UNIVERSIDADE FEDERAL DE PERNAMBUCO
CENTRO DE CIÊNCIAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM PATOLOGIA

IMPACTO DA MÁ-NUTRIÇÃO PRECOCE NA RESPOSTA INFLAMATÓRIA CRÔNICA E SUAS IMPLICAÇÕES SOBRE O EFEITO DA INDOMETACINA EM RATOS *WISTAR*

THIAGO DE OLIVEIRA ASSIS

RECIFE
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Dissertação apresentada ao Programa de Pós-graduação em Patologia do Centro de Ciências da Saúde da Universidade Federal de Pernambuco, para obtenção do título de mestre em Patologia.

Orientadora: Profa. Dra. Maria Bernadete de Sousa Maia.
Co-orientadora: Profa. Dra. Silvia Regina Arruda de Moraes.

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RESUMO

Introdução: Estudos epidemiológicos sugerem que a má-nutrição materna prejudica o desenvolvimento fetal, bem como predispõe a prole a uma maior incidência de doenças. **Objetivo:** Investigar os impactos da má-nutrição precoce na severidade da artrite induzida por adjuvante de Freund e suas implicações sobre o efeito da indometacina em ratos *Wistar*. **Material e Métodos:** A partir do 1º dia de gestação, ratas *Wistar* foram divididas em dois grupos: 1) Normonutridos, que continuaram a receber dieta padrão de biotério e 2) mal nutridos, que passaram a receber dieta multicarencial (Dieta Básica Regional – DBR). A manipulação dietética foi realizada durante todo o período de gestação, associada ou não a lactação. Imediatamente após a gestação, ou lactação, todas as mães e suas respectivas proles passaram a receber a dieta padrão do biotério. No sexagésimo dia de vida, as proles (ratos machos) provenientes de mães normonutridas e más nutridas foram divididas em 06 grupos (n=05 animais/grupo): 1) Mal nutrido durante a vida intra-uterina (MIST); 2) Mal nutrido durante a gestação e tratado com indometacina (2 mg/Kg; v.o.) (MITI), 3) Normonutrido durante a vida intra-uterina (NIST), 4) Normonutrido durante a gestação e lactação tratado com indometacina (2 mg/Kg; v.o.) (NITI), 5) Mal nutrido durante a gestação e lactação (MILST); 6) Mal nutrido durante a gestação e lactação tratado com indometacina (2 mg/Kg; v.o.) (MILTI). Todos os animais dos grupos receberam injeção intraplantar de adjuvante completo de Freud (0,2 mL) na pata traseira direita. Os grupos MITI, NITI MILTI receberam indometacina (2 mg/Kg; v.o) durante 28 dias. No final do experimento amostras de sangue foram retirados para avaliação das variáveis hematológicas e bioquímicas. **Resultados:** A má-nutrição intra-uterina promoveu retardo do crescimento fetal, refletido no baixo peso ao nascimento (4,15 ± 0,15), bem como redução da prole (8,8 ± 0,83) quando comparados ao grupo normonutrido (6,23 ± 0,27 e 11,2 ± 0,83, respectivamente). A intensidade da resposta inflamatória, expressa pelo aumento do volume das patas, nos primeiros 14 dias após a indução da artrite experimental, foi em torno de 1,35 vezes menor no grupo MIST e cerca de 1,49 vezes menor no grupo MILSTI, ambas ($p < 0,05$) em relação à prole NIST. Entretanto, a partir do 21º dia não foi exibida nenhuma diferença estatisticamente significante entre os grupos MIST e NIST, enquanto que no grupo MILSTI foi verificado, ainda, redução da resposta inflamatória de cerca de 1,1 vezes menor que àquela observada no NIST. As concentrações séricas de albumina (3,54 ± 0,27 para o grupo MIST e 3,4 ± 0,23 no grupo MILSTI) e proteína C reativa (PCR) (11,04 ± 0,22 para o grupo MIST e 10,78 ± 0,32 no grupo MILSTI) foram estatisticamente diferentes daquelas verificadas no grupo normonutrido (4,3 ± 0,17 e 11,78 ± 0,35, respectivamente). A contagem de leucócitos (totais e diferenciados) foi semelhante nos grupos estudados. Observou-se que os efeitos da indometacina foram mais pronunciados no grupo NITI quando comparado com os demais grupos mal nutridos. **Conclusão:** A má-nutrição durante a vida intra-uterina ou durante a gestação e lactação promoveram redução da prole e baixo peso ao nascimento e redução das concentrações séricas de albumina e PCR. A dieta multicarencial empregada interferiu na intensidade da resposta inflamatória sistêmica e o efeito antiinflamatório da indometacina em ratos *Wistar*.

PALAVRAS-CHAVE: Má-nutrição precoce; Inflamação crônica; Indometacina.

ABSTRACT

Introduction: Epidemiological studies suggest that poor maternal nutrition, affect fetal development and predispose the offspring to a higher incidence of diseases. **Objective**: To investigate the impact of early malnutrition on the severity of arthritis induced by Freund's adjuvant and its implications on the effect of indomethacin in *Wistar* rats. **Materials and Methods**: From the 1 st day of pregnancy, *Wistar* rats were divided into two groups: 1) Nourished, which continued to receive Labina diet and 2) Undernourished, who also received diet multi deficiency (Regional Basic Diet - RBD). The dietary manipulation was performed during the entire period of gestation or without the period of lactation. Immediately after pregnancy or lactation all mothers and their offspring began to receive the Labina. The sixtieth day of life, the offspring (male rats) from nourished mothers and undernourished were divided into 06 groups (n = 05 animals / group): 1) Undernourished during intrauterine life (MIST), 2) Undernourished during pregnancy treated with indomethacin (2 mg / kg) (MITI), 3) Nourished during intrauterine life (NIST), 4) Nourished during pregnancy and lactation treated with indomethacin (2mg/Kg) (NITI), 5) Undernourished during pregnancy and lactation treated with indomethacin (2mg/Kg) (MILT). All groups received intraplantar injection of complete Freund´s adjuvant (0,2mL) in the right hind leg. The group MITI, NITI, MILT and given indomethacin (2 mg / kg)for 28 days. At the end of the experiment blood samples were taken for evaluation of hematologic and biochemical variables. **Results**: The malnutrition promoted intra-uterine fetal growth retardation, reflected in low birth weight (4.15 ± 0.15) and as reduction in the number of offsprings (8.8 ± 0.83) when compared with nourished group (6,23 ± 0,27 and 11.2 ± 0.83, respectively). The intensity of the inflammatory response, expressed by increase in the volume of the feet in first 14 days after induction of experimental arthritis, was about 1.35 times lower in MIST and groups 1.49 times lower in the MILSTI, group both in relation to NIST. However, after 21 days was not shown any statistically significant difference between the MIST and NIST, while the MILSTI it was verified, a reduction of the inflammatory response of about 1.1 times lower than that observed in NIST group. Serum albumin concentration (3.54 ± 0.27 for the MIST group and 3.4 ± 0.23 for the MILSTI) and C-reactive protein (CRP) (11.04 ± 0.22 for the MIST group and 10.78 ± 0.32 for the MILSTI) were statistically different from those found in the nourished group (4.3 ± 0.17 and 11.78 ± 0.35, respectively), while the leukocyte count (total and differentiated) was similar in both groups. The effects of indomethacin were more pronounced in the offspring than in other NITI malnourished groups verified by a larger percentage of inhibition of edema throughout the experimental course **Conclusion**: Malnutrition during intrauterine life or during pregnancy and lactation promotes: reduction of offspring and low birth weight, reduction of serum albumin and CRP, also influence the intensity of the inflammatory response and anti-inflammatory effects of the indomethacin in *Wistar* rats.

Keywords: Early malnutrition; Chronic inflammation, Indomethacin.

SUMÁRIO

1 APRESENTAÇÃO **11**

2 REVISÃO DA LITERATURA **14**

2.1 Artigo de revisão **14**

3 MATERIAL E MÉTODOS **29**

3.1 Animais **29**

3.2 Manipulação nutricional **29**

3.3 Delineamento experimental **30**

3.4 Análise estatística **32**

3.5 Considerações bioéticas **32**

4 RESULTADOS **34**

4.1 Artigo Original 1 **34**

5 CONSIDERAÇÕES FINAIS **62**

6 REFERÊNCIAS **64**

ANEXOS **71**

APRESENTAÇÃO

1 APRESENTAÇÃO

O crescente número de indivíduos com deficiência nutricional é um problema mundial emergente que implica em importantes conseqüências econômicas e de saúde pública. Nos últimos anos, estudos epidemiológicos sugerem que a má-nutrição materna prejudica o desenvolvimento fetal, bem como predispõe a prole a uma maior incidência de doenças tais como: doenças metabólicas e obesidade; diabetes tipo 2; doença cardíaca coronariana; hipertensão arterial; alterações no ciclo circadiano. Estas podem desenvolver-se ao longo de toda a vida pós-natal (BARKER et al., 1993; ERIKSSON et al., 2000; JONES, 2002; KENNAWAY, 2002).

O grau de comprometimento que a má-nutrição intra-uterina pode trazer para o feto depende da fase de desenvolvimento que este se encontra bem como o órgão afetado, sendo os efeitos tanto mais intensos e permanentes quanto mais precocemente ocorrer à má-nutrição e mais tarde for iniciada a recuperação nutricional (GURMINI et al., 2005).

No tocante ao processo inflamatório, estudos como o de Deitch (1990) realizados com animais experimentais na fase adulta têm demonstrado que a deficiência nutricional predispõe a um baixo grau de resposta inflamatória local e sistêmica, que podem prejudicar a resposta do hospedeiro aos estímulos inflamatórios, tornando-o assim mais suscetível a infecções e danos teciduais. O órgão afetado pelo processo inflamatório pode sofrer lesão funcional grave. A terapêutica antiinflamatória, nestes casos, apresenta-se como uma forma de controle da intensidade da resposta inflamatória. Nesse aspecto, os antiinflamatórios não esteróides (AINEs) se apresentam como os medicamentos mais amplamente prescritos no mundo, embora estejam associados a uma elevada taxa de complicação incluindo danos gastrintestinais (ROCHA et al., 2007). A Indometacina é um derivado

metilado do ácido indolacético pertencente à classe dos AINEs comumente utilizada para reduzir dor, rigidez articular e edema, circulando no organismo preferencialmente através de fracas ligações com proteínas plasmáticas, sobretudo a albumina, e eliminado principalmente pelas vias urinária (60%) e biliar (33%). Atua inibindo a enzima ciclo-oxigenase (COX) que converte o ácido araquidônico em prostaglandinas que são importantes mediadoras da inflamação como também estão relacionadas a homeostase do organismo.

Poucos estudos tem investigado uma relação entre a má-nutrição intrauterina e suas repercussões nas respostas inflamatórias local e sistêmica e suas relações com efeitos de antiinflamatórios não esteróides, sobretudo a utilização da indometacina. Nesse contexto, os objetivos desta pesquisa foram o de revisar na literatura os efeitos da má-nutrição intra-uterina na resposta inflamatória local e sistêmica em ratos *Wistar*; Estudar o impacto da má-nutrição intra-uterina na resposta inflamatória crônica e suas implicações sobre o efeito da indometacina em ratos *Wistar*. O presente estudo foi realizado no Laboratório de Farmacologia da Universidade Federal de Pernambuco, tendo como orientadora a Profa. Dra. Maria Bernadete de Sousa Maia e como co-orientadora a Profa. Dra. Sílvia Regina Arruda de Moraes. Este trabalho originou dois artigos científicos: O primeiro intitulado: **Effects of malnutrition intrauterine on the inflammatory response in *Wistar* rats adults: A systematic review,** e o segundo artigo: **Impact of early malnutrition in chronic inflammatory response and its implications on the effect of indomethacin on *Wistar* rats.** Ambos foram submetidos ao periódico Advanced Anatomic in Pathology.

REVISÃO DA LITERATURA

2 REVISÃO DA LITERATURA

2.1 ARTIGO DE REVISÃO

Title: Effects of malnutrition intrauterine on the inflammatory response in *Wistar* rats adults: A systematic review

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ABSTRACT

Purpose: To review the literature on the effects of fetal programming in the inflammatory response in *Wistar* rats. **Methods:** We performed a search in the following databases: PubMed, SciELO, MEDLINE, PUBMED, SCIENCE DIRECT, SCOPUS, LILACS, SpringerLink. The main search terms were malnutrition and inflammation in Portuguese and English. We included original articles involving albino rats. We excluded review articles as well as those involving humans and animals other than rats as well as articles that were related to malnutrition were not the intrauterine. Those items that were presented in more than a database operated, were counted only once. **Results:** There were 16 in PUBMED, 16 in SCOPUS, 4 in MEDLINE, 341 in SCIENCE DIRECT, 8 SciELO, 1 in LILACS and 77 in SPRINGERLINK, totaling 463 articles. Of these, after application of the criteria for inclusion and exclusion, 4 were selected for analysis. **Conclusion:** The malnutrition intrauterine seems to interfere with the inflammatory response in the adult offspring of *Wistar* rats, but its mechanisms remain uncertain.

Keywords: Fetal Programming; Inflammatory response; Malnutrition; Rats *Wistar*.

INTRODUCTION

The malnutrition is provoked by a quantitative and qualitative unbalance of nutrients in the organism. Those unbalances are sustained mainly by the lack of proteins, fats and carbohydrats that weaken the physiologic processes of the organism [1]. The protein-energy malnutrition is the most frequent reaching about 800 million people in the world [2].

Countless epidemic studies suggest that the intra-uterine atmosphere is extremely important in the determination of the future of the individual health [3]. The concept of "fetal programming" suggests that the fetus can be programmed during the intra-uterine development to develop diseases in the adult age [4]. In agreement with that theory, alterations in the maternal nutritional state, contemplated in the weight when being born, they determine the development of diseases in the adult phase [5,6]. The effects of the intra-uterine malnutrition depends on the development phase in that it is the fetus or the organ, being the effects so much more intense and permanent the more earlier to happen the malnutrition and later it is initiate the nutritional recovery [1,6]. Although in adult or smaller degree for the descendants, the intra-uterine malnutrition, even in phases different from the gestation, it can affect the product pregnancy harming the growth and the development of the several organs and apparel. Studies with animals have been trying to correlate the state nutritional in the beginning of the life and the susceptibility for diseases in the adult phase including diabetes mellitus, cardiovascular diseases, changes in renal function, arterial hypertension and alterations in the cycle circadian [7,8].

The process of inflammatory response is important to guarantee the integrity of the organism. It is the capacity of the organism in answering to noxious incentives

guaranteeing like this the balance between the systems and homeostasis. Though, the beneficial effects are influenced by factors as malnutrition, genotype, pré-existent inflammation and chronic intoxication [9]. The maternal malnutrition during the gestation and nursing can result in endocrine immune dysfunction in the axis hypothalamus-pituitary-adrenal altering the concentration permanently of hormones adrenocorticotropic and glucocorticoids that promote changes in the levels of leptina front the sharp and chronic inflammations contributing to affect the endocrine and immunological systems [10]. Studies have shown that the intrauterine malnutrition affects the vascular endothelium compromising the efficacy of macrophage endothelial transmigration [11]. The local inflammatory response was quite influenced by the malnutrition, there was a delay in the repairing tissue in mice, because the exsudate of the animals with regular nutrition presented fibroblasts with deposition of collagen and the undernourished ones no [9]. In that context, the objective of this study is it of revising in the literature the effects malnutrition intrauterine in the inflammatory response in rats *Wistar*.

METHODOLOGY

To conduct this study were asked the following databases: SciELO (Scientific Electronic Library Online), MEDLINE (U.S. National Library of Medicine), PUBMED (National Library of Medicine and The National Institute of Health); SCIENCE DIRECT, SCOPUS, LILACS (Latin American and Caribbean Health Sciences) and SPRINGERLINK. The search strategy involved the following databases with their respective search terms: In SciELO as "Malnutrition and inflammation”. MEDLINE as "malnutrition" [Subject descriptor] and "inflammation" [Subject descriptor] and "rats

Wistar" [Subject descriptor]. PUBMED as "early malnutrition and chronic inflammatory response" and "Early malnutrition and inflammatory response and rats" for a second search. In SCIENCE DIRECT as "intrauterine malnutrition and inflammation and rats". In SCOPUS as "early malnutrition and inflammation AND rats" and "fetal programming and inflammation and rats" for a second search. In LILACS as "malnutrition" [Subject descriptor] and "inflammation" [Subject descriptor]. In SPRINGERLINK as "malnutrition intrauterine and inflammation and rats".

Were included original articles involving albino rats. Were excluded review articles as well as those involving humans and animals other than rats as well as articles that were related to malnutrition were not the uterus and not involve the concept of fetal programming. Those items that were presented in more than a database explored, were counted only once.

At the base of SciELO, PubMed and LILACS were not established limits, while in MEDLINE, SCOPUS, Science Direct, SPRINGERLINK were explored and published articles in the following periods, 1966-2009, 2004-2009, 1996-2009, 1996-2009 respectively. Found 16 items in PUBMED, 16 in SCOPUS, 4 in MEDLINE, 341 in SCIENCE DIRECT, 8 in SciELO, 1 in LILACS and 77 in SpringerLink, totaling 463 articles. Of these, after application of the criteria for inclusion and exclusion, four were selected for analysis.

Selected articles were critically analyzed through an interpretation guide used to avaliate their individual merits, based on study [12] and adapted from [13]. Items for assessing quality of the articles are expressed in the form of scores in Table 1.

Table 1. Quality of studies on intrauterine malnutrition and inflammation

	Items of evaluation criteria*												
Studies	1	2	3	4	5	6	7	8	9	10	11	12	Total (%)
Maristella et al 2008[6]	2	2	2	2	2	1	2	1	2	1	1	1	79,1
Torrens et al 2009[14]	2	2	2	2	2	2	2	2	1	1	1	2	87,5
Maristella et al 2007[15]	2	2	2	2	2	1	2	2	0	1	1	2	79,1
Maristella et al 2005[16]	2	2	2	2	2	1	2	2	2	1	1	1	83,3

* Evaluation criteria: 1. Detailed bibliographic review of relevant funds. 2. Exclusion criteria. 3. Specific assumptions. 4. Appropriate scope of malnutrition in relation to inflammation. 5. Sample size. 6. Follow-up. 7. Authors referenced, performance methodology. 8. Standardization of measurement techniques. 9. Data presented for each hypothesis. 10. Adequate statistics. 11. Appropriate estimate of statistical error. 12. Valid conclusions and clinical recommendations.

RESULTS

Table 2 shows the search results after application of the criteria for inclusion and exclusion.

Table 2 – Studies evaluating the effects of intrauterine malnutrition on the inflammatory response in *Wistar* rats.

Study	Sample characteristics	Experimental model	Main results
Maristella et al 2008[6]	*Wistar* rats undernourished during pregnancy	Induction of inflammation in the lungs	Low birth weight of offspring malnourished (P <0.001). The intrauterine malnutrition reduces the pulmonary allergic inflammatory response.
Torrens et al 2009[14]	*Wistar* rats undernourished during pregnancy	Effects of inflammation in the endothelium vascular	The intrauterine malnutrition leads to endothelial dysfunction with the predisposition of inflammation..
Maritella et al 2007[15]	*Wistar* rats undernourished during pregnancy	Induction of the inflammatory response to factor necrosis tumor in the scrotum	Marrow hypocellularity, decreased the level of LTB4 and L-selectin, change the composition of the basement membrane with a reduction of collagen IV, reduction of leukocyte migration.
Maritella et al 2005[16]	*Wistar* rats undernourished during pregnancy	Induction paw edema by Zimozan®	Leukopenia, a reduction of ICAM-1, Selectin-P and L-Selective in rats undernourished during pregnancy.

DISCUSSION

A nutritional imbalance during the intrauterine development has predisposed to offspring disease in adult life. It has been a low birth weight of those offspring [17,18,19] when the maternal diet is restricted, the availability of nutrients for the transport of placenta is decreased, reducing the supply of nutrients and limiting the growth of the fetus [20]. Diseases such as hypertension, type 2 diabetes, and disorders in renal hemodynamics has been well studied [21].

Few studies have shown the effects of intrauterine malnutrition on the inflammatory response, especially in relation to inflammatory cells, however, has been reported that red bone marrow may suffer reduced efficacy of cell morphogenesis mainly white cell defense [15].

The inflammatory response is a defense mechanism the body's beneficial and non-specific reaction, it means that, any factor from the activation of factors such as an activated globulin (Hageman factor) as the actual tissue injury of any kind can evoke signals that trigger the inflammatory response. The degree effectiveness of inflammation requires a good margination of leukocytes into the vascular endothelium, good aggregation in the endothelium as well as an effective targeting of leukocytes to inflammatory foci [15,16].

The leukocyte migration into the inflammatory focus involves several steps that require a reduction in the rate of local blood flow, favoring the process of adhesion to endothelium [22]. The increased permeability and vascular dilation associated with an increase in platelet aggregation and hemoconcentration corroborate a slowdown of the circulation in the affected area. In such circumstances, the figurative elements that

would normally pass through the center of the bloodstream are distributed throughout the liquid column and the endothelium, facilitating leukocyte adhesion (Accession and surface) in the endothelium with subsequent migration to the interstitial region (Leukocyte extravasation) and direction to the inflammatory focus (chemotaxis). The paving process is related to the ionic charge of the endothelium and the presence of proteins (adhesins): Selectins, integrins and immunoglobulins that can be produced by leukocytes as well endothelial cells. The mobilization of polymorphonuclear cells to the inflammatory focus was deficient in malnourished individuals creating a deficit in the inflammatory process [23]. The malnutrition in early lactation in *Wistar* rats produces permanent changes programming secretion of insulin and glucocorticoids [10]. Other studies have shown that changes in the development of inflammatory response occurring in insulin-deficient state [24]. These amendments also limited the production of a good acute inflammatory response in adulthood. These responses appear to be due to deficiencies in relation leukocyte-endothelium, especially in the paving process, as the malnourished animals had a lower expression of immunoglobulin ICAM-1 (inter-cellular-adhesion-molecules). In studies of to intrauterine malnutrition [16], promoted in the adult offspring of rats reduced significant expressions of ICAM-1, P-Selectin, L-Selectin, leukopenia, which reduces leukocyte migration to the inflammatory focus malnutrition predisposes offspring to infections. ICAM-1 is an immunoglobulin that plays an important role in the process of leukocyte adhesion and surface and subsequent transendothelial migration during inflammation under the regulation of various chemical mediators and cytokines that modulate the intensity of its expression. This reduced expression may decrease the ability of leukocytes, particularly neutrophils, to adhere to the endothelium, reducing the effectiveness of the inflammatory response [25]. In studies of [26] the insulin reduced the expression of

ICAM-1 by increasing the expression of nitric oxide. Since L-selectin plays an important role in regulating the speed of rolling leukocytes, faced with a reduction in its expression can slow the migration process [27].

Intrauterine malnutrition significantly reduced the production of antigen specific immunoglobulin E (IgE), production and infiltration of inflammatory cells in the airways, mucus secretion, production of leukotriene B4 (LTB4), in addition to increased levels of corticosterone in the offspring adult [6]. Decreased production of LTB4 affects the functions of adhesion, decreasing inflammatory mechanism. It is well established that malnutrition affects the axis hypothalamic-pituitary-adrenal increasing activities culminating with adrenal hypertrophy followed by overproduction of corticosteroids, other studies with rats, these changes also affect the metabolic homeostasis compromised the innate defense mechanism [28]. High levels of glucocorticoids inhibit the action of several inflammatory mediators, reduce the synthesis of chemokines and cytokines, and disrupt the activation of leukocytes, attenuating the inflammatory process [10].

Other substances have been studied as mediators of inflammatory response, the leptin is a protein produced mainly by adipose tissue with pleiotropic functions, regulating metabolism, endocrine and immune. Its receptors are homologous to GP-130, factor signal transduction subunit of the interleukin 6. This similarity has led to studies suggesting that it could be classified as a cytokine. Leptin levels decrease during acute inflammation may be a component of defense in the host organism, on the other hand, a deficiency of leptin has increased the susceptibility of organisms to infection and inflammation. It is known that leptin deficiency can disrupt the process of hematopoiesis in addition to lymphocyte functions and may interfere in the chronic inflammatory response [29].

CONCLUSION

The malnutrition intrauterine seems to interfere with the inflammatory response in the adult offspring of *Wistar* rats, but its mechanisms remain uncertain. This suggests that further studies aimed at understanding the factors that trigger this process.

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MATERIAL E MÉTODOS

3 MATERIAL E MÉTODOS

3.1 Animais

Foram utilizadas ratas albinas primíparas da linhagem *Wistar* (250-300g) e suas descendências, compostas por 30 ratos machos, provenientes da colônia de criação do Departamento de Nutrição da Universidade Federal de Pernambuco. Os animais foram acasalados na proporção de três fêmeas para um macho em gaiolas de polipropileno com dimensão de 430x430x200 mm num ambiente com temperatura de 23 ± 1°C com livre acesso à água filtrada. O estado de prenhez foi determinado através da observação da presença de espermatozóides na secreção vaginal.

3.2 Manipulação nutricional

A partir do 1º dia de gestação, as ratas foram divididas em dois grupos: 1) Normonutridas, alimentados *ad libitum* com a dieta padrão de biotério (Labina ® Purina do Brasil), composta de 23% de proteína, 74,4% de carboidratos e 2,5% de gorduras e 2) Mal nutridas, alimentadas *ad libitum* com a dieta multicarencial (Dieta Básica Regional – DBR) com baixo teor de proteínas (10,4%), gorduras (1,86%) e carbohidratos (82,3%) e deficiente em vitaminas e sais minerais [11].

Segundo a manipulação nutricional a prole foi dividida em três grupos, cada um com 10 animais: 1) Normonutridos durante a vida intra-uterina e sem tratamento (NIST), constituído pela prole das nutrizes que receberam a dieta padrão de biotério durante a gestação e 2) Mal nutridos durante a vida intra-uterina e sem tratamento (DIST), composto por filhotes provenientes das nutrizes que receberam a DBR durante a gestação e 3) Mal nutridos durante a vida intra-uterina e lactação e sem tratamento (DILST), composto por filhotes provenientes das nutrizes que receberam a DBR durante a gestação e lactação. Os pesos das proles foram mensurados

utilizando-se uma balança (ACCULAB® VI-400) previamente calibrada, ao nascimento e nos 21° (Desmame), 60° e 88° (fim dos experimentos) dias de vida.

Após o parto, as ratas dos grupos NIST e DIST receberam dieta padrão de biotério para a recuperação nutricional, enquanto que as ratas do grupo DILST continuaram sendo alimentadas pela DBR. No 21° dia de vida, as proles NIST, DIST e DILST foram desmamadas e a prole DILST passou a ser alimentada pela dieta padrão de biotério para sua recuperação nutricional. No sexagésimo dia de vida os animais foram então submetidos aos diferentes ensaios biológicos.

3.3 Delineamento experimental

Para avaliação do impacto da má-nutrição intra-uterina na intensidade da resposta inflamatória crônica e suas implicações sobre o efeito antiinflamatório da indometacina, os animais foram divididos em seis grupos (n=5 animais/grupo) e dois experimentos foram realizados:

Experimento A - Para avaliação do impacto da má-nutrição intra-uterina na intensidade da resposta inflamatória crônica, os animais NIST, bem como da prole DIST e DILST receberam na região subplantar da pata traseira direita uma injeção de 0.2 mL de Adjuvante Completo de Freund (ACF) (SIGMA, USA) para indução da artrite experimental [12].

Experimento B - Outros grupos (n=5/grupo) da prole normonutrida, mal nutrida durante a gestação e mal nutrida durante a gestação e lactação, foram submetidos ao mesmo procedimento acima descrito, e subdivisões em grupos que foram tratados diariamente com indometacina (PRODOMI; 2 mg/kg; v.o), originando os grupos NITI (Normonutridos tratados com indometacina), DITI (Mal nutridos durante a vida intra-uterina tratados com indometacina) e DILTI (Mal nutrido na vida intra-uterina e

lactação tratados com indometacina). O tratamento foi realizado com uma dose diária, durante 28 dias consecutivos. Os volumes das patas de todos os grupos foram mensurados pletismograficamente (pletismômetro Ugo Basile, Italy) imediatamente antes da injeção do ACF e no 7°, 14°, 21° e 28° dias subseqüentes a administração do ACF.

O percentual de aumento no volume das patas (para avaliar a intensidade da resposta inflamatória) nos grupos DIST e DILST comparando-se com os grupos não tratados, enquanto que o efeito antiinflamatório da indometacina foi determinado através da comparação dos volumes das patas entre os grupos NITI e NIST; DITI e DIST e DILTI e DILST de acordo com a fórmula abaixo:

Percentagem de inibição (%I) = 1- Vt/Vc X 100, onde Vt e Vc correspondem à média das diferenças resultantes das medidas das patas nos grupos tratados com indometacina e controle (não tratados), respectivamente.

Determinação de parâmetros bioquímicos e hematológicos

No final dos experimentos, foram obtidas amostra de sangue de todos os animais através do plexo orbital para determinação das variáveis bioquímicas (albumina sérica e proteína C reativa (PCR); e hematológicas: leucócito total e contagem diferencial. A contagem do número total de leucócitos foi realizado em analisador de células hematológicas Coulter TKS®. Esfregaços sanguíneos, corados pelo método de May-Grunwald-Giemsa, foram usados para as contagens diferenciais dos leucócitos (segmentados, eosinófilos, linfócitos e monócitos). As análises foram realizadas no Laboratório Central do Hospital das Clínicas da Universidade Federal de Pernambuco. Em seguida todos os animais foram eutanasiados em câmara de CO2.

3.4 Análise estatística

Os dados foram armazenados em bancos de dados nos softwares Excel (*Microsoft Office*® 2007) e Bioestat® (v. 5.0). A normalidade dos dados foi verificada através do teste de Shapiro-wilk, as variáveis quantitativas foram analisadas segundo a estatística descritiva para determinação das médias e respectivos desvios-padrões. Quando necessário, foi empregado o teste t de Student para amostras independentes para comparação entre as médias dos grupos controle e experimental estabelecendo $p<0,05$ para significância dos dados.

3.5 Considerações bioéticas

Os procedimentos adotados no manejo dos animais seguiram as recomendações do Colégio Brasileiro de Experimentação Animal – COBEA (BRASIL, 1979).

O projeto foi enviado e aprovado junto ao Comitê de Ética em Experimentação Animal da Universidade Federal de Pernambuco (UFPE) (Processo No: 230760070027/2008-19).

RESULTADOS

4 RESULTADOS

4.1 ARTIGO ORIGINAL

Impact of early malnourishment on the chronic inflammatory response and its implications in the effect of indomethacin in Wistar rats

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ABSTRACT

Introduction: Epidemiological studies suggest that maternal malnourishment impairs fetal development, also predisposing the adult offspring to a higher disease incidence. **Objective:** to investigate the impacts of early malnourishment on the severity of arthritis induced by Freund's Adjuvant and its implications in the effect of indomethacin in Wistar rats. **Methods:** From the 1st day of pregnancy, female Wistar rats were divided into two groups: 1) Nourished, which continued to receive the laboratory standard diet and 2) malnourished, that started to receive a devoid diet (Regional Basic Diet – RBD). The diet manipulation was done during the whole gestational period associated or not to the lactation period. Immediately after the gestation or the lactation all the mothers and their respective offsprings begun to receive the laboratory standard diet. On the sixtieth day of life the litters male rats from both nourished and malnourished mothers were divided into 06 groups (n=05 animals per group): 1) Malnourished during intra-uterine life (MIST); 2) Malnourished during pregnancy and treated with indomethacin (2 mg/Kg; orally) (MITI); 3) Nourished during intra-uterine life (NIST); 4) Nourished during gestation and lactation and treated with indomethacin (2 mg/Kg; orally) (NITI). 5) Malnourished during gestation and lactation (MILST); 6) Malnourished during gestation and lactation and treated with indomethacin (2 mg/Kg; orally) (MILTI). All the groups received intraplantar injection of complete Freund's adjuvant (0,2 mL) on the right hind paw during 28 days. By the end of the experiment blood samples were extracted for evaluation of hematologic and biochemical variables. **Results:** Intra-uterine malnourishment retarded the fetal growth, seen by the low birth weight (4,15 ± 0,15), as well as by the litter reduction (8,8 ± 0,83) when compared to the nourished group (6,23 ± 0,27 e 11,2 ± 0,83, respectively). The intensity of inflammatory response, expressed as a percentage of paw volume increase, during the first 14 days after the experimental arthritis induction, was around 1,35 times lower on the MIST group and about 1,49 times lower on the MILSTI group, both in comparison to the NIST group. From the 21st day, however, no significant statistical difference was observed between the MIST and NIST litters, whereas a reduction in the inflammatory response around 1,1 times lower than observed on the NIST group was yet seen on the MILSTI group. The serum albumin (3,54 ± 0,27 to the MIST e 3,4 ± 0,23 MILSTI groups) and C-reactive protein (PCR) (11,04 ± 0,22 to the MIST e 10,78 ± 0,32 to the MILSTI groups) concentrations were statistically different from the ones verified on the nourished (4,3 ± 0,17 e 11,78 ± 0,35, respectively) group, while the leukocyte (total and undifferentiated) count was similar on the studied groups. The effects of indomethacin were more pronounced in the offspring than in other NITI malnourished offspring verified by a larger percentage of inhibition of edema throughout the experimental course. **Conclusão:** Malnourishment during intra-uterine life or during both the gestation and the lactation causes: reduction in litter size and low birth weight; decrease in the proteins of the acute phase of inflammation (albumin and PCR), influences in the intensity of systemic inflammatory response and the anti-inflammatory power of indomethacin in Wistar rats.

Keywords: Early malnourishment; Chronic inflammation; indomethacin.

INTRODUCTION

The growing number of individuals with nutritional deficiency is a problem emerging worldwide that implies important economical and public health consequences [1]. On the last years epidemiological studies suggest that maternal malnourishment impairs fetal development, also predisposing the litter to a higher disease incidence during adult life – being the compromising degree as intense and permanent as precocious is the malnourishment and as late begins the nutritional recovery [1,2,3].

About the inflammatory process, researches done with animals during adult phase have shown that the nutritional deficiency predisposes to a low degree of local [2,3] and systemic [4] inflammatory responses, which can weaken the host's response to stimuli, making it more susceptible to infections and tissue damage [5]. Both the humoral and cellular immunities find themselves compromised in the presence of protein-energetic malnutrition, consequently contributing to inflammatory response abnormalities [6].

The systemic inflammatory response represents an important group of coordinated physiological actions that serves to combat infections and other kinds of injuries to which the organism is exposed. The systemic inflammatory response, together with its chemical mediators, performs an essential role on the physiopathology of various chronic diseases, amongst them being the rheumatoid arthritis [7]. Several studies have reproduced with success chronic arthritis in animals through the arthritis model induced by Freud's complete adjuvant, which contains a fragment of a Mycobacterium that causes in the host an inflammatory response similar to rheumatoid arthritis [8]. On the majority of circumstances, an intact inflammatory response increases the probability of success after acute lesion or infection. On a

healthy individual, the inflammatory response to tissue lesion or infection is fast and efficient, with specific resolution occurring even before the immune system involvement. However, when the inflammatory stimulus is intense or the individual is malnourished or has a chronic disease, a series of inter-related events occur, among them the interaction of immune cells, phagocytes, and the release of cytokines that affect the usage of nutritional substances in the body [6].

The anti-inflammatory therapy, in these cases, is essential. In this aspect, the non-steroid anti-inflammatories (AINE's) are the most prescribed medicaments in the world [9]. Indomethacin is a methyl-derived indoleacetic acid pertaining to the AINE's class, commonly used to reduce pain, joint stiffness and edema. Its pharmacokinetic characteristics point out Pka variation from 4,1 to 4,9 with preferential absorption in the gastrointestinal tract, especially in sites with low pH, flowing by the organism mainly through weak plasmatic protein bonds, mainly albumin, being eliminated via urine (60%) and bile (33%). It inhibits the cyclooxigenase (COX) enzyme, which converts the arachidonic acid into prostaglandins, which are important inflammation mediators – also being related to the organism homeostasis [10].

It is evident that the understanding of inflammatory response in individuals with intra-uterine nutritional deficiencies – considering the numerous factors involved in the inflammatory responses like Interleukins (IL), Tumoral Necrosis Factor (TNF), albumin, C-reactive protein (PCR), leukocytes and others – may favor the development of new strategies to predict the susceptibility to diseases, accompany therapies and even develop new approaches to the prevention and treatment of diseases in which the inflammatory responses have physiopathologic implications (rheumatoid arthritis, arteriosclerosis, intestinal inflammatory diseases…).

The objective of this study is, therefore, to evaluate the impacts of early malnutrition on the chronic inflammatory response and its implications on the anti-inflammatory effect of indomethacin on the adult offspring of Wistar rats.

MATERIAL AND METHODS

Experimental animals

Primiparous albino Wistar rats (250-300g) and their litters, consisting of 30 male rats, from the breeding colony of the Departamento de Nutrição da Universidade Federal de Pernambuco were used. The animals mated in the proportion of three female to one male in polypropylene cages with dimensions of 430x430x200 mm in an environment with temperature of 23 ± 1°C and free access to filtered water. The pregnancy state was verified by the observation of the presence of spermatozoids in the vaginal secretion.

Nutritional manipulation

From the 1st day of gestation the female rats were divided into two groups: 1) Nourished, fed ad libitum with the laboratory standard diet (Labina Purina do Brasil), composed of 23% protein, 74,4% carbohydrates and 2,5% fat and 2) Malnourished, fed ad libitum with the devoid diet (Regional Basic Diet – RBD) with low protein (10,4%), fat (1,86%) and carbohydrate (82,3%) levels and devoid of vitamins and mineral salts [11].

The litters were divided into three groups according to nutritional manipulation, each having 10 animals: 1) Nourished during intra-uterine life and without treatment (NIST), consisting of the offspring of the mothers that received the standard laboratory diet during gestation and 2) Malnourished during intra-uterine life and without treatment (MIST), consisting of cubs from the mothers that received the RBD during gestation and 3) Malnourished during intra-uterine life and lactation and without treatment (MILST), consisting of cubs from the mothers that received the RBD during

gestation and lactation. The weights of the litters were measured by using a previously-calibrated scale (ACCULAB® VI-400) on birth, weaning, 60th and 80th days of life.

After the delivery, the female rats of the NIST and MIST groups received the laboratory standard diet for nutritional recovery, whereas the female rats of the MILST group continued to be fed with the RBD. On the 21st day of life, the litters from the NIST, MIST and MILST groups were weaned and the MILST litter was fed with the laboratory standard diet, from this day on, for nutritional recovery. On the sixtieth day of life the animals underwent the different biological studies.

Experimental protocol

To evaluate the impact of intra-uterine malnutrition on the intensity of chronic inflammatory response and its implications on the anti-inflammatory effect of indomethacin, the animals were divided into six groups (n=5 animals/group) and two experiments were done:

Experiment A – to evaluate the impact of intra-uterine malnutrition on the intensity of chronic inflammatory response, the NIST animals, as well as the MIST and MILST litters received on the subplantar region of the right hind paw an injection (0.2 mL) of Freud's complete adjuvant (ACF; SIGMA, USA) to induce the experimental arthritis [12]..

Experiment B – Other groups (n=5/group) – the nourished, malnourished during gestation and malnourished during gestation and lactation litters underwent the same procedure previously described being, moreover, treated daily with indomethacin (PRODOMI; 2 mg/kg; orally), creating the NITI (Nourished, treated with indomethacin), MITI (Malnourished during intra-uterine life, treated with indomethacin) and MILTI

(Malnourished during intra-uterine life and lactation, treated with indomethacin) groups, being the treatment done in one dose per day, during 28 consecutive days to evaluate the implications of malnutrition on the anti-inflammatory effect of indomethacin. The volume of the paws of all groups was measured through plethysmography (plethysmograph Ugo Basile, Italy) immediately before the injection of ACF and on the 7th, 14th, 21st, and 28th days subsequent to the administration of ACF.

The paws volume increase percentage (inflammatory response intensity) on the MIST and MILST groups was compared to that of the NIST group, whereas the anti-inflammatory effect of indomethacin was determined through the comparison of the paws volumes between the NITI and NIST groups; the MITI and MIST groups and the MILTI and MILST groups, according to the following formula:

Inhibition percentage (%I) = 1- Vt/Vc X 100, where Vt and Vc correspond to the mean values of the resultant measurement differences of the paws in the groups treated with indomethacin and control (non-treated), respectively.

Determination of biochemical and hematologic parameters

By the end of the experiments, blood samples from every animal were obtained through the orbital plexus for the determination of the biochemical (serum albumin, C-reactive protein) and hematologic (total and differential leukocytes) variables. The total leukocyte number was counted by the Coulter TKS® hematologic cells analyzer. Blood smears, stained by the May-Grunwald-Giemsa method, were used for the leukocytes differential counting (segmented, eosinophil, lymphocyte and monocyte). The analyses were done in the Laboratório Central of the Hospital das Clínicas da Universidade Federal de Pernambuco. After that all the animals were euthanized in CO2 chamber.

Statistical analysis

The data were stored in data banks in the Excel (Microsoft Office® 2007) e Bioestat® (v. 5.0) softwares. The normality of the data was verified through the Shapiro-wilk test. The quantitative variables were analyzed according to descriptive statistics in order to determine the means and respective standard deviations. When necessary, the Student's t test was used for independent samples in order to do the comparison between the mean values of the control and experimental groups, establishing $p<0,05$ for data significance.

Bioethical considerations

The procedures for animal management followed the recommendations from the Colégio Brasileiro de Experimentação Animal – COBEA (BRASIL, 1979).

The project was sent to and approved by the Comitê de Ética em Experimentação Animal da Universidade Federal de Pernambuco (UFPE) (Process No: 230760070027/2008-19).

RESULTS

Characteristics of the malnourished and nourished litters

Maternal malnutrition caused fetal growth retardation, verified by the low body weight on birth, as well as a numerical reduction in the malnourished litters when compared to the nourished one. On weaning, statistically significant differences were verified on the MIST group weight in relation to the NIST group ($p< 0,05$) and on the MILST group, when compared to the NIST group ($p<0,05$), being the latter difference is still observed on the 60th day of life, fact that is not verified on the MIST and NIST groups comparison (Table 1).

Table 1. Parameters (Mean (X) ± standard deviation (SD) of the malnourished *Wistar* rats litters when compared to the nourished litter.

GROUPS	NIST		MIST		MILSTI	
	n	X ± SD	n	X ± SD	n	X ± SD
Number of cubs per litter	-	11,2 ± 0,83	-	8,8 ± 0,83*	-	8,8 ± 0,83*
Litter weight (g)						
At birth	10	6,23 ± 0,27	10	4,15 ± 0,15*	10	4,5 ± 0,15*
On weaning	10	50,43 ± 1,48	10	43,42 ± 3,29	10	25,5 ± 7,7*
On the 60th day	10	240 ± 36,9	10	238,1 ± 21,06	10	186,8 ± 24*
End of the experiments	10	293,4 ± 38	10	282,2 ± 30,9	10	263 ± 40,5

*The Student's t test was applied: *$p< 0,05$, when compared to the NIST control group.

Determination of biochemical and hematological parameters (Total and differential)

The concentrations of albumin and PCR were significantly reduced on the MIST (3,54 ± 0,27 g/dL e 11,04 ± 0,22 mg/dL, respectively) and MILST (3,4 ± 0,23 g/dL e

10,78 ± 0,32 mg/dL, respectively) groups in comparison to the NIST (4,3 ± 0,17 g/dL e 11,78 ± 0,35 mg/dL, respectively) group. About the number of total and differential leukocytes, however, no statistical differences were found between the malnourished and nourished groups (Table 2).

Table 2. Biochemical and hematological parameters on the adult *Wistar* rats MIST and MILST groups offspring when compared to the NIST group.

	NIST (n=5)	**MIST** (n=5)	**MILST** (n=5)
Albumin (g/dL)	4,3 ± 0,17	3,54 ± 0,27*	3,4 ± 0,23*
PCR (mg/dL)	11,78 ± 0,35	11,04 ± 0,22*	10,78 ± 0,32*
Leukocytes (/mm³)			
Total	16740 ± 3566,9	17625 ± 377,4	17360 ± 901,6
Differential			
Neutrophil	3051,4 ± 1274,8	2258 ± 1013,8	1929 ± 1103
Eosinophil	251 ± 178,6	141 ± 39,4	128 ± 33,8
Basophil	870,4 ± 1592,9	872 ± 628,6	821 ± 603,9
Lymphocyte	12520,8 ± 3099	14262,7 ± 1333,4	14284 ± 952,8
Monocyte	44,2 ± 60,6	88,2 ± 90	97,3 ± 82,9

* $p < 0,05$ when compared to the control (NIST) group by the Student's t test.

Influence of early malnourishment on the inflammatory response intensity

As can be seen on table 3, the inflammatory response intensity, expressed by the percentage of increase in the paws volume, was about 1,35 times lower on the MIST litter and 1,49 times lower on the MILST litter, when compared to the NIST litter. From the 21st day after the induction of experimental arthritis, no difference was verified between the inflammatory response shown by the MIST animals, whereas the

MILSTI group still exhibited an inflammatory response about 1,1 times lower in comparison to the NIST group.

Table 3. *Wistar* rats mean paw volume on the NIST, MIST and MILST groups that had arthritis induced by Freund's complete adjuvant.

	7th Day	14th Day	21st Day	28th Day
Animals				
NIST	4,10 ± 0,12 (315%)	4,51 ± 0,36 (416%)	3,39 ± 0,12 (261%)	3,60 ± 0,20 (277%)
MIST	2,91 ± 0,57* (233%)	3,16 ± 0,54* (253%)	3,30 ± 0,35 (264%)	3,39 ± 0,46 (271%)
MILST	2,68 ± 0,17* (198%)	2,88 ± 0,16* (213%)	2,97 ± 0,20* (220%)	3,01 ± 0,17* (222%)

(%) percentage of increase in paws volumes (Intensity of inflammatory response). *p <0.05 by the Student's t test when the MILSTI and MIST groups are compared to the control group NIST.

Influence of malnourishment during gestation and lactation on the indomethacin anti-inflammatory effects

The indomethacin anti-inflammatory effects were studied in terms of edema inhibition percentage. Table 4 shows the difference of paws volume in relation to the initial volume (before the induction of experimental arthritis) in different time intervals. Statistically significant differences were verified during the 28 days of treatment in the NITI group (p<0,05). On the MITI group, however, statistically significant differences were observed from the 14th day of treatment while on the MILTI group this difference was observed from the 21st day of treatment on (Table 4).

Table 4. Effect of indomethacin (2mg/kg; orally) on the arthritis induced experimentally by ACF in *Wistar* malnourished and nourished rats.

	Paws volume				
	Day 0	7th Day	14th Day	21st Day	28th Day
Groups					
NIST	1,30 ± 0,15	2,80 ± 0,19	3,20 ± 0,40	2,10 ± 0,25	2,30 ± 0,25
NITI	1,28 ± 0,17	2,28 ± 0,28*	2,16 ± 0,19*	1,46 ± 0,15*	1,64 ± 0,21*
(%I)	-	18,57	32,50	30,47	28,69
MIST	1,25 ± 0,15	1,66 ± 0,48	1,91 ± 0,44	2,05 ± 0,25	2,10 ± 0,39
MITI	1,28 ± 0,05	1,51 ± 0,12	1,35 ± 0,19*	1,56 ± 0,19*	1,59 ± 0,32*
(%I)	-	9	29,31	23,90	24,28
MILSTI	1,35 ± 0,1	1,33 ± 0,18	1,53 ± 0,18	1,64 ± 0,23	1,66 ± 0,19
MILTI	1,34 ± 0,05	1,19 ± 0,23	1,33 ± 0,24	1,30 ± 0,13*	1,27 ± 0,19*
(%I)	-	10	13	20,7	23

(%I) Edema inhibition percentage.
Statistically significant differences for the Student's t test between the NITI, MITI and MILTI groups treated with indomethacin (2 mg / kg orally) in comparison to their respective non-treated controls NIST, MIST and MILSTI: *p<0,05.

DISCUSSION

It is well-established in literature that a serious maternal protein restriction predisposes the offspring to diseases on its adult life, including renal [13] and cardiac [14] diseases, arterial hypertension [15], diabetes [16], hypothalamic-pituitary-adrenal axis function alterations [17] and leukocyte reduction for the inflammatory site [18]. In our study, we tried to understand the differences between the malnourished (during gestation and lactation) and nourished litters about an experimental model of chronic arthritis induced by the Freud's complete adjuvant, as well as the implications of the anti-inflammatory aspects of the indomethacin. Our early malnourishment protocol resulted in a low birth weight as described in previous studies [1, 13, 17, 19], as well as significant litter size reduction [1, 17]. It is known that mothers' nutritional state is a determinant factor for the nutritional reserves offered to the litter and for the production and maintenance of lactation [20]; the lack of protein reduces the amino acids mobilization to the litter during the gestation, limiting its organs and body growth [21], which justifies the low birth weight. Our data reveal that the litter that was malnourished during gestation and lactation showed an even stronger limitation to its growth, reflected on the low weight at birth and after weaning. For Pine and Jessop [20], diets with low protein level given to the mothers during the gestation and lactation periods jeopardize the volume and composition of the milk in female rats, reflecting negatively on the cub's growth process.

We also observed that a deficient nutritional state during intra-uterine life did not result, on the adult life, in statistically significant differences about the number of systemic total and differential leukocytes. Differently from other studies [22], in which a significant reduction in the number of these cells, supposedly to the detriment of a reduction in adhesion proteins like selectins – and, above all, the L-selectin, which is

believed, besides other functions, to participate in the release of progenitor cells from the hematopoietic areas to the blood.

Albumin is the most abundant protein in the blood plasma, which transports several substances like a great number of drugs [9,22,23]. The nutritional state has been associated to the hypo-albuminemia, more precisely to a protein-calorie malnourishment [24]. The measurement of serum albumin levels has been frequently used as a malnourishment index [25,26]. Our data reveal that the albumin levels were statistically reduced on the MIST and MILSTI groups in comparison to the NIST group by the end of the experiments ($p<0,05$). This fact, however did not represent a malnourishment index because, on the 88th day of life, when blood samples were collected in order to determine the serum albumin levels, the MIST offspring had already recovered weight, verified through the comparison with the NIST control group.

It is known that the serum albumin is also greatly influenced by other factors apart from malnourishment. The presence of inflammatory cytokines inhibits its synthesis [25]. In our experimental model, besides the malnourishment protocol, we induced chronic arthritis by using Freud's complete adjuvant – a disease model mediated by T cells and caused by the fragment of Mycobacterium, which is very similar to the rheumatoid arthritis [10], resulting in signs and symptoms characteristics of the disease, including the histopathologic alterations, leukocyte migration, hypersensibility and local edema [27,28]. The chronic inflammation induced by the adjuvant associated with the malnourishment increase the risk of diseases, as well as the cytokine levels, resulting in albumin synthesis inhibition [29].

The PCR is an acute [30] and chronic inflammatory response marker that participates in the systemic inflammatory response with inflammatory and anti-

inflammatory pleiotropic functions. Depending on the circumstance, it may strengthen or weaken the inflammatory response [31]. By the end of our experiments we verified that the PCR levels were significantly influenced by the intra-uterine malnourishment, being lower on the MIST ($p< 0,05$) and MILSTI ($p< 0,05$) groups in relation to the NIST group, contributing to a decrease in the inflammatory response identified in the malnourished litters. However, our data do not allow us to state the mechanism to explain such phenomenon, since it is known that PCR is synthesized from the stimulation of IL-6 is secreted during the inflammatory events mainly by monocytes and macrophages, whose differential counted, this study does not show significant differences. Perhaps a dose systemic IL-6 would help to clarify this mechanism.

It is well-established in literature that inflammatory response is an unspecific reaction defense mechanism of the body which may be activated by any factors that predict tissue damage, occurring in three MISTinct phases: (1) transitory acute phase, characterized by local vessel dilatation and capillary permeability increase, which results in the formation of a local inflammatory exsudate; (2) late sub-acute phase, characterized by the infiltration of white and phagocytary cells and (3) chronic phase, in which fibrosis and tissue degeneration occurs. In the present study, the inflammatory response intensity was significantly impaired on the malnourished groups MIST ($p< 0,05$) and MILSTI ($p< 0,05$) when compared to the nourished group NIST during the first 14 days after the arthritis induction by the adjuvant. In the studies of Barja-Fidalgo [32], animals that underwent malnutrition showed lower inflammatory response intensity when induced by carrageenin, supposedly as a consequence of reduced leukocyte adhesion to the vascular endothelium with consequent perivascular migration and lower edema formation. Landgraf et al. [22,33] reported that a litter that undergoes intra-uterine malnourishment has, during adult life, important reduction in

the lipoxygenase pathway of the arachidonic acid metabolism which results mainly in significant decrease in leukotriene B4 production. The capability of developing the initial stages of inflammatory response is reduced this way, compromising effective vessel permeability with consequent reduction in the edema formation, as well as reducing the leukocyte chemotaxis and impairing the sub-acute stage of inflammation.

It is also known that the effectiveness degree of the inflammatory response requires an effective leukocyte margination, effective endothelium aggregation, endothelial transmigration, as well as effective leukocyte aiming to the inflammatory site [33]. The leukocyte migration depends on the circulating leukocytes availability, and our data reveal that there were no statistically significant differences in the number of total nor differential leukocytes, thus the number of leukocytes was not affected by the intra-uterine-only or intra-uterine and lactation malnourishment. Experimental models of chronic arthritis induced by Freud's adjuvant [34] have shown facilitation in this migration due to the induction of the production of adhesion molecules like Vascular Cell Adhesion Molecule-1 (V-CAM-1) and higher circulating leukocytes availability as a reaction to the presence of this substance. Intra-uterine malnourishment caused reduction in leukotriene B4 production causing, as well, functional deficits in both adhesion and chemotaxis [22]. Alterations of the basal membrane with type-IV collagen were identified in the malnourished litter during the gestation in the Mahomoodian studies [35], which interfered in the adhesion and endothelial transmigration processes. Activation, circulation and mononuclear cell migration to inflammatory sites are regulated by adhesion molecules like Inter-Cellular-Adhesion-Molecules (ICAM-1), VCAM-1, E-selectin [23,26] and L-selectin [37]. In the Landgraf et al. [37] works, intra-uterine malnourishment caused, on the adult rats

offspring, significant reduction in the ICAM, P-selectin, L-selectin expressions, as well as leucopenia, decreasing the leukocyte migration to the inflammatory site.

About the indomethacin anti-inflammatory effects, our data revealed that the MITI and MILTI groups, when compared respectively to their MIST and MILSTI controls, showed a lower capability of inhibiting the edema in relation to the NITI animals, compared to their NIST control group during the whole experimental course. Statistically significant differences on the MITI litter in relation to its control group MIST was only verified from the 14th treatment day on, whereas the MILTI, in comparison to its control group MILSTI, statistically significant differences were only verified from the 21st treatment day. Our data shows that the malnutrition factor seems to interfere in the anti-inflammatory effects of indomethacin, revealing yet that the longer it takes to begin the nutritional recovery, the lower is the individual's capacity of responding with efficiency to the indomethacin pharmacological therapy when facing a chronic inflammatory process during adult life. For Lavy et al. [38] the efficiency of edema (induced by Freud's adjuvant) reduction in rats is associated with significant reduction in the total leukocyte number caused by the indomethacin action. This drug binds itself mainly to albumin through weak bonds that work as a kind of storage from which it is gradually released. Only the indomethacin that is free in the blood plasma is capable of going through the endothelial membranes of the circulatory system, diffusing to the tissues [38,39]. In the present study, we have shown that albumin was significantly reduced on the malnourished litters – what does not explain the lower expression of indomethacin anti-inflammatory activities in this group. Other pharmacokinetic factors like the absorption of this drug by the gastrointestinal tract could be possible factors to justify this phenomenon. The indomethacin behaves like an weak acid (Pka = 4,1 to 4,9), being better absorbed in low pH sites [39] like the stomach. For Weaver [40],

however, isolated pre-natal protein malnourishment does not cause long-lasting negative effects on the growth and gastrointestinal tract function, being these repaired before adult age. In relation to the intestinal tract, studies have shown that the number and height of intestinal villi as well as the number of enterocytes are significantly reduced in the malnourished-during-gestation-rats' offspring on the first 15 days of life, not being extended to adult life, except for the number of enterocytes, which remain reduced. These alterations make possible intestinal absorption deficits, which is dependent on the number of enterocytes and their absorption capability [1]. Several pharmacodynamic factors could be involved in the mechanism of reducing the effects of indomethacin in early malnourished offspring, however, our data do not support any such considerations. Posterior studies may help further clarify how early malnourishment influences AINE's pharmacokinetic and pharmacodynamic, mainly the indomethacin, and how that interferes in its anti-inflammatory effects.

CONCLUSIONS

Our results let us conclude that:

- Early malnourishment reduces the systemic inflammatory response in Wistar rats done by the experimental model of arthritis induced by Freud's complete adjuvant;

- Serum concentrations of albumin and PCR are reduced in the adult animals that underwent intra-uterine malnourishment and lactation;

- The leukocyte population (total or differentiated) was not altered on the animals that underwent intra-uterine malnourishment nor on the animals that underwent gestation and lactation malnourishment;

- The anti-inflammatory effects of indomethacin were less pronounced on the malnourished offspring during the gestation as well the malnourished during gestation and lactation litter.

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CONSIDERAÇÕES FINAIS

5 CONSIDERAÇÕES FINAIS

De acordo com os resultados obtidos concluímos que:

● Segundo a literatura a má-utrição intra-uterina parece interferir na resposta inflamatória na prole adulta de ratos *Wistar*, no entanto seus mecanismos ainda permanecem incertos. Sugerem-se mais estudos voltados para a compreensão dos fatores que desencadeiam tal processo.

● A má-nutrição intra-uterina e a má-nutrição durante a gestação e lactação reduz a capacidade de resposta inflamatória sistêmica de ratos *Wistar* frente ao modelo experimental de artrite induzida por Adjuvante Completo de Freund;

● As concentrações séricas de albumina e PCR estão reduzidas na prole adulta dos animais submetidos a má-nutrição intra-uterina e mal nutridos durante a gestação e lactação;

● A população de leucócitos (totais ou diferenciais) não foram alteradas nos animais submetidos má-nutrição intra-uterina nem nos animais submetidos à má-nutrição durante a gestação e lactação em relação aos normonutridos;

● Os efeitos anti-inflamatórios da indometacina foram menos pronunciados na prole mal nutrida durante a gestação bem como na prole mal nutrida durante a gestação e lactação.

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Ofício nº 50/08

Da Comissão de Ética em Experimentação Animal (CEEA) da UFPE
Para: **Profa. Maria Barnadete de Souza Maia**
Departamento de Fisiologia e Farmacologia – CCB
Universidade Federal de Pernambuco
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Os membros da Comissão de Ética em Experimentação Animal do Centro de Ciências Biológicas da Universidade Federal de Pernambuco (CEEA-UFPE) avaliaram seu projeto de pesquisa intitulado ***" Impacto da desnutrição na intensidade da resposta inflamatória e as implicações sobre o efeito de antiinflamatórios não esteroides em ratos Wistar".***

Concluímos que os procedimentos descritos para a utilização experimental dos animais encontram-se de acordo com as normas sugeridas pelo Colégio Brasileiro para Experimentação Animal e com as normas internacionais estabelecidas pelo National Institute of Health Guide for Care and Use of Laboratory Animals as quais são adotadas como critérios de avaliação e julgamento pela CEEA-UFPE.

Encontra-se de acordo com as normas vigentes no Brasil, especialmente a Lei 9.605 – art. 32 e Decreto 3.179-art 17, de 21/09/1999, que trata da questão do uso de animais para fins científicos.

Diante do exposto, emitimos **parecer favorável** aos protocolos experimentais realizados.

Atenciosamente,

Profa. Maria Teresa Jansem
Presidente do CEEA
UFPE

Observação:
Origem dos animais: Biotério do Departamento de Fisiologia e Farmacologia
Animais: Ratos; Wistar; machos ; com 60 dias de vida
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Baixar livros do Conselho Nacional de Educação - CNE
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