Antiperoxidative properties of oil mixes of high ratio Omega-9:Omega-6 and low ratio Omega-6:Omega-3 after molar extraction in rats

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ABSTRACT

PURPOSE: To evaluate the antioxidant and antiperoxidative effects of oil mixes of high ratio Omega-9:Omega-6 and low ratio Omega-6:Omega-3 in the third day after tooth extraction in rats.

METHODS: Thirty-two male Wistar rats (270-310g) were randomly distributed in two groups: Control (n=24) and Test (n=8). Control group was divided into three subgroups (n=8): G1: Sham-Saline; G2: Saline; G3: Isolipid. G1 and G2 animals received NaCl 0.9% while G3 rats were treated with an isolipid mixture (alpha-linolenic acid – ALA) containing -6/-3 oils (8:1 ratio) and -9/-6 (0.4:1 ratio). Test group animals (G4) received oily mixtures (alpha-linolenic acid – ALA, docosahexaenoic acid – DHA, eicosapentaenoic acid – EPA) of -6/-3 (1.4:1 ratio) and -9/-6 (3.4:1 ratio). Saline and oils were administered by gavage during four days before and three days after first mandibular molar extraction. Following, samples (arterial blood and alveolar mucosa) were collected for glutathione (GSH) and thiobarbituric acid reactive substances (TBARS) assays.

RESULTS: Oil mixes induced a significant decrease in GSH and TBARS tissue and plasma concentrations in the third day post-surgery.

CONCLUSION: Gavage administration of oil mixes of high ratio Omega-9:Omega-6 and low ratio Omega-6:Omega-3 after molar extraction in rats induces a significant decrease in lipid peroxidation.

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Introduction

Oxidative stress can be defined as imbalance between production and collection of reactive oxygen species (ROS). The oral supplementation of antioxidants is well indicated in several situations, such as cancer, sepsis, preoperative of major surgeries and malnourished or critically ill patients. The omega-3 is one of the most used in research immunonutrients.

Currently, the adequacy of the balance of dietary lipids has motivated numerous investigations. In patients with changes in metabolic responses, the balance between dietary lipids aims to control oxidative stress and increased inflammatory response, through the relationship between the types of polyunsaturated fatty acids ingested, affecting the synthesis of eicosanoids that act as intermediate messengers of growth factors controlling the growth and differentiation of epithelial cells.

Protective effects of oils mixes against ischemia/reperfusion injury have been demonstrated. Pinheiro et al. investigated the effects of pre-conditioning with mixtures of oils containing high and low ratio ω-6/ω-3 and ω-9/ω-6 relationship in an experimental model and concluded that these oil combinations protects brain neurons against ischemia-reperfusion injury. The data found in the literature vary widely as to the best combination of antioxidants, the dosage, route of administration, the levels to be ideally achieved, the best time and how long they have to be administered in order to provide an effective protection against the oxidative injury. Despite the fact that there is no consensus regarding those topics, an early study has recommended that therapy should be instituted before the establishment of oxidative injury.

The hypothesis of this study is that the use of combinations containing different proportions of omega 3, 6 and 9 amino acids may have a noticeable antioxidant effect during the inflammatory phase of the wound healing after dental extraction.

Methods

Approval for experimental use of laboratory animals was obtained from the local Ethics Committee on Animal Use (CEUA, former CEPA) (protocol 73/2011, February 29, 2012) and is in compliance with the Federal Law No. 11794 of October 8, 2008, and the Decree nº 6,689, July 15, 2009 that regulated the law in 11,794, available from http: www.planalto.gov.br/ccivil03/Ato2007-2010/2008Lei11794.htm. The study was designed to minimize the number of animals required for the experiments.

Study design

Wistar rats provided by the Faculty of Medicine Small Animals Breeding Facility (UFC) In this controlled experimental study, after one week of acclimatization, 32 male Wistar rats provided by the Faculty of Medicine Small Animals Breeding Facility (UFC), weighing 270-310g, were randomly distributed in two groups: Control (n=24) and Test (n=8). Control group was divided into three subgroups (n=8) as follows: G1: Sham-Saline; G2: Saline; G3: Isolipid. G1 and G2 animals received NaCl 0,9% while G3 rats were treated with an isolipid mixture (alpha-linolenic acid – ALA) containing -6/-3 oils (8:1 ratio) and -9/-6 (0.3:1 ratio), by gavage for four days before and three days after surgical procedure. Test group animals (G4) received oily mixtures (alpha-linolenic acid – ALA, docosahexaenoic acid – DHA, eicosapentaenoic acid – EPA) of -6/-3 (1.4:1 ratio) and -9/-6 (3.4:1 ratio). Saline and oils were administered by gavage during four days before and three days after first mandibular molar extraction.

<table>
<thead>
<tr>
<th>TABLE 1 – Composition of isolipid and oil mixes preparations.</th>
<th>Composition</th>
<th>Source</th>
<th>ω-3</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Isolipid</strong></td>
<td>ω-6 + ω-3</td>
<td>ALA</td>
<td>ω-6: ω-3 = 8: 1</td>
<td></td>
</tr>
<tr>
<td>Corn oil</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Soybean oil</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>Test oil mixes</strong></td>
<td>ω-9 + ω-6 + ω-3</td>
<td>ALA (35%)</td>
<td>ω-9: ω-6 = 0.3: 1</td>
<td></td>
</tr>
<tr>
<td>Olive oil</td>
<td>EPA (39%)</td>
<td></td>
<td>ω-6: ω-3 = 1.4: 1</td>
<td></td>
</tr>
<tr>
<td>Canola oil</td>
<td>DHA (26%)</td>
<td></td>
<td>ω-9: ω-6 = 3.7: 1</td>
<td></td>
</tr>
<tr>
<td>Fish oil</td>
<td></td>
<td></td>
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</tbody>
</table>

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On the third day, G1 and G2 rats, anesthetized with a fresh-prepared mixture of ketamine 90mg.kg$^{-1}$ + xylazine 10mg.kg$^{-1}$ injected intramuscularly, underwent a surgical molar extraction. G1 animals underwent sham operation. Rats were kept under controlled environmental conditions (24°C relative humidity 40%-60%, 12-hour alternate light–dark cycles, food and water ad libitum).

Samples (arterial blood and alveolar mucosa) were collected on the third post-operative and the animals were killed by cervical dislocation at the end of the experiment.

**Laboratory parameters**

Thiobarbituric acid reactive substances (TBARS) and glutathione (GSH) concentrations were assayed to evaluate the oxidative stress, using methods described in the literature. Lipid peroxidation was assayed by measuring malondialdehyde as TBA-reactive substances$^8$. GSH levels were estimated by the method of Sedlak and Lindsay$^9$.

**Statistical analysis**

Data distribution was analyzed by the Shapiro-Wilk test. All data were expressed as mean ± SEM. SPSS (Statistical Package for the Social Sciences) was used for statistical analysis. One-way ANOVA was performed to determine differences among groups. A probability value of $p<0.05$ was considered to indicate statistical significance.

**Results**

**TBARS Assay**

Blood TBARS concentrations decreased significantly in G4 rats treated with test mixes oil (0.25 ± 0.06 vs. 0.10 ± 0.02, $p<0.05$), compared with saline control group (Figure 1A). Also, there was a significant decrease in tissue TBARS levels in G4 rats compared with saline-treated group tissue of the group treated with test oil mixes (0.27 ± 0.04 vs. 0.11 ± 0.02, $p<0.05$) (Figure 1B).

![FIGURE 1 - Thiobarbituric acid reactive substances (TBARS) levels in blood (A) and local tissue (B); Glutathione (GSH) levels in blood (C) and local tissue (D) three days post dental extraction. *$p<0.05$; ***$p<0.001$; Test ANOVA/Tukey.](image-url)
GSH Assay

There was a significant reduction in GSH blood levels in G4 animals treated with oil mixes (10.84 ± 00:32 vs.6:10 ± 0.45, p<0.001) compared with the group treated with Isolipid mix and with the group treated with saline (12:46 ± 00:24 vs.6:10 ± 0.45, p<0.001) (Figure 1C). However, GSH blood and tissue concentrations increased significantly (p<0.001) in rats treated with saline, compared with animals submitted to a simulated (sham) procedure (Figure 1C/D). There were significant reductions in tissue GSH levels in animals treated with test oil mixes compared with saline group (21.10 ± 0.69 vs.12:42 ± 7.90, p<0.001) and isolipidic mix (21.10 ± 0.69 vs.19:58 ± 12:27, p<0.001) (Figure 1C/D).

Discussion

Research studies concerning the relationship between the repair and the possible factors that affect the healing process after tooth extraction in rats were initiated in early 192310-11. The healing process post dental extraction has been studied in several animal models, and its sequence is relatively understood and divided into three phases: (a) initial phase: inflammatory between one and five days, (b) middle phase: during bone formation five to 20 days, (c) final phase: bone remodeling phase from 20 to 60 days12. Local and systemic factors may affect rat socket healing, including salivary gland hypofunction, osteoporosis, osteopetrosis, diabetes mellitus and treatment with calcitonin or cortisol13-17. In this work it was found that oil mixes administered by gavage before and after dental extraction may provide protection from lipid peroxidation in the third post-operative day, (Figure 1)

Fatty acids are used in a selective synthesis of phospholipids of cell membranes and their organelles. Essential fatty acids can determine structural and functional alterations of membrane phospholipids, including cells of the immune system, modifying its permeability, activity of receptors and enzymes, transport, regulatory functions and cellular metabolism18. Moreover, activate intracellular signaling routes for the formation of biologically active molecules that act as second messengers. Thus, may interfere with physiological events related to hemodynamic19, oxygenation20, inflammation21 and organic defense22.

Omega-9 (oleic acid) confer protection against lipid peroxidation of different polyunsaturated fatty acids such as linolenic acid, EPA, DHA containing 4, 5 and 6 double bonds respectively and because that are much less stable. The use of compositions rich in monounsaturated lipids as compared to the use of polyunsaturated showed low inflammatory response and low production of free radicals rich in monounsaturated formula. Membranes that are rich in monounsaturated fatty acids (MUFA) are less susceptible to oxidation by free radicals that membranes rich in saturated fatty acids, presumably because the greater number of unsaturations increase the likelihood of double bonds than in reactive oxygen species23. This may explain the antiperoxidative protection of oil mixes test shown in Figure 1A and 1B.

Olive oil is rich in α-9 fatty acids and vitamin E and is known for its antioxidant properties. The fact that the molecular structure of oleic acid have only one double bond, together with the presence of vitamin E confers greater protection against lipid peroxidation. Antioxidants decrease the accumulation of ROS24, which can reduce local tissue damage and accelerate the healing process25.

In this study, the use of an oil mix containing alpha-linolenic acid – ALA, docosahexaenoic acid – DHA, eicosapentaenoic acid – EPA of -6/-3 (1.4:1 ratio) and -9/-6 (3.4:1 ratio) promoted a decrease in GSH levels, an issue showing that GSH becomes “lesser necessary” and, in this way, an antioxidant action. Generally, cells react to oxidative stress with a increase in GSH pool as part of their adaptive answer to the potential oxidative lesion.20 Therefore, a lower GSH level indicates a less aggressive potential oxidative stress.

Conclusion

This study shows the antiperoxidative effects of oil mixes containing alpha-linolenic acid – ALA, docosahexaenoic acid – DHA, eicosapentaenoic acid – EPA of -6/-3 (1.4:1 ratio) and -9/-6 (3.4:1 ratio) acids when administered by gavage before and after rat tooth extraction.

References


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