when incubations were performed in pH 5.5 medium compared to much higher cytokine levels from cells incubated in medium with a pH of 7.4. In the present study the PNP and PNP/CLP rats exhibited arterial, intraperitoneal and subperitoneal acidosis, suggesting intracellular acidosis. West et al.[32] have proposed relative intracellular acidosis as the mechanism by which the decrease in cytokines is exerted[29]. Redmond et al.[30] showed that circulating monocytes obtained from patients after laparoscopic cholecystectomy exhibited reduced TNF release compared to those from patients who had open cholecystectomy. They also reported that peritoneal macrophages derived from animals undergoing CO_2 laparoscopy released less TNF in response to lipopolyssacharides (LPS) than those undergoing air laparoscopy[31]. West et al.[32] have shown that murine peritoneal macrophages exposed to CO2 in vitro exhibit inhibition of LPS-stimulated IL-1 and TNF cytokine release, suggesting that this effect is related to the influence of the CO2 environment. These findings contribute to explain the reduction of cytokine release when animals and patients are operated under effect of CO_2 pneumoperitoneum. Whereas these effects might be considered beneficial from the standpoint of inflammation following elective surgery, one experimental study suggests that CO_2 induced immunossuppression might be deleterious in the setting of infection[13]. The clinical significance of these findings remains unknown. In studies where laparoscopy was compared to open surgery for

peritoneal infection such as appendicitis, there was no clear augmentation of infectious complications associated with the use of CO_2 pneumoperitoneum[33].

Gas analysis - The effect of pneumoperitoneum on hemodynamics and blood gas variables has been studied extensively in nonseptic animals[34], and to a lesser degree in acute models of sepsis. In the present study, pneumoperitoneum alone and associated with CLP sepsis induced alterations of the acid-base balance, such as fall of pH, and elevation of pCO_2 , in arterial blood, intraperitoneal and subperitoneal exsudate, without correlation to pO_2. The decrease in pH, that was more accentuated with CLP, was similar to what has been reported[25]. These findings were substantiated by other investigators, who found that intraabdominal pH diminishes with application of a CO_2 pneumoperitoneum. CO_2 used as an insufflation gas appeared to lower peritoneal, blood, and subcutaneous pH more than helium, which induced smaller changes[35]. Gandara et al[36] stated that, in addition to CO_2 absorption, this might be a phenomenon of tissue hypoperfusion. Nevertheless, in our study pO_2 was not affected by pneumoperitoneum in septic and non septic rats. This finding can be explained by the fact that pneumoperitoneum was performed with 3 mmHg pressure, sufficient to keep a normal and spontaneous respiration in rats. This pressure was used in rats by other investigators[37]. By the way, Kuntz et al[35]. showed that, after insufflation with CO_2, intraperitoneal pH was inversely related to the intraabdominal pressure.

Leukocytes - All rats subjected to CLP were found at autopsy having darkish, foul-smelling peritoneal fluid consistent with gross fecal contamination of the abdominal cavity. In the present study the total white cell count and circulating neutrophil were significantly lower following laparoscopic CLP using CO_2 than following LAP/CLP. Laparotomic CLP produced a significant reduction in lymphocyte count compared to laparoscopic CLP using CO2, corroborating with data from other authors[37]. Another work has shown a profound drop in white blood cells in animals subjected to laparotomy or pneumoperitoneum under intraperitoneal inoculation of *Escherichia coli*, without difference between them. These data suggest that some results are conflicting, but there is a tendency to leucopenia and lymphopenia after laparoscopic CLP.

Conclusions

The CO_2 pneumoperitoneum reduced the inflammatory response in an animal model of peritonitis with respect to intraperitoneal cytokines, white blood cell count and clinical correlates of sepsis. The pneumoperitoneum produced hypercarbic acidosis in septic rats.

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4 COMENTÁRIOS, CRÍTICAS E CONCLUSÕES

As vantagens da cirurgia bariátrica no tratamento da obesidade têm sido muito difundidas nos últimos anos. Estudos enfatizam que o tratamento cirúrgico permite um controle de peso a médio e longo prazo, reduzindo as comorbidades. Isto possibilitou um maior conhecimento da eficácia, como também das alterações orgânicas provocadas pelos diversos procedimentos, em particular, as atribuídas ao BGYR.

Dado o crescente volume de procedimentos bariátricos realizados nos tempos atuais, sobretudo após o advento da videolaparoscopia, é mister salientar a necessidade de acompanhamento desses pacientes. Para tanto, a realização de exames cintilográficos pós-operatórios não está descartada.

Partindo destes princípios, julgamos importante utilizar a técnica do *bypass* gástrico e observar, essencialmente, se havia ou não alterações na biodistribuição do pertecnetato de sódio no pós-operatório.

Araújo-Filho *et al.* (2007) observaram alterações na biodistribuição do pertecnetato de sódio após intervenção cirúrgica de grande porte, conhecida desvio biliopancreático com *switch* duodenal, um tipo misto de cirurgia bariátrica, ocasião em que não foram utilizados ratos obesos[25].

Dando continuidade aos estudos nessa linha de pesquisa, desenvolvemos um modelo experimental utilizando, desta vez, a técnica do BGYR, a mais utilizada atualmente no tratamento da obesidade mórbida.

Além do trabalho principal aqui descrito, participamos ativamente da elaboração de outros estudos durante o período de dois anos no Programa de Pós-graduação em Ciências da Saúde, todos publicados em periódicos com indexação internacional, o que trouxe como contribuição o enriquecimento do

ponto de vista crítico e científico da autora, correspondendo às expectativas e possibilitando o cumprimento de um cronograma prévio.

A presente tese teve como mérito a criação de um modelo experimental de cirurgia bariátrica que contribuirá para futuras pesquisas e publicações nesta área, possibilitando à autora o seu aperfeiçoamento científico nos aspectos inter e multidisciplinar.

Durante a trajetória na Pós-graduação houve sempre a participação de alunos de Iniciação Científica e de outros colegas pós-graduandos na execução dos trabalhos experimentais, contribuindo sobremaneira para o crescimento pessoal em todos os sentidos, bem como dos demais colegas da Pós-graduação e Graduação.

Dentre as dificuldades encontradas, houve o fato de se tratar de uma intervenção cirúrgica de grande porte, realizada em pequenos animais, o que necessitou do uso de microscópio e instrumental cirúrgico especial para realização dos procedimentos.

Um projeto piloto com pelo menos três séries de experimentos foi necessário, até que se conseguisse sistematizar a técnica com baixa morbi-mortalidade e observar os animais dentro do período pré-estabelecido.

Além disso, trabalhando com material radioativo, utilizamos equipamentos de radioproteção como aventais de chumbo, que dificultaram as manobras operatórias. O caráter mutilante da técnica empregada limitou o tempo de observação pós-operatório.

No presente estudo foi possível determinar alterações pós-operatórias na biodistribuição do pertecnetato de sódio em órgãos do aparelho digestivo e à distância. Contudo, possíveis justificativas para as alterações descritas ficam

na fase de hipóteses, uma vez que tais explicações não foram o objetivo principal deste experimento.

Outros aspectos pós-operatórios necessitam ser analisados a respeito da cirurgia bariátrica, com o objetivo de explicar as mudanças decorrentes do catabolismo excessivo, alterações imunológicas, moleculares, hormonais, dentre outras. Estudos subseqüentes serão realizados na busca do esclarecimento sobre quais repercussões da cirurgia bariátrica empregada podem explicar as alterações na biodistribuição radioativa observadas no presente trabalho.

O primeiro ensaio experimental anexado a esta tese tem como contribuição adicional alertar sobre possíveis distúrbios na biodistribuição do pertecnetato na avaliação pós-operatória de pacientes submetidos à cirurgia bariátrica, especialmente àqueles que venham a utilizar exames cintilográficos. Tal fato pode implicar na proteção contra repetição de exames, interpretação duvidosa de resultados e redução de custos.

4.1 Perspectivas de trabalhos na mesma linha de pesquisa

Como perspectivas de estudos posteriores, algumas perguntas podem ser respondidas em trabalhos futuros relacionados com a cirurgia bariátrica, tais como: as técnicas restritivas ou disabsortivas isoladamente podem ter o mesmo comportamento do *bypsass* gástrico? Até que ponto tais alterações seriam significativas, com potencial para produzir implicações clínicas em humanos operados? Somente ensaios clínicos futuros com uso de exames de imagem da medicina nuclear podem contribuir para elucidar a dúvida.

A linha de pesquisa terá continuidade, a procurar explicações em estudos futuros para as alterações de biodistribuição observadas, através de parâmetros laboratoriais, estudos histológicos e microbiológicos.

4.2 Conclusão

A partir do modelo experimental utilizado no presente estudo, os dados permitem concluir que a cirurgia do *bypass* (desvio) gástrico em Y de *Roux* altera a biodistribuição do pertecnetato de sódio no fígado, estômago e osso de ratos operados.

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Abstract

The Roux-en-Y gastric bypass (RYGB) is the surgical technique used in the treatment of morbid obesity. This reduces the volume of the stomach and small intestine length, generating structural and metabolic changes that may influence the results of scintigraphic examinations of patients. In order to evaluate the postoperative biodistribution of sodium pertechnetate ($Na^{99m}Tc$) in organs of rats subjected to the technique of Roux-en-Y gastric bypass (RYGB), we used 12 rats randomly divided into treatment group (n = 6), submitted to surgery RYGB and the control group (C, n = 6). On the 15th day after surgery was 0.1 mL administered of $Na^{99m}Tc$ by orbital plexus of both groups, with mean activity of 0.66 MBq. After 30 minutes, the rats were killed and fragments of thyroid, heart, lung, liver, stomach, kidney and femur were removed. The samples were washed with 0.9% saline solution, weighed and submitted to the 1470 Gamma Counter, Perkin-Elmer WizardTM-Finland to determine the percentage of total radioactivity per gram (% ATI / g) of each organ. We applied the Student t test for statistical analysis, with a confidence interval of 95%. Significant reduction in mean% ATI / g was observed in the liver, stomach and femur of animals underwent surgery RYGB compared to the control group ($p < 0.05$). In other organs there was no statistically significant difference between the groups. In conclusion, surgery RYGB in rats modified the biodistribution of $Na^{99m}Tc$ in some organs and may have clinical implications in the interpretation of scintigraphic examinations. This study was a multidisciplinary involving researchers at the Experimental Surgery, Pharmacy, Radiobiology, Nuclear Medicine and Statistics.

Keywords: Biological Availability. Bariatric Surgery. Sodium Pertechnetate Tc 99m. Radiopharmaceuticals. Gastric Bypass. Anastomosis Roux-en-Y.

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