Rev Dor. São Paulo, 2011 oct-dec;12(4):337-41

ORIGINAL ARTICLE

Anti-inflammatory effect of dietary supplementation with omega-3 fatty acids in rats*

Efeito anti-inflamatório da suplementação dietética com ácidos graxos ômega-3, em ratos

Tamirys Ribeiro Mesquita[1], Adriana Aparecida de Souza[2], Elton Constantino[3], Naira Correa Cusma Pelógia[4], Irimar de Paula Posso[5], Oscar César Pires[6]

* Received from the Basic Institute of Biosciences, University of Taubaté. Taubaté, SP.

SUMMARY

BACKGROUND AND OBJECTIVES: Several studies have shown the beneficial effects of Omega-3 fatty acids on health: on the lipid metabolism promoting plasma triglycerol levels decrease, increase in HDL cholesterol and anti-inflammatory action by decreasing arachidonic acid derivatives synthesis: prostaglandin E2 (PGE3), thromboxane A2 (TXA2), prostacyclin (PGI2) and leukotriene B4. So, it is supposed that supplementation with EPA and DHA (ω-3) fatty acids may attenuate inflammatory process effects by decreasing eiocosanoids synthesis as well as the indiscriminate use of anti-inflammatory drugs. This study aimed at comparing the analgesic / anti-inflammatory effect of dietary supplementation with omega-3 fatty acid (ω-3) and tenoxicam in rats.

METHOD: Participated in this study 18 male Wistar rats, weighing between 220 and 300 g, distributed in 3 groups (n = 6): Control group (CG), tenoxicam group (TG) and Omega group (OG), to receive, respectively 0.2 mL saline, 1 $mg.kg^{1}.day^{-1}$ tenoxicam and 200 $mg.kg^{-1}.day^{-1}$ omega-3 fatty acid per day by gavage. Formalin test was performed after two weeks of treatment and nociceptive response was observed, since release of local endogenous mediators is attributed to the second phase, which generates local inflammatory response, responsible for the sensitization of primary afferents and of medullar neurons subsequent to the activation of nociceptors. Statistical analysis of results was performed with the SAS JMP program, adopting significance level of 5%.

RESULTS: Tenoxicam and omega-3 groups were not statistically different when compared in the modified formalin test phase. However, they showed less painful response, with statistical significance, in the second formalin test phase as compared to CG.

CONCLUSION: Results have shown comparable anti-inflammatory effect between tenoxicam and dietary supplementation with omega-3 fatty acid, suggesting that dietary supplementation with omega-3 fatty acid may be very useful, especially for chronic processes where the use of non-steroid anti-inflammatory drugs is associated to higher morbidity especially of the digestive and renal systems.

Keywords: Inflammation, Omega-3, Pain, Rats.

RESUMO

JUSTIFICATIVA E OBJETIVOS: Diversos estudos têm demonstrado os efeitos benéficos dos ácidos graxos ômega-3 à saúde: no metabolismo lipídico, promovendo redução nos níveis plasmáticos dos triacilgliceróis, aumento de HDL colesterol e, ação anti-inflamatória ao reduzir a síntese de derivados do ácido araquidônico: prostaglandina E2 (PGE2), tromboxano A2 (TXA2), prostaciclina (PGI2) e leucotrieno B4. Portanto, há de

1. Graduated Nutritionist by the University of Taubaté. Taubaté, SP, Brazil.
2. Student of Biology, University of Taubaté. Taubaté, SP, Brazil.
3. Anesthesiologist, Assistant Professor of Anesthesiology, University of Taubaté. Taubaté, SP, Brazil.
4. Pharmaceutist, Doctor Professor of Physiology, University of Taubaté. Taubaté, SP, Brazil.
5. Anesthesiologist, Associate Professor of Anesthesiology, School of Medicine, University of São Paulo; Professor of Pharmacology, University of Taubaté. Taubaté, SP, Brazil.
6. Anesthesiologist, Doctor Professor of Pharmacology, University of Taubaté. Taubaté, SP, Brazil.

Correspondence to:
Oscar César Pires, MD.
Avenida Itália, nº 1551 – R4 – Rua 13, n° 821
Jardim das Nações
12030-212 Taubaté, SP.
E-mail: ocpires@uol.com.br

se supor que a suplementação com ácidos graxos EPA e DHA (ω-3), pode atenuar os efeitos do processo inflamatório a partir da diminuição da síntese dos eicosanoides, assim como a utilização indiscriminada dos anti-inflamatórios. O presente estudo teve como objetivo comparar o efeito analgésico e anti-inflamatório, entre a suplementação dietética com ácido graxo ômega-3 (ω-3) e tenoxicam em ratos.

MÉTODO: Participaram do estudo 18 ratos Wistar machos, pesando entre 220 e 300 g, distribuídos em três grupos (n = 6): Grupo controle (GC), Grupo tenoxicam (GT) e o Grupo ômega-3 (GO) para receberem respectivamente 0,2 mL de solução fisiológica, 1 $mg.kg^{-1}.dia^{-1}$ de tenoxicam e 200 $mg.kg^{-1}.dia^{-1}$ de ácido graxo ômega-3 diariamente, por gavagem. Após duas semanas de tratamento, foi realizado o teste da formalina e observação da resposta nociceptiva, já que à segunda fase do teste se atribui liberação de mediadores endógenos locais, que geram resposta inflamatória local, responsável pela sensibilização de aferentes primários e de neurônios medulares subsequente a ativação de nociceptores. A análise estatística dos resultados obtidos foi realizada utilizando o programa JMP do SAS, adotando o nível de significância de 5%.

RESULTADOS: Os grupos tenoxicam e ômega-3 não apresentaram diferenças estatisticamente significantes quando comparados entre si nas fases do teste da formalina modificado. Não obstante, apresentaram menor resposta álgica, com significância estatística, na segunda fase do teste da formalina quando comparados com o GC.

CONCLUSÃO: Os resultados demonstraram efeito anti-inflamatório comparável entre o emprego de tenoxicam e a suplementação dietética com ácido graxo ômega-3, sugerindo que o uso da suplementação dietética com ácido graxo ômega-3 poderá ser de grande valia, principalmente nos processos crônicos, onde o emprego de anti-inflamatórios não esteroides se relaciona a maior morbidade principalmente no sistema digestivo e renal.

Descritores: Dor, Inflamação, Ômega-3, Ratos.

INTRODUCTION

The indiscriminate use of anti-inflammatory drugs by the population is a reality, especially in low-income countries where people have no easy access to health services[1]. Many of these drugs have adverse effects, such as gastropathies and nephropathies, in addition to immunosuppressive action, so, their prolonged use and / or high doses, may decrease immune responses and bring consequences to health[2].

Polyunsaturated fatty acids are classified according to the location of the first double binding, as from the molecule terminal methyl group, in omega-3 (ω-3), omega-6 (ω-6), omega-7 (ω-7) and omega-9 (ω-9). Polyunsaturated fatty acids – linoleic acid (ω-6) and α-linolenic acid (ω-3) – when metabolized form other polyunsaturated fatty acids, such as arachidonic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA; ω-3), respectively.

Mammals are able to synthesize saturated fatty acids of the ω-7 e ω-9 series, but cannot synthesize linoleic (ω-6) and α-linolenic (ω-3) PUFA, which are considered essential fatty acids and shall come from the diet[3]. Major linoleic acid sources are: soybean, sunflower and corn oil, and from α-linolenic acid they are: linseed and canola oil, and fish[3,4].

Several studies have shown beneficial ω-3 effects for health: in lipid metabolism by decreasing triglycerides plasma levels, total cholesterol and LDL, vasodilator action and possible action in cancer prevention and/or treatment (breast, prostate and colon), depression and Alzheimer's disease, in addition to decreased incidence of arteriosclerosis, anti-inflammatory, anticoagulant and antiplatelet activity.

Omega-3 fatty acids are also indispensable for neonates because they represent one third of brain lipids structure, where the lack of such substances may decrease the production of enzymes related to learning functions[5,6]. In addition, studies in humans and animals have shown a negative association between the ingestion of MIFA and PUFA and the incidence of cardiovascular diseases[7,8]. On the other hand, studies have shown that excessive consumption of oils rich in linolenic acid (ω-6) or saturated fatty acids is not recommended due to their pro-inflammatory, pro-thrombotic and pro-atherogenic effects[9,10]. However, ω-3 fatty acids have also anti-inflammatory action for decreasing the synthesis of arachidonic acid derivatives: prostaglandin E2 (PGE2), thromboxane A2 (TXA2), prostacyclin (PGI2) and leukotriene B4 (LTB4)[11].

So, it is supposed that supplementation with EPA and DHA (ω-3) fatty acids may attenuate inflammatory process effects by decreasing eicosanoids synthesis as well as the indiscriminate use of anti-inflammatory drugs.

Faced to what has been exposed, this study aimed at identifying and comparing the analgesic-anti-inflammatory effect of dietary supplementation with omega-3 fatty acids (EPA/DHA) and of tenoxicam in male Wistar, young adult rats using the modified formalin test.

METHOD

After the Institution's Animal Experiments Ethics Committee approval (Process 014/2009) this study was carried out with 18 male Wistar rats weighing between 220 and 300 g, keeping three animals per compartment where they remained for at least 20 days before the experiment was started, for appropriate adaptation, being treated with commercial balanced feed and water "ad libitum", 12-hour light-dark cycles and room temperature varying from 22 ± 3° C (19 to 25° C).

Animals were distributed in three groups (n = 6), as follows: control group (CG), tenoxicam group (TG) and omega-3 group (OG). CG animals received daily 0.2 mL saline by gavage. GT animals received 1 $mg.kg^{-1}.day^{-1}$ tenoxicam diluted in saline administered by gavage with insulin syringe in a volume equivalent to 0.2 mL. GO animals received 200 $mg.kg^{-1}.day^{-1}$ ω-3 polyunsaturated fatty acids (PUFA) supplementation (EPA: 180.0 mg/g and DHA: 120.0 mg/g), administered by gavage with insulin syringe in a volume equivalent to 0.2 mL.

All animals were treated just once a day for 20 days[12,13]. Before formalin test, animals were individually placed in a glass chamber of 25 x 25 x 25 cm for 15 minutes for adaptation to the study site. A mirror was placed behind the chamber to help visualization of flinches in all directions[14]. Pain was induced with 50 µL of 2% formalin solution in the dorsal region of the right hind paw[15]. For the analysis of pain response to formalin injection, all flinches unrelated to gait were considered.

Counting was continuous for 60 minutes with partial flinch numbers recorded every 5 minutes. Nociceptive response to formalin is biphasic: there is a short initial period (phase I, lasting 5 to 10 minutes); then, after a short behavior remission period, phase II is started consisting in a longer period (20 to 60 minutes) of sustained activity. In general, the initial response is attributed to direct nociceptors activation, while phase II is associated to local endogenous mediators release, which generates local inflammatory response, responsible for primary afferents and medullar neurons sensitization, subsequent to nociceptors activation[14,16].

SPSS (Statistical Package for Social Sciences) software version 17.0 was used for statistical analysis of results, with statistical significance level below 5% ($p < 0.05$).

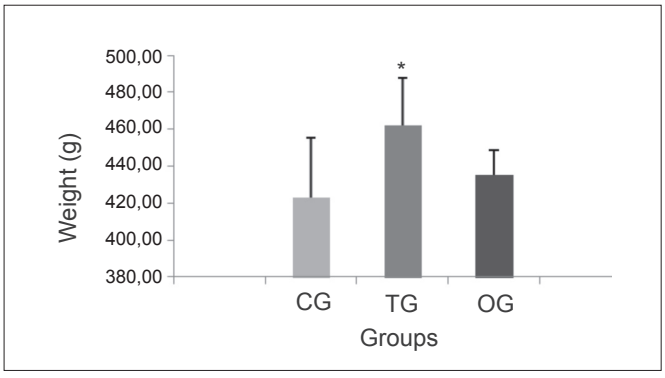
RESULTS

Kruskal-Wallis test was used to compare weight and number of flinches in phases I, intermediate and II among the three groups, and when differences were found ($p = 0.025$), Mann-Whitney test was applied to identify which groups were different among themselves. There has been no difference among groups when initial weight, formalin test phase I and intermediate phase weight were compared ($p > 0.05$). However, there has been significant difference among groups when animals' weights at study completion (Table 1 and Graph 1) were compared to phase II of the formalin test ($p < 0.05$).

Table 1 – Mean weights at beginning and completion of experiment

Group	Initial weight	SD	Final weight	SD
CG	384.0	41.54	423.01	32.16
TG	389.8	11.97	461.83*	26.09
OG	411.0	22.10	435.16	13.64
P Value	0.2463		0.0484	

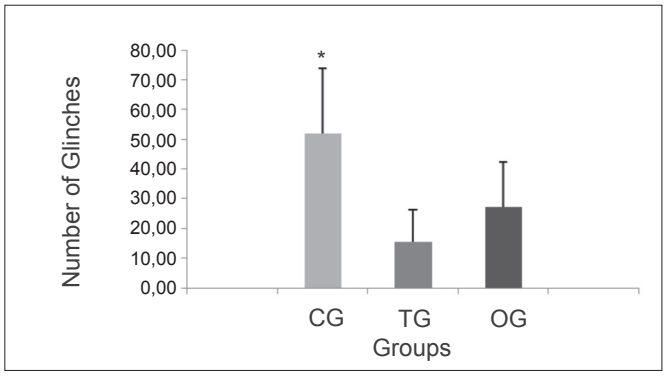
CG = control group; TG = tenoxicam group; OG = omega-3 group
* Statistically significant difference.



Graph 1 – Means and standard deviation of animals' weights, in groups, at treatment completion.
CG = control group; TG = tenoxicam group; OG = omega-3 group
* Statistically significant difference ($p = 0.0004$).

To check possible differences among the three observation phases, when simultaneously compared, Friedman test was applied. Since there has been statistically significant difference among the three observation phases ($p < 0.05$), Wilcoxon Signaled Posts test was applied and the result was that in the three studied groups, the intermediate phase was different from the other two ($p < 0.05$) which were statistically similar ($p > 0.05$).

For phase II, since there has been statistically significant difference among groups, Mann-Whitney test was applied to identify which groups were different among themselves when compared pair by pair. A significant difference was found between CG ($p < 0.05$) as compared to OG and TG, which were not different between them (Graph 2).



Graph 2 – Means and standard deviation of the number of flinches, in groups, during phase II of the formalin test.
CG = control group; TG = tenoxicam group; OG = omega-3 group
* Statistically significant difference (p = 0.0484).

DISCUSSION

The analysis of mean weights has shown that TG had higher weight gain with a final mean gain of 72.03 g, statistically significant difference as compared to other groups, followed by CG with 39.01 g gain. OG has shown the lowest weight gain with mean of 24.16 g.

A possible explanation for this difference is that tenoxicam, a non-steroid anti-inflammatory drug (NSAID) may cause sodium and water retention, resulting in weight gain. It is known that some NSAIDS side-effects are nephropathies and cardiovascular disorders, documented in large prospective clinical and observational trials, which have shown cardiovascular risks such as blood pressure increase and heart failure due to the inhibition of PGs formation changing sodium and water exchanges at the renal tubular level[17].

Omega-3 fatty acids supplementation (Table 1) has not caused significant weight gain changes in animals of this group, in line with other authors' findings[12,18].

The intermediate formalin test phase has shown statistically significant difference as compared to the other two phases, which were statistically similar in all groups, as expected. This characterization of behaviors during phases making them different among themselves are also described by other authors[14,19,20].

Notwithstanding, OG had efficacy comparable to TG in producing analgesia during phase II, characterized by central sensitization and/or acute inflammation, because they had lower flinches mean, which was statistically different as compared to CG.

In line with other studies, it was shown that formalin test is an effective, valid and reliable test for the biphasic observation of the pain process, evidencing nociceptors activation in phase I, central modulation in the intermediate phase and inflammatory response in phase II[19-21].

CONCLUSION

The anti-inflammatory efficacy of omega-3 fatty acid, which is comparable to TG and superior to CG, with statistical significance, suggests that supplementation with omega-3 fatty acids may be very useful, especially for diseases requiring chronic treatment with anti-inflammatory drugs.

REFERENCES

1. Piotto FRSB, Nogueira RM, Pires OC, et al. Prevalência da dor e do uso de analgésicos e anti-inflamatórios na automedicação de pacientes atendidos no Pronto-Socorro Municipal de Taubaté. Rev Dor 2009;10(4):313-7.
2. Lapointe BM, Frenette J, Côte CH. Lengthening contraction-induced inflammation is linked to secondary damage but devoid of neutrophil invasion. J Appl Physiol 2002;92(5):1995-2004.
3. Calder PC. Long-chain n-3 fatty acids and inflammation: potential application in surgical and trauma patients. Braz J Med Biol Res 2003;36(4):433-46.
4. James MJ, Gibson RA, Cleland LG. Dietary polynsaturated fatty acids and inflammatory mediator production. Am J Clin Nutr 2007;71(1 Suppl):343S-8S.
5. Nestel PJ. Fish oil and cardiovascular disease: lipids and arterial function. Am J Clin Nutr 2000;71(1 Suppl):228S-31S.
6. Moraes FP, Colla LM. Alimentos funcionais e nutracêuticos: definições, legislação e benefícios à saúde. Rev Eletr Farm 2006;3(2):109-22.
7. Lima FEL, Menezes TN, Tavares MP, et al. Ácidos graxos e doenças cardiovasculares: uma revisão. Rev Nutr 2000;13(2):73-80.
8. Martin CA, Almeida VV, Ruiz MR, et al. Ácidos graxos poliinsaturados ômega-3 e ômega-6: importância e ocorrência em alimentos. Rev Nutr 2006;19(6):761-70.
9. Covington MB. Omega-3 fatty acids. Am Fam Physician 2004;70(1):133-40.
10. Murakami KTT, Pinto M, Lima VMF. Ácidos graxos ômega-3 e imunologia de frangos de corte. Rev Vet Zoo 2008;15(2):112.
11. Kremer JM. ω-3 fatty acid supplements in rheumatoid arthritis. Am J Clin Nutr 2007;71(1 Suppl):349S-51S.
12. Haidamus LLA. A suplementação com ácidos graxos poliinsaturados ômega-3 reduziu a concentração plasm-

ática de eicosanóides pró-inflamatórios, da enzima lactato desidrogenase e de lesões musculares em ratos submetidos a sessões de natação. Campinas. Tese (Mestrado em Ciências da Alimentação). Departamento de Engenharia de Alimentos, Universidade de Campinas; 2007.
13. Watanabe ALC, Watanabe LM. Efeitos do tenoxicam sobre a cicatrização da parede abdominal: estudo experimental em ratos. Acta Cir Bras 2005;20(2):140-3.
14. Souza AM, Franco PAB, Ashmawi HA, et al. Efeito analgésico local do tramadol em modelo de dor provocada por formalina em ratos. Rev Bras Anestesiol 2008;58(4):371-9.
15. Pires OC, Ashmawi HA, Constantino E, et al. Antagonistas serotoninérgico e noradrenérgico por via subaracnoidea aumentam a resposta álgica em ratos. Rev Bras Anestesiol 2011;61(2):202-10.
16. Ting E. Participação da histamina e do receptor histamínico H1 na nocicepção em ATM de ratos. Piracicaba. Tese (Mestrado em Odontologia). Faculdade de Odontologia de Piracicaba, Universidade Estadual de Campinas; 2003.
17. Cheng HF, Harris RC. Cyclooxygenases, the kidney, and hypertension. Hypertension 2004;43(3):525-30.
18. Song JH, Miyazawa T. Enhanced level of n-3 fatty acid in membrane phospholipids induces lipid peroxidation in rats fed dietary docosahexaenoic acid oil. Atherosclerosis 2001;155(1):9-18.
19. Yamamoto T, Nozaki-Taguchi N. The role of cyclooxygenase-1 and -2 in the rat formalin test. Anesth Analg 2002;94(4):962-7.
20. Xie YF, Wang J, Huo FQ, et al. Validation of a simple automated movement detection system for formalin test in rats. Acta Pharmacol Sin 2005;26(1):39-45.
21. Pitcher GM, Henry JL. Second phase of formalin-induced excitation of spinal dorsal horn neurons in spinalized rats is reversed by sciatic nerve block. Eur J Neurosci 2002;15(9):1509-15.

Presented in August 12, 2011.
Accepted for publication in November 24, 2011.