UNIVERSIDADE FEDERAL DO RIO GRANDE DO NORTE

CENTRO DE CIÊNCIAS DA SAÚDE

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

AVALIAÇÃO DA BIODISTRIBUIÇÃO DO PERTECNETATO DE SÓDIO EM RATOS *WISTAR* INFECTADOS COM *Trypanosoma cruzi* OU TRATADOS COM BENZONIDAZOL

VANESSA SANTOS DE ARRUDA BARBOSA

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A minha filha Júlia, ao meu esposo Marcelo e aos meus pais Marlindo e Nanci, que com amor e compreensão encorajaram-me a enfrentar todos os obstáculos dessa jornada.

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SUMÁRIO

RESUMO

O objetivo do presente trabalho foi avaliar a biodistribuição do radiofármaco pertecnetato de sódio ($^{99m}TcO_4$), utilizado em exames cintilográficos, em ratos *Wistar* infectados experimentalmente com a cepa *Y* do *Tripanosoma cruzi,* parasito causador da doença de Chagas e em ratos tratados durante 30 dias com o medicamento anti-*T. cruzi* benzonidazol. Para isso foi determinado em um contador gama automático o percentual de radioatividade por grama (%ATI/g) de vários órgãos como: cérebro, coração, esôfago, estômago, intestino delgado, intestino grosso, baço, fígado, músculo e sangue. Comparando-se os grupos controle com o teste, observou-se que a biodistribuição do $^{99m}TcO_4$ não se alterou nos órgãos dos animais tratados com benzonidazol. Os animais infectados pelo *T. cruzi* tiveram aumento da captação do $^{99m}TcO_4$ no sangue e diminuição no cólon, com mudanças na histopatologia desse último que é órgão alvo do parasito. Em conclusão, os dados permitem afirmar que o tratamento com benzonidazol em ratos não altera a biodistribuição de $^{99m}TcO_4$, mas a infecção pelo *T. cruzi* a modifica, podendo resultar em potenciais implicações clínicas e diagnósticas. A realização desse estudo teve caráter multidisciplinar com o envolvimento de biólogos, médicos, farmacêuticos e estatísticos.

1. INTRODUÇÃO

A doença de Chagas é uma enfermidade de caráter endêmico, causada pelo protozoário flagelado *Trypanosoma cruzi*. É um problema médico-social grave no Brasil e em diversos países da América Latina, atingindo cerca de 10 milhões de indivíduos, com 300 mil novos casos por ano[1,2].

Possui quadros clínicos variados, dentre eles, as formas indeterminada (assintomática), digestiva (megacólon e o megaesôfago) e cardíaca, que podem se apresentar isoladas ou associadas, sendo o acometimento cardíaco a principal causa de mortalidade e morbidade na doença de Chagas[3, 4].

O benzonidazol é um quimioterápico com atividade específica anti-*T. cruzi* utilizado por milhares de pessoas para tratamento da doença de Chagas. Apresenta baixa eficácia e alta toxicidade e seu tratamento pode durar até 90 dias, de acordo com as manifestações clínicas e tolerância do organismo[5, 6, 7, 8.]

O radiofármaco pertecnetato de sódio é amplamente utilizado em nosso país em exames de medicina nuclear, como as cintilografias, sendo a radiação gama emitida pelo tecnécio-99m, captada por uma gama câmara. Este composto se constitui na forma química, obtida em sistema gerador Molibdênio-99/Tecnécio-99m, e quando administrado no paciente tem uma distribuição padrão pelos órgãos. A alteração de sua biodistribuição pode ser interpretada como indicativo de doença, nos exames cintilográficos. Porém vários fatores como uso de fármacos naturais e sintéticos, dieta, cirurgias e tabaco podem alterar a biodistribuição normal de um radiofármaco[9, 10, 11].

A proposta desse projeto foi avaliar a alteração da biodistribuição do radiofármaco pertecnetato de sódio em duas situações: em ratos *Wistar*

tratados com o medicamento benzonidazol com ação anti-*T. cruzi* e em ratos *Wistar* infectados experimentalmente com a cepa *Y* do *T. cruzi*. Portanto, um dos nossos objetivos foi verificar se o fármaco benzonidazol, utilizado no tratamento da doença de Chagas, pode alterar a biodistribuição do radiofármaco. A importância disso se dá pelo fato de que milhares de pessoas utilizam o benzonidazol e em algum momento durante o tratamento poderiam fazer um exame cintilográfico, podendo o medicamento gerar alteração da biodistribuição do radiofármaco. Isso levaria a um erro de diagnóstico ou a repetição de exames e, conseqüentemente, a exposição desnecessária do paciente à radiação. Outra proposta do projeto foi avaliar se a biodistribuição do pertecnetato de sódio pode ser alterada em ratos infectados pelo *T. cruzi* na fase aguda da infecção. Isso é importante para que se possa detectar precocemente eventuais alterações orgânicas, por meio de radiofármacos, já que o tratamento imediato é fundamental para a cura parasitológica[3], além de verificar se a presença do parasito na corrente sanguínea poderia influenciar a fixação do radiofármaco aos elementos sanguíneos.

2. REVISÃO DE LITERATURA

A doença de Chagas, desde a sua descoberta em 1909, continua a despertar o interesse da comunidade científica por ser a maior endemia da América Latina, não ter cura e por possuir muitos aspectos da biologia do parasito *Trypanosoma cruzi* e da fisiopatologia da doença, ainda desconhecidos[8, 12].

São formas de transmissão do *T. cruzi*, as dejeções dos insetos vetores, os triatomíneos, via congênita, transfusional e oral. O parasito pode invadir e se multiplicar em diferentes células do hospedeiro, incluindo as do sistema fagocítico mononuclear, medula óssea, músculo liso e estriado, fibroblastos e células do sistema nervoso central, gerando quadros clínicos variados**,** dentre eles o megacólon, megaesôfago e a cardiopatia chagásica[3, 4, 13].

Observam-se duas fases distintas na doença de Chagas, uma aguda e outra crônica. Os sinais típicos da fase aguda são a parasitemia (presença do tripomastigota no sangue) e a proliferação de amastigotas em vários tecidos. A parasitemia desenvolve-se por uma fase indetectável (período pré-patente), outra detectável e crescente e uma terceira, detectável e decrescente. As alterações tissulares predominantes nessa fase dependem do potencial da cepa em determinar uma reação necrótico-inflamatória nos diversos órgãos, bem como da resposta imune inata desenvolvida pelo hospedeiro[12].

Na fase crônica são conhecidas as formas indeterminada, digestiva (megacólon e o megaesôfago) e cardíaca (cardiopatia chagásica) que podem se apresentar isoladas ou associadas, sendo o acometimento cardíaco a principal causa de mortalidade e morbidade na doença de Chagas[3, 4].

Vários autores têm demonstrado variações na morfologia e no grau de parasitemia durante o curso da infecção por diferentes cepas do *T. cruzi* em animais de experimentação; a cepa *Y*, por exemplo, dá origem a parasitemias muito intensas e fatais com rápida multiplicação intracelular e elevado parasitismo nas lesões inflamatórias. Há evidências comprovadas da influência desta cepa na resposta terapêutica aos quimioterápicos ativos sobre o *T. cruzi*[12, 14].

O benzonidazol (Bz), comercialmente conhecido por Rochagan®, é um fármaco com atividade específica anti-*T. cruzi in vivo* e *in vitro,* disponível no Brasil desde a década de 70. É um N-benzil-2-nitro-1-imidazolacetamida que atua diretamente sobre a síntese de macromoléculas por ligação covalente ou outras interações dos intermediários de nitroredução com componentes celulares. Apresenta baixa eficácia e alta toxicidade, especialmente na fase crônica da doença e seu tratamento pode durar até 90 dias, de acordo com as manifestações clínicas e tolerância do organismo[13]. O Ministério da Saúde indica o uso do Bz nas seguintes situações: fase aguda da infecção, forma congênita, reativação associada com imunossupressão e em situações de transfusões ou transplante de órgãos e em alguns pacientes nas formas indeterminada e crônica com fraco envolvimento cardíaco[15]. O esquema mais utilizado de tratamento é feito na dose de 5 mg/Kg/dia em adultos e menos de 10 mg/Kg/dia em crianças, por 60 dias[6, 16].

O diagnóstico dos diversos quadros clínicos da doença de Chagas geralmente se faz através de exames radiológicos, eletro e ecocardiograma, cintilografias e endoscopias[12, 17].

As cintilografias são ferramentas diagnósticas frequentemente utilizadas nas análises anatomofuncionais de órgãos e sistemas**,** em pacientes com doenças tropicais**,** mediante o uso de radiofármacos, que têm como radionuclídeo o tecnécio-99m[18].

O tecnécio-99m (^{99m}Tc) é um dos radionuclídeos mais utilizados na medicina nuclear devido a vários fatores, tais como: fácil obtenção a partir de um sistema gerador $^{99}Mo/^{99m}Tc$ a custo relativamente baixo, emissão de fóton gama único com energia de 140 KeV adequada para detecção externa, meia-vida física de seis horas, dosimetria favorável e baixo impacto ambiental. Todos esses fatores, associados à possibilidade do ^{99m}Tc poder ser reduzido da valência +7 (valência esperada no eluato na forma química de pertecnetato) para estados de oxidação mais baixos, em presença de um agente redutor como o cloreto estanoso ($SnCl_2$), permite a obtenção de diferentes radiofármacos a partir da simples reconstituição de conjuntos de reativos liofilizados (“kits”) e a marcação de grande variedade de moléculas e células[19, 20].

O pertecnetato de sódio ($Na^{99m}TcO_4$) quando injetado por via endovenosa é distribuído através dos líquidos vasculares e intersticiais e utilizado na obtenção de imagens diagnósticas do estômago, glândulas salivares, glândulas tireóide e paratireóides, plexo coróide, cérebro e estudos de refluxo esofágico e de fluxo sanguíneo[19, 20].

A distribuição, fixação e eliminação de radiofármacos em um organismo dependem de vários fatores, tais como o fluxo sanguíneo, o metabolismo tecidual e a ligação destes aos elementos sanguíneos. A biodistribuição, definida como grau de concentração e a distribuição do elemento radioativo

nos diversos órgãos e tecidos, obedece a um padrão de captação que pode ser traduzido em normalidade ou doença[19].

Há dados na literatura mostrando que a biodistribuição de um radiofármaco pode ser alterada por: processos patológicos, cirurgias, uso de tabaco, dietas e fármacos sintéticos ou naturais[10, 21, 22, 23, 24].

O pertecnetato de sódio teve alteração de sua captação pelo estômago e tireóide de ratos com restrição de proteínas na dieta[21] e na mama, fígado, intestino, ovário, útero e vagina de ratas tratadas com o fármaco antineoplásico paclitaxel[22]. A alteração da biodistribuição do pertecnetato de sódio também foi demonstrada em cirurgia de derivação biliopancreática, onde ocorreu alteração da captação de pertecnetato de sódio na tireóide, pulmão, pâncreas, baço e músculo de ratos[24].

O Glucantime®, fármaco com atividade anti-*Leishmania* aumentou a captação do radiofármaco ácido metilenodifosfônico, marcado com tecnécio-99m (^{99m}Tc-MDP), no baço, rins, testículos, coração e fígado e diminuiu na bexiga de ratos[25]. O mesmo radiofármaco também teve sua biodistribuição alterada em vários órgãos de ratos pelos fármacos antimaláricos artemisinina e mefloquina[26]. Ficou demonstrado que o extrato da planta medicinal *Ginkgo biloba* pode alterar a captação do pertecnetato de sódio nos rins, fígado e duodeno[27] e que o extrato da erva de São João (*Hypericum perforatum*) diminuiu a captação do mesmo radiofármaco no osso, músculo e tireóide e aumentou sua fixação no pâncreas[28]. Ocorreu aumento da captação de $Na^{99m}TcO_4$ no fígado de ratos tratados com o flavonóide rutina[29] e com extrato de berinjela (*Solanum melongena*)[30].

Pode-se explicar a alteração da biodistribuição de um radiofármaco pela ação de um fármaco devido a vários fatores, como: o fármaco poder modificar a natureza química do radiofármaco, ou o meio bioquímico do alvo no qual o radiofármaco está exposto, alterar o meio bioquímico do não alvo para o radiofármaco ou interferir na ligação do radiofármaco com as proteínas plasmáticas e elementos sanguíneos[11].

Até o presente momento, não há registro na literatura sobre a influência de parasitos na ligação do ^{99m}Tc aos elementos sanguíneos.

3. INDEXAÇÃO DE ARTIGO

3.1 ARTIGO PUBLICADO

Effect of Tripanosomicide Benznidazole (Rochagan®) on the Biodistribution of Sodium Pertechnetate ($Na^{99m}TcO_4$) in Wistar Rats

Vanessa Santos de Arruda Barbosa, Cecília Maria de Carvalho Xavier Holanda, Roseane Pereira da Silva, Daniel Pereira de Oliveira, Maurício Ferreira da Silva Júnior, Elias Herculano de Oliveira, Maria Helena Constantino Spyrides, Aldo Cunha Medeiros

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Effect of Tripanosomicide Benznidazole (Rochagan®) on the Biodistribution of Sodium Pertechnetate ($Na^{99m}TcO_4$) in Wistar Rats

Vanessa Santos de Arruda Barbosa[1*], Cecília Maria de Carvalho Xavier Holanda[1,2], Roseane Pereira da Silva[1], Daniel Pereira de Oliveira[2], Maurício Ferreira da Silva Júnior[2], Elias Herculano de Oliveira[2], Maria Helena Constantino Spyrides[3] and Aldo Cunha Medeiros[1,4]

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ABSTRACT

Benznidazole, a drug with specific anti-Trypanosoma cruzi activity, is used in the treatment of Chagas' disease. The radiopharmaceutical sodium pertechnetate ($Na^{99m}TcO_4$) is used to obtain diagnostic images of the stomach, thyroid, parathyroids, salivary glands, brain and in the study of esophageal reflux and blood flow. This study aimed at evaluating in vivo the influence of benznidazole treatment on the sodium pertechnetate biodistribution in Wistar rats. The percentage of radioactivity per gram (%ATI/g) of various organs (brain, heart, esophagus, stomach, small intestine, large intestine, spleen, liver, muscle and blood) was determined. Comparing the treated rats with the controls, we observed that sodium pertechnetate biodistribution did not change when administered to rats treated for thirty days with benznidazole.

Keywords: Antiparasite, Radiopharmaceutical, Technetium-99m, Benznidazole, Biodistribution, *Trypanosoma cruzi*

INTRODUCTION

Chagas' disease is an endemic disorder caused by the flagellate protozoan *Trypanosoma cruzi*. It is a serious medical and social problem in Brazil and several Latin American countries, affecting 18 million individuals, with 300 thousand new cases every year (WHO, 2002). *T. cruzi* can invade multiple host cells, generating megacolon, megaesophagus and chagasic heart disease (Santos et al., 2005; Texeira et al., 2006). Benznidazole (Bz), commercially known as Rochagan®, is a drug with specific anti-*T. cruzi* activity *in vivo* and *in vitro*, available in Brazil since the 1970s (Coura and Castro, 2002). It contains N-benzyl-2-nitro-1-imidazolacetamida, which acts directly on the

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macromolecule synthesis by a covalent link with cellular components. It has demonstrated low efficiency and high toxicity, especially in the chronic phase of the disease and its treatment can last for up to 60 days, depending on clinical manifestations and host tolerance (Cançado, 2005).

The most widely used treatment scheme is 10 mg/kg/day in adults and less than 5 mg/kg/day in children (Castro et al., 2000; Cançado, 2002; Urbina and Docampo, 2003; Dias, 2004). Following oral administration, Bz is absorbed by the intestine, and is bound to plasma proteins and red blood cells to be distributed throughout the body. Maximum plasma concentrations are achieved in two to four hours. The half-life of plasma elimination is approximately twelve hours (Morilla et al., 2005).

Among the many diagnostic tools that can be used in tropical diseases, scintigraphy is widely used in the anatomic and functional analyses of organs and systems (Braga, 2002). Technetium-99m (^{99m}Tc), in the form of sodium pertechnetate ($Na^{99m}TcO_4$), is a radionuclide that connects to a wide variety of molecules and cells (Thrall and Ziessman, 2003; Saha, 2004). When injected intravenously, it is distributed through the veins and interstitium and is used to obtain diagnostic images of the stomach, thyroid, parathyroids, salivary glands, brain and in the study of esophageal reflux and blood flow (Saha, 2004; Owunwanne et al., 1995; Thrall and Ziessman, 2003).

Several drugs can interfere with the biological behavior of radiopharmaceuticals used in scyntigraphic examinations. They can change the biological effect of the radiopharmaceutical and their interaction can lead to hypo or hyper uptake of radiopharmaceuticals in a particular organ, causing incorrect diagnosis or misinterpretation of results. Repeated scintigraphy may result in unnecessary radiation for patients (Bernardo-Filho et al., 2005; Gomes et al., 2002). Although Bz is the drug currently recommended by the National Foundation of Health, Brazil, and is used by thousands of people, little is known about its action mechanism, its effects on host cells or its toxicity. Thus, it is important to study the effect of Bz on the biodistribution of the radiopharmaceutical sodium pertechnetate in laboratory animals subjected to chronic treatment with this drug. The aim of this work was to assess *in vivo* the influence of Bz on the biodistribution of sodium pertechnetate in rats.

MATERIALS AND METHODS

Twelve male *Wistar* rats weighing 200-250g from the Centro de Ciências da Saúde, Universidade Federal do Rio Grande do Norte (UFRN), Natal–RN, Brazil were used. The protocol was conducted in accordance with Brazilian College of Animal Experimentation guidelines and was approved by the Research Ethics Committee of Onofre Lopes Hospital-UFRN (08/2007). The animals had free access to water and standard food for rodents (Labina Purina ®) and were randomly allocated to 2 groups: control and treated. The treated group (n=6) received 5 mg/kg/day of Bz diluted with sorbitol, by gavage. The control rats (n=6) received only sorbitol. The animals were treated for 30 days. On the last day of treatment the rats received 0.1 mL of $Na^{99m}TcO_4$ (3.7 MBq) via orbital plexus.

The $Na^{99m}TcO_4$ was eluted in a $^{99}Mo/^{99m}Tc$ generator (Instituto de Pesquisas Energéticas e Nucleares, São Paulo, Brazil). After 60 minutes, all animals were quickly killed under anesthesia with xylazine (20 mg/kg) and ketamine (50 mg/kg), by intraperitoneal via. Samples were harvested from the brain, heart, esophagus, stomach, small intestine, large intestine, spleen, liver, muscle and blood. The tissue samples were washed in 0.9% saline, weighed on a precision scale (Mark 160®, Bel equipment, Italy) and the percentage of radioactivity per gram of tissue (%ATI/g) was determined in an automatic gamma counter (Wizard 1470, Perkin-Elmer, Finland). The efficiency of the gamma counter was 86%, as specified by the manufacturer. The biochemical and hematological dosages were performed in automated equipment TermoKonelab 60i, Abbot and Cell-Dyn 3500R, Abbot, respectively. Data were presented as mean ± standard deviation. The percentage of radioactivity per gram (%ATI/g) was determined by dividing the percentage of total radioactivity of each sample by its weight in grams. The ATI%/g was compared using the non-parametric Mann-Whitney test and the biochemical and hematological parameters by Student's t-test, considering the level of statistical significance at $p<0.05$ in both tests. Statistica 6.0 software was used.

RESULTS

Table 1 shows the relationship between the controls and the Bz-treated rats. No statistically significant difference ($p>0.05$) in biodistribution of sodium pertechnetate was observed in any of the organs.

Table 2 shows significant differences in alanine aminotransferase (ALT) dosage and the percentage of neutrophils and lymphocytes, when comparing the treated and control rats. All the other parameters such as iron, total protein, aminotransferase aspartate (AST), creatinine, glucose, red blood cells, hemoglobin, hematocrit, white blood cells and platelets showed no significant differences, when the two groups were compared ($p>0.05$).

Table 1 - Effect of benznidazole treatment on $Na^{99m}TcO_4$ biodistribution in *Wistar* rats.

Organs	% ATI	
	Controls	Treated
Spleen	0.00211 ± 0.00076	0.00199 ± 0.00039
Brain	0.00175 ± 0.00392	0.00020 ± 0.00007
Heart	0.00158 ± 0.00039	0.00192 ± 0.00053
Esophagus	0.00349 ± 0.00123	0.00330 ± 0.00084
Stomach	0.02576 ± 0.00761	0.02276 ± 0.01016
Liver	0.00379 ± 0.00105	0.00402 ± 0.00080
Small Intestine	0.00390 ± 0.00132	0.00299 ± 0.00169
Large Intestine	0.00149 ± 0.00071	0.00163 ± 0.00043
Muscle	0.00050 ± 0.00011	0.00063 ± 0.00014
Blood	0.00683 ± 0.00158	0.00811 ± 0.00165

Mean ± SD. No difference was observed between the two groups ($p>0.05$).

Table 2 - Effect of benznidazole treatment on biochemical and hematological parameters.

Biochemical and hematological dosages	Groups	
	Controls	Treated
Iron (µg/L)	363.0 ± 24.69	308.5 ± 65.56
Total proteins (g/dL)	6.50 ± 0.303	6.55 ± 0.327
ALT (UI/L)	116.0 ⊥ 32.44	98.2 ⊥ 23.60
AST (UI/L)	73.16 ± 8.495	56.66* ± 9.667
Creatinine (mg/dL)	0.375 ⊥ 0.055	0.381 ⊥ 0.040
Glucose (mg/dL)	122.000 ± 19.193	117.666 ± 10.557
Red blood cells (u/mm^3)	6653333 ⊥ 372487.1	6578333 ⊥ 526931.4
Neutrophils (%)	57.5 ± 2.949	34.0* ± 8.786
Lymphocytes (%)	34.5 ⊥ 2.428	58.6* ⊥ 7.890
Hemoglobin (g/%)	12.183 ± 0.598	11.450 ± 0.831
Hematocrit (%)	52.133 ⊥ 3.669	51.616 ⊥ 3,023
Platelets (u/mm^3)	716833.3 ± 14770.47	718333.3 ± 00059.32
Leukocytes (u/mm^3)	3195.0 ± 1126.7	2206.6 ± 646.3

Mean±SD . *, $p<0.05$.

DISCUSSION

The biological behavior of radiopharmaceuticals used for diagnosis in nuclear medicine is well established in the scientific literature. The interaction between a drug and a radiopharmaceutical may alter its biodistribution and result in an unexpected effect (Gomes et al., 2002, Bernardo-Filho et al., 2005).

It is of critical importance to know which drugs can interfere with the normal biodistribution of radiopharmaceuticals, to avoid the misinterpretation of scintigraphic images. Several authors have shown that radiopharmaceutical

biodistribution can be altered by natural and synthetic products, radiotherapy, surgery and diet (Gomes et al., 2002; Xavier-Holanda et al., 2002; Passos *et al,* 2002; Bernardo et al., 2004; Moreno et al., 2005; Santos-Filho and Bernardo-Filho., 2005; Holanda et al., 2006; Araújo-Filho et al., 2007).

Glucantime, an anti-*Leishmania* drug, increased the uptake of the radiopharmaceutical methylene disphosphonic acid, labeled with technetium-99m (^{99m}Tc-MDP) in the spleen, kidney, testicles, heart and liver of rats (Xavier-Holanda et al., 2002). The biodistribution of ^{99m}Tc-MDP was also changed in various organs of rats treated with the antimalarials artemisinin and mefloquine (Holanda et al., 2006). Araujo-Filho et al. (2007) reported changes in sodium pertechnetate biodistribution in the thyroid, lung, pancreas, spleen and muscle after biliopancreatic bypass surgery with duodenal switch in rats. Moreno et al. (2005) showed that *Ginkgo biloba* extract can change the sodium pertechnetate biodistribution in the kidneys, liver and duodenum. Santos-Filho and Bernardo-Filho. (2005) showed that *Hypericum perforatum* extract reduced the uptake of the radiopharmaceutical in bone, muscle and thyroid.

Some studies show that Bz can have adverse effects on adrenal cortex, esophagus and colon cells in rats and that it exerts a mutagenic and carcinogenic effect (Diaz, 2000, Castro et al., 2006, de Castro et al., 2003). Because of its antigenic and toxic effect, Bz can cause several undesirable reactions, mainly in the nervous system and gastrointestinal tract (Cançado, 2002).

During benznidazole treatment (30 to 60 consecutive days), skin reactions, gastrointestinal disorders, nausea, paresthesia, or symptoms of peripheral polyneuritis may occur, especially after prolonged treatment or with excessive doses of Bz. The depression of the bone marrow with neutropenia, thrombocytopenic purple and hepatotoxicity are serious effects of Bz that demand caution when they are being used (Cançado, 2005). In our study, neutropenia was found in Bz-treated rats, but no hepatic and intestinal mucous membrane damage was observed. Although Bz can cause these several undesirable reactions, our results were shown positive in relation to interaction Bz-radiopharmaceutical. Both Bz and sodium pertechnetate are bound to plasma proteins, but it seems that there is no competition between them for the same connection sites (Coura and Castro, 2002; Owunwanne et al., 1995). Bz also, probably, does not interfere in the intestinal mobility and does not alter blood flow. The alteration of these factors could modify the biodistribution of the radiopharmaceutical (Owunwanne et al., 1995). Data from biochemical and hematological parameters also lead us to believe that Bz, administered in equivalent dose to treat Chagas' disease in humans, had no toxic effect on the organs studied, nor did it cause enough tissue damage to promote a change in the uptake of $Na^{99m}TcO_4$.

In this study we demonstrated that the administration of benznidazole didn't alter sodium pertechnetate biodistribution to important target organs, what demonstrate that this result is satisfactory for its use in patients with Chagas' disease. In spite of, this experimental were performed in W*istar* rats.

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RESUMO

O benzonidazol é um quimioterápico com atividade específica anti-*T. cruzi* utilizado por milhares de pessoas para tratamento da doença de Chagas. O radiofármaco pertecnetato de sódio ($Na^{99m}TcO_4$) é utilizado na obtenção de imagens diagnósticas do estômago, tireóide, paratireóides, glândulas salivares, plexo coróide, cérebro e de estudos de refluxo esofágico e de fluxo sanguíneo. Esse trabalho objetivou avaliar *in vivo* a influência do tratamento crônico com o benzonidazol na biodistribuição do radiofármaco pertecnetato de sódio em ratos *Wistar*. O percentual de radioatividade por grama (%ATI/g) de vários órgãos (cérebro, coração, esôfago, estômago, intestino delgado, intestino grosso, baço, fígado, músculo e sangue) foi determinado. Comparando-se o grupo controle e o tratado observou-se que o pertecnetato de sódio não possui sua biodistribuição alterada quando administrado em ratos tratados por trinta dias com a droga

benzonidazol, mostrando não prejudicar a interpretação de diagnósticos por imagem.

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3.2 MANUSCRITO SUBMETIDO

Biodistribution of Technetium-99m Pertechnetate in Rats Infected with *Trypanosoma cruzi*

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Experimental Parasitology

Biodistribution of Technetium-99m Pertechnetate in Rats Infected with *Trypanosoma cruzi*

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ABSTRACT

With the aim of investigating the biodistribution of technetium-99m pertechnetate ($^{99m}TcO_4^-$) in rats infected with Y strain of *Tripanosoma cruzi*, at the peak of parasitemia, (14th day of infection), Wistar rats were injected with 0.1 ml of $^{99m}TcO_4^-$ (3.7MBq). After 60 minutes, the percentage of radioactivity per gram was counted in several isolated organs and blood, using the gamma counter 1470 Wizard-PerkinElmer, Finland. The uptake of $^{99m}TcO_4^-$ increased significantly in blood and decreased in colon of infected animals ($p<0.05$). Significant reduction in serum iron and red blood cells and significant increase in total proteins, leukocytes and lymphocytes in the infected rats were observed, comparing with controls ($p<0.05$). A reduction in thickness of the muscular layer of colon and mononuclear inflammation were observed. These results conclusively demonstrate that *T. cruzi* infection was associated with changes in the biodistribution of $^{99m}TcO_4^-$ and in the morphology of colon, with potential clinical implications.

Index Descriptors: Technetium, Pertechnetate, Bioavailability, *Trypanosoma cruzi*; Chagas disease.

INTRODUTION

Chagas disease is endemic and is caused by the protozoan *Trypanosoma cruzi (T. cruzi)*. It is a serious medical and social problem in Brazil and in Latin America, reaching approximately 10 million individuals, and 40 million of people are at risk of infection (Schofield et al. 2006). At least 300 thousand new cases occur each year (WHO, 2002). *T. cruzi* can proliferate in different host cells, including the mononuclear

phagocytes system, bone marrow, striated and smooth muscle, fibroblasts and cells of the central nervous system, causing various clinical conditions, including megacolon, megaesophagus and chagasic cardiopathy (Santos et al. 2005; Texeira et al. 2006; Coura and Castro. 2002). The clinical diagnosis is performed by laboratory tests, radiological examination, electrocardiogram, echocardiogram, myocardial scintigraphy and endoscopy (Andrade 2005; Kamiji and Oliveira 2005). The scintigraphy is often used for anatomofunccional analysis of organs and systems in patients with tropical diseases, by using the radiopharmaceuticals which have the radionuclide technetium-99m (Braga 2002).

The technetium-99m pertechnetate ($^{99m}TcO_4^-$) is a radiopharmaceutical with the ability to connect to a variety of molecules and cells. When injected intravenously, it is distributed through the vascular and interstitial spaces, and is used to obtain diagnostic images of the stomach, salivary glands, thyroid and parathyroid glands, choroid plexus, brain, and studies of esophageal reflux and blood flow (Saha 2004; Thrall and Ziessman 2003). The distribution, elimination and fixation of radiopharmaceuticals in the body depend on several factors, such as blood flow, tissue metabolism and there binding to the blood elements. The biodistribution, defined as the concentration and distribution of radioactive elements in organs and tissues, follows a standard uptake that can mean normal or disease (Saha 2004).

Information is still scarce regarding changes in the biodistribution of $^{99m}TcO_4^-$ in an organism infected by *T. cruzi* in the acute phase of infection. It can detect early some organic changes by means of radiopharmaceuticals, and determine if the bloodstream parasites can influence the uptake of $^{99m}TcO_4^-$ in plasma proteins. Therefore, the objective of this study was to evaluate, in vivo, the biodistribution of $^{99m}TcO_4^-$ in Wistar healthy rats and in rats infected with *T. cruzi* during the peak parasitemia.

MATERIAL AND METHODS

We used 12 male Wistar rats, weighing 200±23g, from the vivarium of the Center of Health Sciences, Federal University of Rio Grande do Norte, Natal / RN, Brazil. The animals had free access to water and standard rodent food (Purina / Labina ®). The protocol was conducted according to international regulations for animal experimentation and approved by the Research Ethics Committee of Hospital Universitário Onofre Lopes - UFRN (08.2007).

The Y strain of *Trypanosoma cruzi* was from the Laboratory of Biology of *T. cruzi*, Department of Parasitology / Institute of Biological Sciences of the UFMG. The animals were randomly allocated into 2 groups. The infected group rats (n = 6) received intraperitoneally a suspension containing $2x10^5$ blood trypomastigotes / mL of the Y strain of *T. cruzi*. The control rats (n = 6) received saline in the same way. The parasitemia of animals was monitored on alternate days and the count was determined by light microscopy. At the peak of parasitemia, (14^{th} day of infection), all the rats were injected with 0.1 ml of $^{99m}TcO_4^-$ (3.7 MBq) via ocular plexus. The $^{99m}TcO_4^-$ was eluted from a generator 99Mo/99mTc produced by the Institute of Energy and Nuclear Research, São Paulo / Brazil. After 60 minutes, all animals were quickly killed by overdose of anesthetic. Samples of blood and several organs (brain, heart, esophagus, stomach, small intestine, intestinal thick, spleen, liver, muscle) were isolated and radioactivity of each organ was determined by means of an automatic gama counter (1470 Wizard-PerkinElmer, Finland) with automatic correction for decay and efficiency of 86%. The percentage of radioactivity per gram (% ATI / g) was calculated dividing the percentage of total radioactivity of each organ, by its weight in grams. Frozen tissue samples were taken from all the organs studied. However, histological examination was performed only in colon, the organ in which the biodistribution of $^{99m}TcO_4^-$ in infected rats was significantly different, from that of non infected rats. The specimens were fixed in 10% formaline, cut as 5 μm tissue sections and stained with hematoxylin and eosin and dehydrated in ethanol and xylene. All specimens were examined by the same accredited pathologist who had no knowledge of the study groups. Morphometric measurements were made using light micrographs (100X) of the stained sections in which the mucosal villi were cut as close to their longitudinal axis as possible. In two pictures, villous height, crypt depth, mucosal thickness, and thickness of the muscularis were measured in two areas, and the mean values from two light micrographs were calculated for each parameter. Inflammation was examined as well. From blood, hematological parameters were measured by Automatic Analyzer Abbot Cell Dyn 3500; biochemical dosages were measured using the Spectrophotometer Konelab 60i, (assay kit from Weiner, São Paulo, Brazil). All data were presented as mean ± standard deviation. The %ATI/g was compared by Mann-Whitney and haematological parameters by T-Student test, considering both $p<0.05$ statistically significant. The software used to obtain the results was the Statistica 6.0.

RESULTS

Figure 1 shows the presence of blood trypomastigotes forms at the peak of parasitemia in rat observed on the 14th day of infection. Table 1 shows the biodistribution of $^{99m}TcO_4^-$ in organs of control group rats and infected group rats. There was a statistically significant increase ($p<0.05$) in the uptake of $^{99m}TcO_4^-$ in the blood and a significant decrease of its uptake in the colon of infected animals, when compared with controls. The $^{99m}TcO_4^-$ had not its biodistribution changed in the other organs. Table 2 shows a significant reduction in the levels of serum iron and a significant increase in total proteins in the infected rats, comparing with controls. There was a decrease in the number of red blood cells and hemoglobin and increase of total leukocytes and lymphocytes in the infected group rats, and the difference was significant when compared to the control group rats ($p<0.05$). The histopathological analysis of the colon showed reduction in thickness of the muscular layer and an inflammatory process with intense nodular mononuclear infiltration in muscle layer, shown in Figure 2. The colon of the control group rats showed no histopathological changes (Fig. 3).

TABLE 1.

Biodistribution of $^{99m}TcO_4^-$ in control Wistar rats and in rats infected with *T. cruzi*.

Organs	% ATI/g	
	Control	Infected
Spleen	0.0029 ± 0.0026	0.0018 ± 0.0005
Brain	0.0004 ± 0.0004	0.0002 ± 0.0001
Heart	0.0043 ± 0.0068	0.0021 ± 0.0008
Esophagus	0.0036 ± 0.0020	0.0027 ± 0.0020
Stomach	0.0270 ± 0.0087	0.0189 ± 0.0181
Liver	0.0040 ± 0.0013	0.0043 ± 0.0012
Small bowel	0.0014 ± 0.0008	0.0009 ± 0.0002
Colon	0.0045 ± 0.0016	0.0023 ± 0.0014*
Muscle	0.0007 ± 0.0006	0.0006 ± 0.0002
Blood	0.0062 ± 0.0017	0.0106 ± 0.0050*

* $p<0.05$, compared with control.

TABLE 2

Biochemical and hematological measures in control Wistar rats and in rats infected with *T. cruzi*.

Biochemical and hematological parameters	Control	Infected
Iron (µg/L)	216.7 ± 46.1	138.5 ± 21.6*
Red blood cells/mm^3	7.373.333 ± 389	5.933.333 ± 618*
Hematocrit (%)	41.2 ± 11	41.5 ± 12
Hemoglobin (mg/dL)	13.0 ± 1.5	11.7 ± 1*
Leukocytes /mm^3	2.676 ± 1.625	7.798 ± 1.253*
Lymphocytes (%)	53.2 ± 16	80.7 ± 8*
Monocytes (%)	2.8 ± 2	3,3 ± 2
Neutrophyls (%)	34.2 ± 17	34.5 ± 30
Platelets /mm^3	543.000 ± 153	561.000 ± 107
Total proteins (g/dL)	5.8 ± 0.37	6.29 ± 0.48*

* $p<0.05$ compared with control.

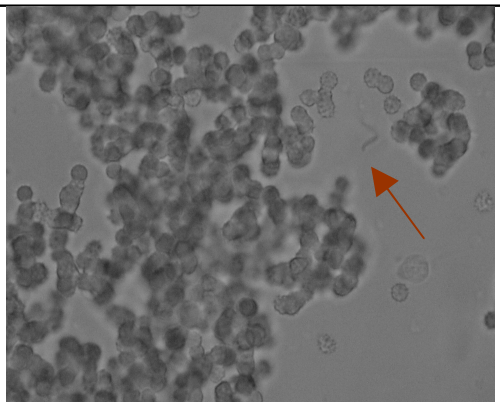

Fig. 1 Trypomastigotes in the blood of infected rat, at the parasitemic peack.

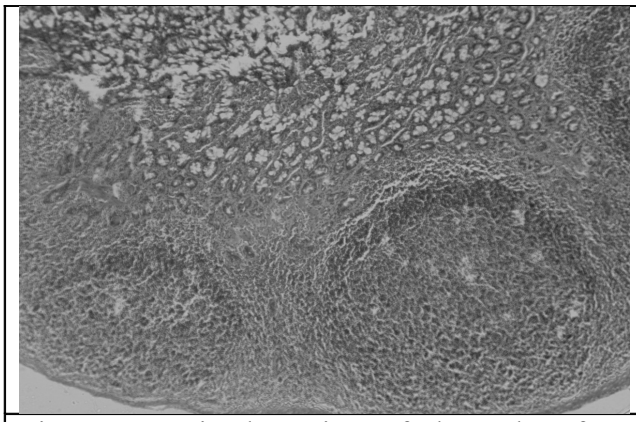

Fig. 2 – Stained section of the colon from infected rat. An inflammatory process with intense and nodular mononuclear infiltration in muscle layers is shown. HE 100x

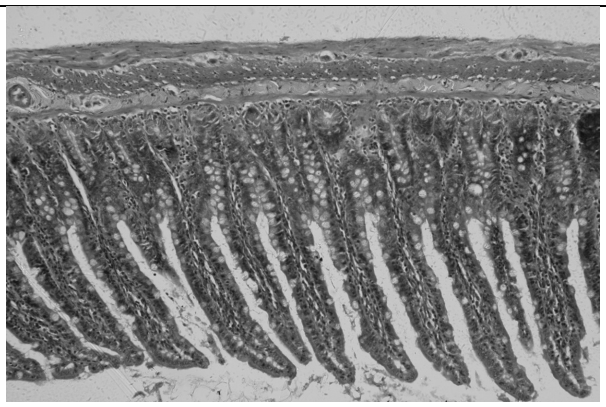

Fig. 3 - Stained section of the colon from control noninfected rat: there is no evidence of inflammatory process in the muscle layers. HE 100x

DISCUSSION

Several experimental models such as mice, wild rodents, rabbits, hamsters, dogs and monkeys, have been used in studies of Chagas' disease (Magalhães-Santos et al. 2004; Ramirez et al. 2000; Chapadeiro et al. 1999). In this study, rats were chosen because they develop lesions similar to those found in human Chagas' disease, including the reproduction of megas (Ramirez et al. 2000). Additionally, it has been successfully used in studies of biodistribution of radiopharmaceuticals for several authors (Xavier Hollanda et al. 2006, Santos-Filho and Bernardo-Filho 2005; Araújo-Filho et al. 2007; Barbosa et al. 2008).

There are evidences in the literature that the biodistribution of radiopharmaceuticals may be altered by diseases, procedures, cigarette smoking, surgery, food or natural and synthetic drugs (Passos et al. 2002; Bernardo-Filho et al. 2005; Xavier-Hollanda et al. 2008; Valencia 2005; Araújo-Filho et al. 2007). In rats with protein restriction diet, the uptake of $^{99m}TcO_4^-$ was altered in stomach and thyroid (Passos et al. 2002). Rats treated with the antineoplastic drug paclitaxel had the biodistribution of pertechnetate altered in breast, liver, intestine, ovary, uterus and vagina (Xavier-Hollanda et al. 2008). Changing on biodistribution of pertechnetate associated to surgical procedure was demonstrated after the bariatric surgery named biliopancreatic bypass. The uptake of pertechnetate was altered in thyroid, lung, pancreas, spleen and muscle of rats. (Araújo-Filho et al. 2007). The treatment of rats

with the extract of *Hypericum perforatum* decreased the uptake of the radiopharmaceutical in bone, muscle and thyroid and increased their attachment to the pancreas. (Santos-Filho and. Bernardo-Filho 2005).

The distribution of pertechnetate takes place due to its connection to proteins, erythrocytes and leukocytes. Its uptake may be altered in diseases like tumors, cysts, inflammation, bleeding, etc, where specific images are generated for diagnosis using scintigraphy (Saha 2004; Braga 2002). In this work we demonstrated the decrease in the uptake of $^{99m}TcO_4^-$ in the colon of chagasic rats, probably due to the inflammatory process and destruction of some histological structures of the organ. The digestive tract is affected in Chagas disease, soon after two weeks of infection with *T. cruzi*, when has been demonstrated focal points of inflammation with necrosis in the organs (Texeira et al. 2006).

The lesions observed in the acute phase are characterized by inflammatory reaction with predominance of mononuclear cells after the rupture of amastigotes pseudocysts. As a result, granulomas usually apear in the muscle tissue, including the heart (Coura 2007). The platelet aggregation, eosinophil degranulation, microvascular disease, edema, thrombosis, ischemia and blood stasis are also shown in the acute phase of infection (Araújo-Jorge 2000). In this work, the granulomatous reaction and reduction in the thickness of the intestinal wall of the colon of infected rats (Fig.2), at least in part, may explain the decrease in uptake of $^{99m}TcO_4^-$ in the colon. We suppose that the blood flow and vascular integrity of the colon may have been compromised, generating ischemia.

It is well demonstrated that hyperviscosity occurs in the blood of rats infected with *T. cruzi,* due to morphological changes in erythrocytes and increased plasma proteins, causing damage on microcirculation and reducing the blood flow in the organs affected by Chagas' disease (Berra et al. 2005). In fact, in this work the infected rats had serum total proteins significantly higher than in controls. Another explanation has been based on the life cycle of the Y strain of *T. cruzi* in mice. Parasites were found in the capillaries of some organs, and it was hypothesized that, as the parasites were larger in diameter than the blood vessels, they could cause blood stasis (Pinto et al. 1999),

Histopathological changes similar to granulomatous mononuclear inflammatory reaction was observed in an experimental model (Pernia-Guillen et al. 2001) and in humans (Silveira et al. 2007). Some studies on the behavior of several strains of *T. cruzi*

in mice also found inflammatory infiltrates in organs such as heart, skeletal muscle and smooth muscle (Silva et al. 2006; Devera et al. 2002; Martins et al. 2003).

The acute-phase of Chagas disease is a set of metabolic changes that include leukocytosis, decreased serum zinc and iron, increase in protein catabolism and glycogenesis, increased total protein synthesis and fever (Araújo-Jorge 2000). The decrease of the iron dosage, the increase in total protein and leukocytosis in the infected rats of this work, happened during the intense inflammatory response observed during the analysis of the biodistribution of the $^{99m}TcO_4^-$. The lymphocytosis found in our results is usually found in the initial inflammatory process. The decrease in the number of red blood cells and serum iron in the infected rats of this study may explain the anemia, which is common in humans, and was demonstrated in experimental models in mice (Cardoso and Brener 1980; Marcondes et al. 2000). However, the mechanisms responsible for this change are not entirely clear. According to Malvezi et al. (2004), cytokines such as TNF-α produced by activated macrophages during the acute phase of infection, may decrease the erythropoiesis. Even with the decrease in red blood cells, we found that the uptake of $^{99m}TcO_4^-$ in the blood of infected rats was higher than in controls, probably due to the fact that the $^{99m}TcO_4^-$ connects for the most part (80%) to plasma proteins (Saha 2004). The increase in the proteinemia and leukocytosis, as evidenced in the infected rats may at least in part, explain the increased uptake of $^{99m}TcO_4^-$ in blood of that animals. Rebello et al. (1994) have shown uptake of $^{99m}TcO_4^-$ by *Schistosoma mansoni*, but there is no evidence whether the $^{99m}TcO_4^-$ is able to connect to the *T. cruzi.* Further studies about the binding of $^{99m}TcO_4^-$ to the parasite are still needed.

In conclusion, at the peak of blood parasitemia, the Y strain of *T. cruzi* affected the biodistribution of $^{99m}TcO_4^-$, specially in the colon and blood of rats. Histopathologic and metabolic changes may have influenced the changing on biodistribution.

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4. COMENTÁRIOS, CRÍTICAS E CONCLUSÕES

O desenvolvimento do presente trabalho teve como base a associação dos conhecimentos de radiobiologia com a doença de Chagas experimental e ao antiparasitário benzonidazol. Sua proposta foi verificar se haveria alteração na biodistribuição do radiofármaco pertecnetato de sódio em ratos tratados com o fármaco benzonidazol e em ratos infectados com o *Trypanosoma cruzi*. A parte experimental foi desenvolvida no Núcleo de Cirurgia Experimental Clóvis Sarinho do Centro de Ciências da Saúde (CCS) e no Laboratório de Ensaios Antiparasitários e Radiobiologia Experimental do Centro de Biociências (CB) da UFRN. O parasito *T. cruzi* utilizado nos experimentos foi doado pelo Laboratório de Biologia do *T. cruzi* do Departamento de Parasitologia/ Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais (UFMG) e o material radioativo pela Liga Norteriograndense contra o Câncer, Natal / RN. Os animais de Experimentação foram provenientes do Biotério do CCS e do CB da UFRN. Todo o protocolo experimental foi aprovado pelo Comitê de Ética e Pesquisa do Hospital Universitário Onofre Lopes / UFRN sob o nº 08/2007.

O protocolo experimental da tese foi iniciado em 2006 com os testes pilotos de biodistribuição do pertecnetato de sódio e de infecção pelo *T. cruzi* nos ratos. O primeiro foi desenvolvido em Recife, no Departamento de Biofísica da Universidade Federal de Pernambuco (UFPE), pois a UFRN ainda não dispunha de um contador gama, que é um aparelho que permite a contagem da radioatividade. Nessa época, a linha de pesquisa em radiobiologia, estava recém-implantada na UFRN pelo Prof. Dr. Aldo da Cunha Medeiros. O fato de irmos para Recife para fazer a contagem da radioatividade dos órgãos gerava

maior dificuldade na execução do experimento e um custo maior. Posteriormente a UFRN adquiriu um contador gama e então pudemos fazer os experimentos dentro da Universidade.

O teste piloto de infecção do *T. cruzi*, foi feito para verificarmos a viabilidade da cepa Y do *T. cruzi* e para conhecermos o pico parasitêmico no rato. Para a infecção dos ratos com o *T. cruzi*, foi necessário o uso de camundongos para a obtenção dos tripomastigotas sanguíneos, que são formas infectantes, o que tornou o experimento mais dispendioso e exigiu capacidade técnica para manipulação de camundongos infectados. Os tripomastigotas provenientes de culturas eram inoculados no camundongo e no pico da parasitemia sanguínea, era feita a sangria desses animais para a obtenção de tripomastigotas sanguíneas que seriam, então, inoculadas nos ratos. O comportamento biológico da cepa Y do *T. cruzi* foi analisado quanto à sua capacidade infectante e quanto ao dia exato do pico parasitêmico. Analisamos também os parâmetros bioquímicos e hematológicos dos ratos infectados. Nesse experimento foram envolvidos alunos de graduação para a confecção de monografia sob a orientação da Profª. Dra Cecília Holanda do DMP. Os resultados dos testes de infecção foram apresentados no 33º Congresso Brasileiro de Análises Clínicas e 6º Congresso Brasileiro de Citologia Clínica, realizado em Curitiba, com o título: “Avaliação Hematológica e Bioquímica em Ratas Wistar infectadas Experimentalmente com Cepa Y do *Trypanosoma cruzi”*. O resumo foi publicado na Revista Brasileira de Análises Clínicas, Rio de Janeiro: Sociedade Brasileira de Análises Clínicas, 2006. v.38. p.91B.

A escolha do rato *Wistar* como modelo experimental para o estudo de biodistribuição do radiofármaco, foi devido a esses animais desenvolverem lesões similares às encontradas na doença de Chagas humana em vários aspectos como: parasitemia, sorologia positiva, alterações eletrocardiográficas, miocardite e miosite, fibrose e pan-infectividade, na fase aguda e ninhos de amastigotas, cardiopatia e megas, na fase crônica[31, 32]. Esse modelo também se mostrou ideal por ser amplamente utilizado em estudos de biodistribuição de radiofármacos por vários autores[21, 25, 26, 27, 28, 30].

Verificada a viabilidade da cepa Y do *T. cruzi* e diante dos dados do dia do pico da parasitemia sanguínea nos ratos, fizemos os testes de biodistribuição do pertecnetato de sódio e separamos os órgãos para análise histopatológica. Os dados mostraram que ocorreram alterações da captação do radiofármaco no cólon e no sangue do grupo infectado e alterações histológicas no cólon. Os resultados parciais desse experimento foram apresentados no XX Congresso Brasileiro de Parasitologia com o título: "Avaliação da biodistribuição do pertecnetato de sódio ($Na^{99m}TcO_4$) em ratos infectados com *Trypanosoma cruzi",* realizado em Recife em 2007.

A partir dos resultados foi gerado o manuscrito: "Biodistribution of Technetium-99m Pertechnetate in Rats Infected with *Trypanosoma cruzi"*, enviado para o periódico Experimental Parasitology (Qualis Internacional B1).

No segundo experimento, os ratos foram tratados com o antiparasitário benzonidazol por 30 dias e posteriormente foi feito o estudo de biodistribuição do radiofármaco. Esse experimento mostrou não haver interação entre a droga e o radiofármaco. Os resultados parciais foram apresentados no VI Congresso da Sociedade Brasileira de Biociências Nucleares, 2008, Cabo Frio, sob o

título: “Efeito do tratamento crônico da droga tripanosomicida benzonidazol (Rochagan) na biodistribuição do pertecnetato de sódio ($Na^{99m}TcO_4$) em ratos wistar”, com o resumo publicado na revista MN Metabólica. São Paulo: Atlântica, 2008. v.10. p.32 – 33.

Os resultados das dosagens bioquímicas e hematológicas no grupo tratado com o benzonidazol foram apresentados no I Simpósio Internacional de Ciências Farmacêuticas do Nordeste do Brasil, realizado na cidade do Natal em julho de 2008, sob o título: “Effect of benznidazole (rochagan) on biochemical and hematological parameters in *wistar* rats”.

O artigo completo foi publicado no periódico, Qualis B2 internacional, Brazilian Archives Biology and Technology com o título: “Effect of tripanosomicide benznidazole (Rochagan®) on the biodistribution of sodium pertechnetate ($Na^{99m}TcO_4$) in *wistar* rats”. Vol. 51, pp. 175-180, 2008.

A relevância dos resultados está pautada no fato de os radiofármacos serem amplamente utilizados como ferramenta diagnóstica através de exames cintilográficos, sendo cada vez mais freqüente em nosso país. Com isso, um número cada vez maior de pessoas tem acesso a esses métodos. O benzonidazol, por ser o único fármaco antiparasitário de escolha no tratamento de pacientes chagásicos no Brasil, é utilizado por um grande número de pessoas durante um período prolongado (30 a 60 dias)[15]. No presente estudo, ficou demonstrado que o radiofármaco pertecnetato de sódio não possui sua biodistribuição alterada em muitos órgãos, quando administrado em ratos tratados cronicamente com a droga Bz, sugerindo não prejudicar a interpretação de diagnósticos por imagem. Devido a esse fato, os resultados mostraram-se satisfatórios, embora constatados em animais de

experimentação. Entretanto, são necessários ainda estudos *in vitro* e clínicos. Os resultados também podem contribuir no conhecimento dos efeitos citotóxicos do medicamento, já que pouco se conhece sobre seus efeitos na célula hospedeira.

Ficou demonstrado também que a biodistribuição do radiofármaco, em ratos infectados com o *T. cruzi*, gera menor captação do pertecnetato de sódio no intestino grosso e maior captação no sangue. O processo inflamatório visualizado no intestino grosso, que é órgão alvo do parasito, e a mobilização do sistema de defesa com aumento da produção de proteínas totais e leucocitose no sangue, talvez possam explicar as alterações de captação do referido radiofármaco nesses sítios. Também não podemos descartar a hipótese de haver ligação entre o ^{99m}Tc e o *T. cruzi* no sangue. Além de possibilitar visualizar alterações teciduais precoces na fase aguda da infecção, podendo ser usado como ferramenta diagnóstica, esse estudo também contribuiu para o conhecimento do modelo rato na doença de Chagas experimental.

Por tudo que aprendi e vivenciei no decorrer do curso de Doutorado, considero que houve uma evolução bastante considerável dos meus conhecimentos. O processo de produção de idéias, o cumprimento de disciplinas obrigatórias e complementares, o desenvolvimento de diferentes protocolos e a interação e convívio com diferentes profissionais, além de contribuir enormemente para meu constante aprendizado, tiveram um valor inestimável no meu processo de formação e atuação. É pautada nessa rede de experiências, idéias e sentimentos que vem sendo desenvolvida a familiaridade com a pesquisa e o amadurecimento científico e profissional que poderão ser

relevantes para ações em futuros projetos de pesquisa, na orientação de alunos de graduação e/ou pós-graduação e na geração de novos conhecimentos.

5. APÊNDICE

5.1. OUTROS ARTIGOS PUBLICADOS

Effect of Paclitaxel (Taxol) on the Biodistribution of Sodium Pertechnetate ($Na^{99m}TcO_4$) in Female Wistar Rat

Cecília Maria de Carvalho Xavier Holanda, Elias Herculano de Oliveira, Louisianny Guerra da Rocha, Vanessa Santos de Arruda Barbosa, Maria Helena Constantino Spyrides, Cícero Flávio Soares Aragão, Aldo Cunha Medeiros

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Effect of Paclitaxel (Taxol®) on the Biodistribution of Sodium Pertechnetate ($Na^{99m}TcO_4$) in Female *Wistar* Rats

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ABSTRACT

The evidence that natural or synthetic drugs can affect the biodistribution of radiopharmaceuticals (radiobiocomplexes) in setting of nuclear medicine clinic is already known. We studied the effect of Paclitaxel, an anti-neoplastic agent for the treatment of solid tumors, on the biodistribution of $Na^{99m}TcO_4$ in female rats. Paclitaxel (1mg/mL/week) was administered into animals in single dose during 3 weeks, with interval of 1 week among them. The control group received NaCl 0.9% solutions by the same via. One hour after the last dose, it was injected $Na^{99m}TcO_4$ in the animals. The percentage of activity per gram (%ATI/g) and biochemical and hematological determinations were performed. A significant increase were found in alanine aminotransferase, aspartate aminotransferase, glucose and in the %ATI/g of some organs (ovaries, uterus, vagina, breasts, large intestine and liver).These results can be associated, probably, to the capacity of paclitaxel to alter the biodistribution of $Na^{99m}TcO_4$ and the metabolism of glucose and hepatic enzymes.

Keywords: paclitaxel, drug interaction, radiopharmaceuticals, $Na^{99m}TcO_4$, antitumoral drug, cancer.

INTRODUCTION

Cancer is a multifactorial disease that results fromthe interaction of multiple genetic and environmental factors. Chemotherapy is usually given early after diagnosis in several cancer subtypes to offer best results (Steinkellner et al., 2001; Vaclavikova et al., 2003). The use of phytotherapeutic products by the world population has greatly increased in the last decades (Briskin, 2000; Ang-Lee et al., 2001; Chan, 2003). Paclitaxel, commercially known by Taxol®, is a compound with intense antitumoral activity extracted from the *Taxus* species. It is presently one of the most important drugs used in cancer

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chemotherapy (Itoh et al. 2004; Feldweg et al., 2005).

Paclitaxel is an unique anticancer agent with tubulin-stabilizing action, and widely used for several malignancies, including ovarian, breast, stomach and non-small cell lung cancers (Sparreboom et al., 1997; Akerley, 2000; Souza, 2004; Itoh et al. 2004; Feldweg et al., 2005).

However, chemotherapeutic drugs are often associated with some degree of toxicities, which are caused by reactive metabolites generated by the biotransformation of anticancer drugs in the liver (Steinkellner et al., 2001; Lahowel and Fillastre, 2004; Choi and Li, 2005). Paclitaxel is mainly metabolized through the liver and undergoes biliary excretion (Itoh et al. 2004; Feldweg et al., 2005).

Traditional noninvasive imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) are used primarily for imaging anatomical and morphological changes associated with diseases (Vallabhajosula, 2007). Historically, CT has been the modality of choice for the diagnosis and staging of malignant disease and for monitoring the response to treatments (Vallabhajosula, 2007).

These screening techniques, however, often lack the necessary sensitivity and specificity for early diagnoses of many cancers and for the detection of subcentimeter neoplasms and preneoplastic disease (Hanahan and Weinberg, 2000; Vallabhajosula, 2007). To develop effective treatment modalities, especially, patient specific treatments, a more sensitive and specific detection of early malignancies is essential. Molecular imaging is the visualization, characterization, and measurement of biological processes at the molecular and cellular levels in a living system (Schlyer, 2003; Vallabhajosula, 2007). Radioisotope based molecular imaging techniques, such as positron emission tomography (PET) (Nutt, 2002; Rajendran and Mankoff, 2007) and single photon emission computed tomography (SPECT), capture functional or phenotypic changes associated with disease (Mankoff et al., 2005). It is a field that aims to integrate patient-specific and disease-specific molecular information with traditional anatomical or structural imaging readouts. The hybrid or fusion-imaging of PET/CT is improving the sensitivity and specificity of clinical PET imaging technique (Schlyer, 2003; Rajendran and, Mankoff, 2007).

The progress in diagnostic nuclear medicine over the years since the discovery of technetium-99m (^{99m}Tc) is indeed phenomenal. The preeminence of ^{99m}Tc is attributable to its optimal nuclear properties of a short half-life (6 hours), metastable radionuclide, radiotracer with gamma photon emission of 140 keV, which is suitable for high-efficiency detection for imaging with gamma cameras used in nuclear medicine and which results in low radiation exposure to the patient (Saha, 2004; Bernardo-Filho et al. 2005). The evidence that natural and/or synthetic drugs can affect the biodistribution of radiopharmaceuticals (radiobiocomplexes) in setting of nuclear medicine clinic is already known (Xavier-Holanda et al., 2002; Saha, 2004; Bernardo-Filho et al. 2005; chemotherapeutic treatment. This fact can to lead a misdiagnosis or unnecessary exposure to radiation during the repetition of these exams. Frequently, this phenomenon is responsible for modification of the biodistribution of the radiopharmaceutical (Xavier-Holanda et al., 2002; Thrall and Ziessman, 2003; Bernardo-Filho et al. 2005; Holanda et al., 2006). The aim of this study was to evaluate the effect of the paclitaxel on the biodistribution of the radiopharmaceutical sodium pertechnetate ($Na^{99m}TcO_4$) labeled with technetium-99m, in female *Wistar* rats and on some biochemical and hematological determinations.

MATERIALS AND METHODS

The animals were obtained from *Centro de Ciências da Saúde, Universidade Federal do Rio Grande do Norte,* Natal-RN, Brasil, were housed in groups with free access to food and water, maintained under constant environmental conditions (23±2°, 12h/12h of light/dark cycle). Studies were performed in healthy female *Wistar* rats (weight range: 180–250 g). Twelve animals were used in this experiment and were randomly divided into two groups (treated and control) of 6 animals each one. These experiments were approved by the Ethical Committee for Using Animals of UFRN, with the number CEA/212/2008.

Paclitaxel was kindly provided by Bristol-Myers-Squibb (30 mg paclitaxel in 6 ml of ethanol: CremophorEL, 50:50) and stored at 4°C during use. Stock solutions of paclitaxel were prepared by dilution in methanol and stored at room temperature for a week.

In the treated group, paclitaxel, dissolved in isotonic saline solution (NaCl 0.9%) was administered by intraperitoneal via (IP) into animals at a dose of 1mg/mL/week, in single dose during 3 weeks, with interval of one week among them. The control group received saline solution by the same way and period. One hour after the last dose, it was injected 0.1 mL of $Na^{99m}TcO_4$ (3.7 MBq) via orbital plexus. $Na^{99m}TcO_4$ was eluted in a $^{99}Mo/^{99m}Tc$ generator (*Instituto de Pesquisas Energéticas e Nucleares, São Paulo, Brasil*). After 60 minutes, all animals were quickly killed under anesthesia with xylazine (20 mg/kg) and ketamine (50 mg/ kg), by IP via. Breast, large intestine, liver, ovary, oviduct, stomach, spleen, Santos-Filho and Bernardo-Filho, 2005; Holanda et al., 2006). These interactions can alter results of thyroid, uterus, vagina and samples of blood were isolated. The tissue were washed in saline, weighed in a balance (Mark 160®, Bel equipment, Italy) and the radioactivity was determined in an automatic gamma counter (Wizard 1470, Perkin-Elmer, Finland) and the percentage of radioactivity per gram of tissue (%ATI/g) was calculated. Before the administration of $Na^{99m}TcO_4$, it was withdrawn 2 mL of whole blood of each animal and the biochemical and hematological determinations were performed in automated equipment TermoKonelab 60i/Abbott and Cell-Dyn 3500R/Abbott, respectively. Data were presented as mean ± standard deviation. The percentage of radioactivity per gram (%ATI/g) was determined by dividing the percentage of total radioactivity of each tissue by its weight in grams. The ATI%/g was compared using the non-parametric Mann-Whitney test and the biochemical and hematological parameters by Student's t-test, considering the level of statistical significance at $p<0.05$ in both tests. Statistica 6.0 software was used.

RESULTS

Table 1 shows the relationship between the uptake (%ATI/g) of the $Na^{99m}TcO_4$ on the paclitaxel-treated group (n=6) and on the saline-control group (n=6), 60 min after administered of the $Na^{99m}TcO_4$. The analysis of the results shows a significant ($p<0.05$) increase of the uptake of radioactivity in breasts, large intestine, liver, ovaries, uterus and vagina.

Table 1 - Effect of paclitaxel treatment on the biodistribution of sodium pertechnetate activity in female *Wistar* rats after 60 min injection of radiopharmaceutical ($Na^{99m}TcO_4$).

Organs	% ATI/g	
	Controls	Treated
Blood	0.030±0.001	0.050±0.001
Breast	0.040±0.000	0.420±0.005*
Liver	0.160±0.002	0.360±0.003*
Large Intestine	0.050±0.000	0.150±0.004*
Ovaries	0.040±0.000	0.160±0.002*
Oviducts	0.080±0.001	0.090±0.002
Spleen	0.080±0.000	0.100±0.004
Stomach	1.110±0.006	1.280±0.008
Thyroid	3.190±0.010	3.280±0.013
Uterus	0.070±0.000	0.210±0.004*
Vagina	0.030±0.000	0.200±0.001*

Mean±SD. *, $p<0.05$

Table 2 shows the effect of the paclitaxel on the biochemical and hematological parameters of the female *Wistar* rats (n=6) and on the control group (n=6), before the administration of the $Na^{99m}TcO_4$. The analysis of the results shows a significant ($p<0.01$) increase of the glucose, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and white blood cells.

Table 2 – Effect of paclitaxel treatment on biochemical and hematological parameters of female *Wistar* rats.

Biochemical and hematological parameters	Groups	
	Controls	Treated
ALT (UI/L)	110.17± 25.18	158.33± 16.91**
AST (UI/L)	111.17± 35.72	157.83± 52.15**
Glucose (mg/dL)	97.83± 14.96	154.67± 33.61**
Hematocrit (%)	29.76± 4.08	31.45± 3.42
Hemoglobin (g/%)	11.62± 1.01	11.88± 1.02
Leukocytes (u/mm^3)	1860.00± 338.20	3040.00± 701.90**
Lymphocytes (%)	53.33± 9.37	69.66± 4.92
Neutrophils (%)	54.83± 7.05	64.83± 5.56
Platelets (u/mm^3)	648167± 106878	669500± 111159
Red blood cells (u/mm^3)	5688333± 889436	5746667± 646209
Total proteins (g/dL)	6.66± 0.78	6.75± 0.52

Mean±SD. **, $p<0.01$.

DISCUSSION

Cancer is a systemic disease resulting from alterations in the interactions between oncogenes and tumor suppressor genes, which under normal physiological conditions control cell maturation, division and migration (Hanahan and Weinberg, 2000; Steinkellner et al., 2001; Vaclavikova et al., 2003).

In our study, we observed alterations on biochemical and hematological parameters and on biodistribution of the radiopharmaceutical sodium pertechnetate in female *Wistar* rats treated with the paclitaxel in some tissues.

Paclitaxel is an antineoplastic agent that has shown great promise in the therapeutic treatment of certain solid tumors including breast cancer. It is also the most promising anti-mitotic agent developed for cancer treatment in the past decade (Rosenblum and Shivers, 2000). The primary mechanism of action of paclitaxel is attributed to its ability to bind to microtubules and prevent their assembly. All treatment regimens for majority of cancers produce a lot of side effects, including hematological or liver toxicities (Steinkellner et al., 2001; Lahowel and Fillastre, 2004).

ALT and AST are diagnostic tumor markers in liver and heart diseases. The decreased activities of these enzymes in liver indirectly indicate the progression of tumor growth as tumor markers are directly associated with the malignancy in the cancerous conditions and is a potential molecular biomarker for assessing exposure to any toxic agents (Boutet et al., 2005). Tissue damage is the sensitive feature in the cancerous conditions so any deterioration or destruction of the membrane can lead to the leakage of these enzymes from the tissues. Hence, the elevation of these liver specific enzymes observed in breast cancer condition may be due to the progression of tumor growth (El-Beshbishy, 2005). Itoh et al. (2004) have reported increased activities of the enzymes (ALT and AST) in plasma and serum of cancer bearing rats, and now, our experiments also demonstrated increased activities of these enzymes (ALT and AST) in the chronic treatment of female rats with paclitaxel.

We have previously shown that the antiparasitic drugs such as glucantime and mefloquine (Xavier Holanda et al., 2002; Holanda et al., 2006) can alter the biodistribution *in vivo* of ^{99m}Tc-methylenediphosphonic acid (^{99m}Tc-MDP) in *Wistar* rats. Besides these studies, it was also observed alterations on the biodistribution of the $Na^{99m}TcO_4$ in organs of *Wistar* rats treated with *Punica granatum* and *Artemisia vulgaris* (Amorim et al., 2003; Holanda et al., 2006). In our experiment, we demonstrated that the paclitaxel increased the uptake of $Na^{99m}TcO_4$ in ovaries, uterus, vagina and breasts of female *Wistar* rats treated with this drug, what suggest an action of this antineoplastic agent in these organs. The %ATI/g also increased in liver and large intestine. This fact probably occurred due to the paclitaxel to

be metabolized through the liver and undergoes biliary excretion (Itoh et al., 2004).

In conclusion, these experimental models permit to study drug interactions and biological activities of vegetal extracts and synthetic drugs. Moreover, these findings could be worthwhile to try to understand and to avoid some pitfalls in the nuclear medicine imaging.

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RESUMO

Já está bem estabelecido na literatura científica que produtos naturais ou sintéticos podem alterar a biodistribuição de radiofármacos. O objetivo desse estudo foi avaliar a influência do paclitaxel, um agente antineoplásico para tratamento de tumores sólidos na biodistribuição do pertecnetato de sódio em ratas *Wistar* e na determinação de componentes bioquímicos e hematológicos. Paclitaxel, comercialmente conhecido por Taxol® (1mg/mL/semana), foi administrado, intraperitoneamente, nos animais do grupo tratado, em dose única, por 3 semanas, mas com intervalo de uma semana entre elas. O grupo controle recebeu solução de NaCl 0,9%. Uma hora após a última dose de paclitaxel, os animais receberam 0,1 mL de $Na^{99m}TcO_4$ (3,7MBq) via plexo orbital. O percentual de radioatividade por grama (%ATI/g) e parâmetros laboratoriais foram determinados. Ocorreu um aumento significativo (p<0,05) do %ATI/g nos ovários, útero, vagina, mamas, intestino grosso e fígado. Os níveis de glicose sangüínea e das enzimas hepáticas (ALT e AST) também aumentaram significantemente (p<0,01). Esses resultados podem estar associados, provavelmente, à capacidade do paclitaxel em alterar a biodistribuição do $Na^{99m}TcO_4$ e o metabolismo da glicose e de enzimas hepáticas.

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Effect of Medicinal Plants on the Parasitemia of *Trypanosoma cruzi* and on the Biodistribution of Sodium Pertechnetate ($Na^{99m}TcO_4$)

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Effect of Medicinal Plants on the Parasitemia of *Trypanosoma Cruzi* and on the Biodistribution of Sodium Pertechnetate ($Na^{99m}TcO_4$)

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ABSTRACT

Artemisia vulgaris (AV) is an antihelmintic and antimalarial drug; Aloe vera(babosa) acts as antidiabetic, laxative and anti-inflammatory;Benznidazole (BZ) is a trypanocidal of Trypanosoma cruzi (TC). Technetium-99m (^{99m}Tc) has been used in nuclear medicine to obtain diagnostic images. This study evaluated the plant effects in TC parasitemia and on the biodistribution of ^{99m}Tc in mice. Twenty mice were infected by TC. At the peak of parasitemia, 5 mice received babosa; 5 received AV and 5 received BZ. The parasitemia was determined at 0, 2, 4 and 6 h of drugs administration. Five infected mice without drugs, 5 mice without TC and the group treated with AV, received ^{99m}Tc. The radioactivity was calculated. Infected mice that received babosa reduced significantly ($p<0.05$) the TC parasitemia. The percentage of activity per gram (%ATI/g) decreased significantly on the AV group. These results indicate that babosa possibly is an anti-TC drug and AV reduces the %ATI/g probably due to its biological effects.

Keywords: *Aloe vera*, *Artemisia vulgaris*, technetium-99m, *Trypanosoma cruzi*, parasitemia, biodistribution

INTRODUCTION

The Chagas' disease is a protozoan infection caused by the parasite *Trypanosoma cruzi* and transmitted by the depositing of metacyclic tripomastigotes, eliminated in the feces and urine of the several species of triatomine bugs, during the hematophagism phase (Cançado, 2005; Coura and Castro, 2002). It is an endemic Latin America parasitosis which affects 18 million individuals, with 300 thousand new cases every year (WHO, 2003) and persists for the lifetime of the human/mammalian host. This disease is

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characterized by an acute phase with detectable parasitemia and a long-lasting asymptomatic phase, generating megacolon, megaesophagus and chagasic heart disease (Santos et al., 2005; Teixeira et al., 2006). Treatment includes eradicating the parasite with Benznidazole, commercially known as Rochagan®, a drug with specific anti-*T. cruzi* activity *in vivo* and *in vitro*, available in Brazil since the 1970's (Coura and Castro, 2002). The use of certain plants as phytotherapy has been a millennial practice in folk medicine. Its use has gained enormous popularity around the world, as modern medicine is beyond the reach of many people. The *Artemisia vulgaris* and *Artemisia annua* are examples of ancient plants in Chinese medicine that has shown to be very effective against *Plasmodium falciparum* and *P. vivax*, malaria parasites in humans (Meschinick. and Dobson, 2001). *A. vulgaris* is metabolized and eliminated rapidly from the human organism and induce a rapid reduction of these species of *Plasmodium* (Meschnick, 1998; Meschinick and Dobson, 2001). However, there are no reports in the scientific literature about its tripanosomicidal action (anti-*T. cruzi*). Another example of phytotherapy, widely used in Brazilian folk medicine, is the *Aloe vera* plant, known as "babosa". It is a tropical or sub-tropical plant from North Africa and has been used over the years to treat various ailments and have been referred to as the "miracle" plant. It has been suggested that the extract of the plant promotes healing of diseases through the complex synergistic interaction of many substances, and some specially prepared *A. vera* extracts possess some biological activities such as antiinflammation, anti-cancer, anti-diabetes, macrophage activation, combat gastrointestinal infections and urinary infections, as an analgesic and more (Reynolds and Dweck, 1999). However, its effect anti-*T. cruzi* is not known until the moment. Studies are being developed to discover drugs that provoke the complete eradication of the *Trypanosoma cruzi*, not only through the elimination of tecidual forms (amastigotes) such as blood (tripomastigotes) and the 100% cure of cases in Brazil.

Nuclear medicine (NM) is the medical specialty that uses radioactive isotopes to diagnose through images or therapy. The role of radionuclide technetium-99m (^{99m}Tc) in the diagnostic field of MN is already well established. This is due to its chemical versatility and nuclear properties such as the emission of a single photon gamma (140 keV) and with 89% abundance, perfect for obtaining the images in gamma cameras used in NM (Saha, 2004; Bernardo-Filho et al. 2005). Its short half-life (6 hours) is enough to acquire excellent studies of images, to prepare radiopharmaceuticals, to minimize the dose of radiation for the patient, to have an almost inexistent environmental impact, besides being an ideal radiotracer. The rapid growth of this field in the last decades is attributed to its ideal physicochemical characteristics and to it being easy to obtain from a portable generator of ^{99}Mo/^{99m}Tc in the form of sodium pertechnetate ($Na^{99m}TcO_4$) and can be lyophilized in *kits* to form labeled compounds with ^{99m}Tc in hospital or radiopharmacy clinic (Banerjee et al., 2001; Saha, 2004).

The aim of this study was to evaluate the effect of medicinal plant extracts on the parasitemia of *T. cruzi* and on the biodistribution of the $Na^{99m}TcO_4$ in mice infected with the *Y* strain of *T. cruzi*.

MATERIALS AND METHODS

Twenty-five male *Swiss* mice weighing 18-20g from Centro de Ciências da Saúde, Universidade Federal do Rio Grande do Norte (UFRN), Natal–RN, Brasil, were used. The protocol was conducted in accordance with Brazilian College of Animal Experimentation guidelines and was approved by the Research Ethics Committee of Onofre Lopes Hospital-UFRN (182/2008). The animals had free access to water and standard food for rodents (Labina Purina®) and were randomly allocated to 2 groups: control and treated. The animals were divided, randomly, in groups of 5 mice each. Twenty mice were infected intraperitoneally, with a suspension containing $1x10^5$ tripomastigotes blood parasites /mL of the *Y* strain of *T. cruzi*. Group 1 was used as the control group, being infected experimentally with *T. cruzi* and not treated. Group 2 was also a control group, but neither infected nor treated with drugs. Groups 3, 4 and 5 were infected with *T. cruzi* and received orally (gavage), respectively, 0.25mL of an aqueous *A. vera* (5mg/mL/day), 0.25mL of Benznidazole (5mg/Kg/day), diluted in sorbitol, and 0.25mL of hydroalcoholic extract of *A. vulgaris* (5mg/mL/day). To evaluate the parasitemia of each animal, whole blood of the mice was used and the parasites were counted according to Brener (1962). The mice were infected with *Y* strain of *T. cruzi* provided by the

René Rachou Research Center (CPqRR), FIOCRUZ, Belo Horizonte-MG. The parasitemic curve of the animals was tracked daily, from the 4° to the 12° day of the parasite infection, with the purpose to observe its growth and, thus, determine the parasitemic peak, which occurred between the 7th and the 9th days. The parasitemia of each animal was determined after 3 measuring in three observations. The number of circulating tripomastigotes was counted according to Brener (1962), which consisted in examining 5 µL of peripheri blood, taken by incision in the tail of each animal, in 50 field microscopes and using lamina and laminula, with increase of 400 times. After the counting of the parasites, the number found was multiplied by a correction factor corresponding to 80 (Brener, 1962). In this study, the parasitemia was achieved during the measure of time: 0 (before administering the drugs) and 2, 4 and 6 hours after its use. The group treated with Benznidazole was considered as the control group owing to its trypanosomicide action, which has been recognized since the 1970's. The results obtained in the parasitemia study were analyzed statistically by the parametric ANOVA test and the level of significance to $p<0.05$. Before the administration of the radiopharmaceutical sodium pertechnetate ($Na^{99m}TcO_4$), heparinized blood was withdrawn from infected and treated animals with each drug, and from infected and untreated animals (control group 1), by cardiac puncture, under anesthesia. The biochemical dosages were performed in automated equipment TermoKonelab 60i, Abbott and analyzed by Student's t-test, considering the level of statistical significance at $p<0.05$ in both tests. Statistica 6.0 software was used. Data were presented as mean ± standard deviation.

After observation of the parasitemia and blood collection, a study of biodistribution of $Na^{99m}TcO_4$ was done. For this, 5 animals infected and treated with *A. vulgaris* were used, as well as, 5 infected and not treated (control group 1) and 5 not infected and not treated (control group 2). All of these animals received, by orbital plexus via, 0.1mL of $Na^{99m}TcO_4$ (3.7MBq), recently eluted from the generator of $^{99}Mo/^{99m}Tc$ (*Instituto de Pesquisas Energéticas e Nucleares,* São Paulo, Brasil). After 60 minutes, all animals were quickly killed under anesthesia. Samples were harvested from the brain, heart, intestines, spleen, liver, bladder, femur, lungs, kidneys and blood. The tissue samples were washed in 0.9% saline, weighed on a precision scale (Mark 160®, Bel equipment, Italy) and the percentage of radioactivity per gram of tissue (%ATI/g) was determined in an automatic gamma counter (Wizard 1470, Perkin-Elmer, Finland). The efficiency of the gamma counter was 86%, as specified by the manufacturer. The results of the study of the biodistribution were compared to their control groups and the statistical analysis was done using the non-parametric Mann-Whitney ($p<0.05$) test. Statistica 6.0 software was used.

RESULTS

Table 1 shows the parasitemia of the animals treated with *A. vera,* compared to those treated with benznidazole (control) and *A. vulgaris*. The analysis of the results shows a significant ($p<0.05$) decrease of counting of the parasites in all the times (0, 2, 4 e 6 hours). The values correspond to the mean±DP.

Table 1 - Parasitemia of mice infected with *Tripanosoma cruzi*, on times 0, 2, 4 and 6 hour, after administration of benznidazole, *A. vera* and *A. vulgaris*.

Hours	Benznidazole (control)	*Aloe vera (babosa)**	*Artemisia vulgaris (Artemisine)**
0	165.80±20.30	150.00±50.80	164.30±13.70
2	198.50±12.00	151.80±38.80	300.00±39.60
4	138.80±17.10	122.00±16.16	392.80±55.20
6	33.50±11.90	25.25±1.14	400.80±82.50

Mean±DP. *, $p<0.05$.

Table 2 shows the effect of the *A. vulgaris* extract on the biodistribution of $Na^{99m}TcO_4$ in infected mice, 60 minutes after administration of the radiopharmaceutical. The values correspond to the mean±DP. A significant increase was observed ($p<0.01$) of the %ATI/g in spleen, brain, femur, liver, lungs and blood and a significant decrease of the %ATI/g ($p<0.01$) in heart, intestines, kidney and bladder, compared to the control groups 1 and 2.

In relation to biochemical dosages, there was a significant ($p<0.05$) decrease of blood levels of glucose and cholesterol in the group treated with

Table 2 – Effect of *A. vulgaris* on the biodistribution of $Na^{99m}TcO_4$ after 60 minutes of radiopharmaceutical administration.

	% ATI/g					
Organs	**Control 1**		**Control 2**		***A. vulgaris****	
Bladder	0.075	± 0.007	0.080	± 0.005	0.030	± 0.005
Blood	0.079	± 0.017	0.065	± 0.074	4.065	± 0.074
Brain	0.027	± 0.008	0.020	± 0.007	0.050	± 0.007
Femur	2.076	± 0.059	2.098	± 0.070	5.098	± 0.070
Heart	1.010	± 0.007	1.047	± 0.014	0.047	± 0.014
Intestine	2.013	± 0.034	2.036	± 0.008	0.036	± 0.008
Kidney	7.000	± 1.052	6.035	± 1.028	4.035	± 1.028
Liver	1.069	± 0.028	2.060	± 0.068	4.059	± 0.068
Lung	0.029	± 0.005	0.022	± 0.086	6.022	± 0.086
Spleen	0.035	± 0.010	0.000	± 0.015	0.086	± 0.015

Mean±DP. *, $p<0.05$.

Table 3 - Effect of *A. vulgaris* and *Aloe vera* extract on biochemical parameters of mice infected with *T. cruzi*.

Biochemical parameters	**Control**	***A. vulgaris***	***Aloe vera***
Cholesterol (mg/dL)	134.70±09.50	100.20±17.50*	68.75 ±4.35*
Glucose (mg/dL)	117.50±23.00	62.00 ±41.90*	113.00±10.58*
AST (U/L)	110.50±40.40	621.00±46.00*	955.00±90.00*
ALT (U/L)	74.70 ±10.40	423.80±10.63*	476.00±74.40*

Mean±DP. *, $p<0.05$.

A. vulgaris. A significant increase ($p<0.05$) was observed in the enzymes Aspartate amino transferase (AST) and Alanine amino transferase (ALT) in this group. The group treated with *A. vera* (babosa) also showed a significant decrease ($p<0.05$) in the dosages of glucose and cholesterol and a significant increase in the enzymes (AST and ALT). These results are observed in Table 3. The values correspond to the mean±DP.

DISCUSSION

For at least 30 years there has not been any new drug for the treatment of Chagas' disease, a fact that has instigated the search for new drugs for the treatment of this disease, envisioning alternatives with fewer side effects and greater effectiveness (Camandaroba *et al*, 2003). *A. vulgaris* or "Mugwort", as it is known in traditional Chinese medicine, is a plant widely used to treat diabetes and menstruation disorders. The infusion of their leaves presents potent action against intestinal parasites (Teixeira da Silva, 2004). In natural medicine (herbal) its extract has been widely used as anti-helmintic, anti-malaric, antiseptic, antispasmodic, antireumatic and antibacterial agent (Duke et al., 2002). The active components of *A. vulgaris* include: flavonoids, cumarinics, terpenes, lactones, volatile oils, inulin and traces of alkaloids (Haider et al., 2003; Teixeira da Silva, 2004; Judzentiene and Buzelyte, 2006). However, this study showed that the extract of *A. vulgaris* was not able to reduce the trypomastigote forms of *T. cruzi* in Swiss mice infected with this parasite,

despite the proven action, both of *A. vulgaris* as well as the *A. annua*, in reducing the parasitemia of another protozoan, the *P. falciparum*, in malaric patients (Meshnick and Dobson, 2001).

It is important to assess the interaction of the extract of *A. vulgaris* with the normal metabolism. Our data showed a high decline in blood glucose in animals treated with *A. vulgaris* extract, and also revealed an increase in liver enzymes (AST and ALT), indicating a possible liver injury induced by this extract. Meanwhile, scientific findings on the liver toxicity of *A. vulgaris* are scarce.

The extract of the plant promotes healing of diseases through the complex synergistic interaction of many substances, and specially prepared *Aloe vera* extracts, possess some biological activities such as antiinflammation, anti-cancer, anti-diabetes and macrophage activation, combat gastrointestinal infections and urinary infections, as an analgesic and more (Reynolds and Dweck, 1999). The data obtained from the *A. vera* (babosa) treatment in this study showed that its extract possibly has higher activity in reducing the parasitemia of animals infected with *T. cruzi* than that of benznidazole, a synthetic anti-*T. cruzi* drug used since the 1970's in Brazil.

The biochemical changes found in serum cholesterol and liver transaminases (AST and ALT) may be related to the biological, metabolic or toxic effects of "babosa".

According to Patel and Mengi (2008), the extract of *Aloe vera* possesses hipolipidemic, hipoglicemic and antitrombotic activities. This finding probably explains the low levels of cholesterol and glucose in mice infected and treated with *A. vera* in our study.

Several authors have demonstrated that the biodistribution of radiopharmaceuticals may be altered by natural and synthetic drugs, diets and surgery (Xavier Holanda et al., 2002; Bernardo et al., 2004; Santos-Filho et al., 2005; Holanda et al., 2006; Araújo-Filho et al., 2007). In this study, there was a significant increase in the %ATI/g of the $Na^{99m}TcO_4$ in the femur of mice treated with *A. vulgaris,* probably induced by the extract of this plant on the hydroxyapatite crystals, or the deposition of calcium phosphate in bone. We also observed a significant increase in the %ATI/g of the $Na^{99m}TcO_4$ in the liver, probably due to the metabolization of *A. vulgaris* in that organ.

Our data showed a significant decrease of %ATI/g of the radiopharmaceutical in the kidneys, bladder and intestines, possibly because these organs are the main route for the excretion of metabolites from *A. vulgaris* extract (Meshnick and Dobson, 2001). The changes found in other organs and tissues probably are due to the biological and metabolic effects of *A. vulgaris*. Further studies are necessary to explain the mechanisms of these effects.

The human American trypanosomiasis, a disease of high morbidity and mortality has been treated with unefficient drugs, and requires much research about new drugs and new measures for prevention and cure. In conclusion, the data of this work suggest that the drugs studied had anti-*T. cruzi* effect and changed the metabolism and biodistribution of pertechnetate in mice.

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RESUMO

A *Artemisia vulgaris* (AV) é uma planta com atividades antihelmíntica e antimalárica. *Aloe vera* (babosa) tem ação antidiabética, laxante e anti-inflamatória. Benzonidazol (BZ) é uma droga tripanosomicida contra o *Trypanosoma cruzi* (TC), agente da doença de Chagas. Tecnécio-99m (^{99m}Tc) tem sido usado na medicina nuclear para obtenção de imagens diagnósticas. Este estudo avaliou o efeito de plantas na parasitemia do TC e na biodistribuição do ^{99m}Tc em camundongos. Vinte camundongos foram infectados por TC. No pico da parasitemia, 5 camundongos receberam babosa; 5 receberam AV e 5 receberam BZ. A parasitemia foi determinada durante os tempos 0, 2, 4 e 6 horas após administração das drogas. Cinco camundongos infectados e não tratados, 5 camundongos não infectados e o grupo tratado com AV receberam ^{99m}Tc, na forma de pertecnetato de sódio. A radioatividade foi calculada. Os animais infectados que receberam babosa reduziram significativamente ($p<0.05$) a parasitemia. A porcentagem da radioatividade por grama (%ATI/g) diminuiu significativamente no

grupo tratado com AV. Estes resultados indicam que a babosa possivelmente é uma droga anti-TC e a AV reduz a %ATI/g provavelmente devido seus efeitos biológicos e/ou metabólicos.

Palavras-chave: *Aloe vera*, *Artemisia vulgaris*, tecnécio-99m, *Trypanosoma cruzi*, parasitemia, biodistribuição

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5.2. RESULTADOS APRESENTADOS EM CONGRESSOS

33º Congresso Brasileiro de Análises Clínicas e 6º Congresso Brasileiro de Citologia Clínica, Curitiba, 2006.

Título: “Avaliação hematológica e bioquímica em ratas wistar infectadas experimentalmente com cepa Y do *Trypanosoma cruzi*”.

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33º CONGRESSO BRASILEIRO DE ANÁLISES CLÍNICAS
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CURITIBA • 04 A 08 DE JUNHO DE 2006
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de
Apresentação de Tema Livre

AVALIAÇÃO HEMATÓGICA E BIOQUÍMICA EM RATAS WISTAR INFECTADAS EXPERIMENTALMENTE COM A CEPA Y DE *TRYPANOSOMA CRUZI*

Autores: MARIA DAS GRAÇAS ARAÚJO DO NASCIMENTO; MARIA MARTA DE ARAÚJO FONTES; MARIA DE FÁTIMA ROCHA DOS SANTOS LIMA; VANESSA SANTOS DE ARRUDA BARBOSA; ANTÔNIA CLÁUDIA JÁCOME DA CÂMARA; CECÍLIA MARIA DE CARVALHO XAVIER HOLANDA

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XX Congresso Brasileiro de Parasitologia, Recife, 2007.

Título: “Avaliação da biodistribuição do pertecnetato de sódio ($Na^{99m}TcO_4$) em ratos infectados com *Trypanosoma cruzi”.*

Resumo publicado nos Anais do XX Congresso Brasileiro de Parasitologia.

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apresentaram o trabalho **"AVALIAÇÃO DA BIODISTRIBUIÇÃO DO PERTECNETATO DE SÓDIO (Na99mTcO4) EM RATOS INFECTADOS COM Trypanosoma cruzi"**, no ***XX Congresso Brasileiro de Parasitologia***, realizado no período de 28 de outubro a 01 de novembro de 2007, no Centro de Convenções de Pernambuco, na modalidade PÔSTER.

Recife, 29 de Outubro de 2007.

Silvana Ferreira
Presidente do Congresso

Zulma Medeiros
Presidente da
Comissão Científica

VI Congresso da Sociedade Brasileira de Biociências Nucleares, Cabo Frio, 2008.

Título: “Efeito do tratamento crônico da droga tripanosomicida benzonidazol (Rochagan) na biodistribuição do pertecnetato de sódio ($Na^{99m}TcO_4$) em ratos wistar”.

Resumo publicado na revista MN Metabólica. São Paulo: Atlântica, 2008. v.10. p.32 – 33.

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Vanessa Santos de Arruda Barbosa, Cecília Maria de Carvalho Xavier Holanda, Roseane Pereira da Silva, Daniel Pereira de Oliveira, Maurício Ferreira da Silva Júnior, Raphaella Cavalcante Alves, Aldo da Cunha Medeiros.

Universidade Federal do Rio Grande do Norte, UFRN, foi apresentado sob forma de painel no "VI Congresso da Sociedade Brasileira de Biociências Nucleares", na cidade de Cabo Frio, Rio de Janeiro, Brasil de 11 a 13 de Dezembro de 2008.

Rio de Janeiro,13 de dezembro, de 2008.

Prof. Dr. Mario Bernardo Filho
Presidente

I Simpósio Internacional de Ciências Farmacêuticas do Nordeste do Brasil, Natal, 2008.

Título: “Effect of benznidazole (rochagan) on biochemical and hematological parameters in wistar rats”.

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Work on EFFECT OF BENZNIDAZOLE (ROCHAGAN®) ON BIOCHEMICAL AND HEMATOLOGICAL PARAMETERS IN WISTAR RATS
HOLANDA, C. M. C. X.; BARBOSA, V. S. A.; SILVA, R. P.; OLIVEIRA, D. P.; OLIVEIRA, E. H.; SILVA-JÚNIOR, M. F.; MEDEIROS, A. C.
July 3-4, 2008.
Natal (RN) - Brasil.

Dr. Adriana Augusto de Rezende
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ABSTRACT

The aim of this study was to evaluate the biodistribution of sodium pertechnetate ($^{99m}TcO_4$), used in scintigraphic examinations, in *Wistar* rats experimentally infected with the *Y* strain of *Trypanosoma cruzi*, the parasite causing Chagas' disease and in rats treated for 30 days with the anti-*T. cruzi* benznidazole. The percentage of radioactivity per gram (%ATI/g) of various organs such as brain, heart, esophagus, stomach, small intestine, colon, spleen, liver, muscle and blood was measured in an automatic gamma counter. Comparing the controls with the treated group, it was observed that the biodistribution of $^{99m}TcO_4$ did not change in the organs of animals treated with benznidazole. The rats infected with *T. cruzi* had increasing uptake of $^{99m}TcO_4$ in the blood and decreasing in the colon. Additionally, histopathological changes in the colon were observed. In conclusion, the data show that treatment with benznidazole in rats does not change the biodistribution of $^{99m}TcO_4$ but the infection by *T. cruzi* does it. This findings may result in potential diagnostic and clinical implications. The completion of this study had multidisciplinary nature with the involvement of biologists, physicians, pharmacists and statisticians.

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