Gastric and renal effects of COX-2 selective and non-selective NSAIDs in rats receiving low-dose aspirin therapy

Abstract: The consumption of low-dose aspirin (LDA) to prevent cardiovascular disease continues to increase worldwide. Consequently, the number of chronic LDA users seeking dental procedures that require complementary acute anti-inflammatory medication has also grown. Considering the lack of literature evaluating this interaction, we analyzed the gastric and renal effects caused by a selective COX-2 inhibitor (etoricoxib) and a non-selective COX-2 inhibitor (ibuprofen) nonsteroidal anti-inflammatory drug (NSAID) in rats receiving chronic LDA therapy. Male Wistar rats were divided into six experimental groups (carboxymethylcellulose (CMC) - vehicle; LDA; LDA + ibuprofen; ibuprofen; LDA + etoricoxib; and etoricoxib) and submitted to long-term LDA therapy with a subsequent NSAID administration for three days by gavage. After the experimental period, we analyzed gastric and renal tissues and quantified serum creatinine levels. The concomitant use of LDA with either NSAID induced the highest levels of gastric damage when compared to the CMC group (F = 20.26, p < 0.05). Treatment with either LDA or etoricoxib alone was not associated with gastric damage. No significant damage was observed on kidney morphology and function (F = 0.5418, p > 0.05). These results suggest that even the acute use of an NSAID (regardless of COX-2 selectivity) can induce gastric damage when combined with the long-term use of low-dose aspirin in an animal model. Additional studies, including clinical assessments, are thus needed to clarify this interaction, and clinicians should be careful of prescribing NSAIDs to patients using LDA.

Keywords: Anti-Inflammatory Agents; Aspirin; Gastritis; Kidney; Rats.

Introduction

In dentistry, clinical care of patients who have been chronic users of low-dose aspirin (LDA, 75–325 mg/day) has increased considerably due to the efficacy of this treatment in preventing cardiovascular problems. LDA inhibits platelet aggregation by blocking COX-1, an isofrom of the cyclooxygenase enzyme (COX). Thus, the prescription of short courses of nonsteroidal anti-inflammatory drugs (NSAIDs) after surgical procedures or pathological conditions has become more frequent in patients already taking LDA. Although, NSAIDs can inhibit both isofroms of COX (COX-1 and COX-2), they are classified as non-selective inhibitors, which act on COX-1 and COX-2 (e.g., ibuprofen), and selective COX-2 inhibitors...
(or coxibs), which are highly selective for the COX-2 isoform (e.g., etoricoxib).3

COX-1 is a constitutive isoform responsible for the homeostasis of organs and tissues, and it plays an important role in the gastric mucosa by promoting mucus production and inhibiting acid secretion.4 Furthermore, this isoform ensures the maintenance of adequate blood flow in the renal system.5 In contrast, COX-2 is typically associated with pathological parameters and promotes healing in the presence of inflammation in the gastric mucosa.4 To a lesser degree, this isoform is constitutively expressed in certain organs, including the renal system, where it maintains an adequate glomerular filtration rate and controls blood pressure by physiological modulation of vascular tone and hydric balance in the kidneys.6

Studies have shown that the inhibition of COX isoforms by NSAIDs, whether selective or non-selective for COX-2, can lead to minor gastric injuries; however, the inhibition of both COX-1 and COX-2 produces an intensified lesion, suggesting that inhibition of a single isoform is insufficient for the induction of significant damage.7, 8

In terms of renal function, the use of NSAIDs alone or in combination can trigger renal alterations, such as fibrosis, necrosis, increased serum creatinine levels, and sodium and water retention.9,10

Previous studies have reported the gastric and renal consequences of NSAID use, selective or non-selective and alone or in combination; however, no studies have evaluated the effects of acute use of these drugs in combination with chronic use of LDA. Therefore, we determined the gastric and renal effects caused by the use of a selective COX-2 inhibitor (etoricoxib) and a non-selective inhibitor (ibuprofen) NSAID in rats receiving long-term LDA therapy.

**Methodology**

Our experimental protocol followed the ARRIVE Guidelines for Animal Research by the National Centre for the Replacement Refinement & Reduction for Animals in Research11 and was approved by the Ethics Committee of Animal Use at the Universidade Estadual de Ponta Grossa–State University of Ponta Grossa–UEPG–Ponta Grossa/PR, Brazil (protocol CEUA 040/2014). The sample size was calculated using G*Power 3.12 software with Type I (α) and Type II (β) errors of 5% and 20%, respectively.

Male Wistar rats (10 weeks of age, 250–280 g) were housed in plastic cages (4 animals/cage) under a 12-hour light/dark cycle at a temperature of 22°C with food and water *ad libitum*. The handling of animals followed the recommendations of the National Council for Animal Experiments Control (CONCEA).

The animals were randomly divided into six groups (8 animals/group). Treatments included long-term (42 days) low-dose aspirin (LDA) and the subsequent administration of a non-selective NSAID (ibuprofen) or a COX-2-selective NSAID (etoricoxib) for three days by gavage (Table).

All drugs were suspended in 0.5% sodium carboxymethylcellulose (CMC) and administered once a day. To mimic the clinical use of this therapy, NSAIDs were administered two hours after LDA gavage.15

### Table. Study groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Duration</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMC</td>
<td>0.5% sodium carboxymethylcellulose (CMC) - vehicle</td>
<td>45 days</td>
<td>-</td>
</tr>
<tr>
<td>LDA</td>
<td>Low-dose aspirin (LDA) 6.75 mg/kg/day</td>
<td>45 days</td>
<td>Akyazi et al., 201335; Ghosh et al., 201136</td>
</tr>
<tr>
<td>LDA+Ibu</td>
<td>LDA 6.75 mg/kg/day + Ibuprofen 40 mg/kg/day</td>
<td>45 days</td>
<td>Liu et al., 2014;18 Bilgin et al., 201319</td>
</tr>
<tr>
<td>Ibu</td>
<td>Ibuprofen 40 mg/kg/day</td>
<td>3 days</td>
<td>-</td>
</tr>
<tr>
<td>Eto</td>
<td>Etoricoxib 10 mg/kg/day</td>
<td>3 days</td>
<td>-</td>
</tr>
<tr>
<td>LDA+Eto</td>
<td>LDA 6.75 mg/kg/day + Etoricoxib 10 mg/kg/day</td>
<td>45 days</td>
<td>Bressan e Tonussi, 2008;20 Dhull et al., 201221</td>
</tr>
<tr>
<td>Eto</td>
<td>Etoricoxib 10 mg/kg/day</td>
<td>3 days</td>
<td>-</td>
</tr>
</tbody>
</table>
Blood collection and euthanasia

Under general anesthesia (90 mg/kg of 10% ketamine and 10 mg/kg of 2% xylazine, intraperitoneal), blood samples were collected from the abdominal aorta (3 mL). The animals were euthanized with an overdose of anesthesia.

Gastric macroscopy

The stomach was removed and opened along the greater curvature to expose the gastric mucosa. This region was rinsed with a 0.9% saline solution and photographed using a digital camera (Nikon D5300, © 2015 Nikon Inc.–Zona Franca de Manaus, Manaus, Brazil). The gastric lesion area was measured (mm²) using ImageJ software (National Institute of Mental Health–Bethesda, Maryland, USA), and the results were expressed as a percentage of the total area.

Renalmacroscopy

The kidneys were removed, longitudinally sectioned into two fragments, and photographed using a digital camera (Nikon D5300). We analyzed the cortical and medullary portions for the presence or absence of edema, hemorrhagic foci, and tubular necrosis using previously described methods with some modifications.

Renal function analysis

As previously described, blood collection was performed immediately prior to euthanasia of the animals to quantify serum creatinine (Cr) levels. Serum was obtained by centrifugation at 735 × g for 10 minutes, and the Cr concentration was determined with the colorimetric method using a specific kit (Creatinina WS, Kovalent, Biochemistry, ReageLabor–São Gonçalo, RJ, Brazil) according to the manufacturer’s instructions.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 6 (GraphPad Software Inc.–San Diego, California, USA). The Kolmogorov-Smirnov test was applied to all groups to assess the distribution of data. All values were normally distributed; thus, all statistical analyses were completed using an analysis of variance (ANOVA) followed by the Tukey’s test. The level of significance was set at 5%.

Results

Gastric evaluation

The use of LDA (LDA group) and etoricoxib (Eto group) alone did not significantly increase gastric lesions when compared to the vehicle controls (CMC group) (F = 20.26, p > 0.05). In contrast, the administration of ibuprofen (Ibu group) significantly increased the development of gastric lesions. An even higher percentage of injured gastric tissue (F = 20.26, p < 0.05) was observed in the groups treated with a combination of LDA and ibuprofen (LDA + Ibu group) and LDA and etoricoxib (LDA + Eto group). The effects of each treatment on the gastric mucosa are shown in Figure 1.

Renal evaluation

Macroscopic and functional renal analyses revealed no statistically significant differences among groups (F = 0.0, p > 0.05 and F = 0.5418, p > 0.05, respectively). Figure 2 shows the serum creatinine concentration for each experimental group.

Discussion

In dentistry, short courses of NSAIDs are commonly prescribed to control the pain and inflammation...
related to clinical procedures. In this context, we analyzed the gastric and renal effects of different NSAIDs in rats receiving long-term LDA therapy. NSAID selection was based on their IC\textsubscript{50} values, which refers to the measurement of the effectiveness of a substance at inhibiting a specific biological or biochemical function. In this case, a higher IC\textsubscript{50} value corresponded to a greater selectivity of the drug for COX-1, whereas a lower IC\textsubscript{50} corresponded to a greater selectivity for COX-2. We used the following two NSAIDs for our study: ibuprofen, a non-selective inhibitor (IC\textsubscript{50} = 15 μg/mL), and etoricoxib, a selective COX-2 inhibitor (IC\textsubscript{50} = 0.0029 μg/mL). 17

The selected doses of ibuprofen (40 mg/kg) and etoricoxib (10 mg/kg) were determined with a two-step method: a review of the doses used in previous animal studies that evaluated the anti-inflammatory properties of these drugs\textsuperscript{18-21} and the calculation of an equivalent dose using the mathematical formula based on body surface area (BSA) suggested by the Food and Drug Administration (FDA).\textsuperscript{22} The doses of ibuprofen and etoricoxib selected for our animal model are equivalent to 400 mg and 100 mg in humans, respectively. These doses are considered to be clinically effective at controlling inflammation and are routinely prescribed by dentists.

A macroscopic analysis was considered sufficient for our current study based on previous reports involving the use of this methodology for observing and quantifying gastric lesions (inflammatory area is calculated as a percentage of the total gastric area).\textsuperscript{15,23} The macroscopic damage observed in rats is considered to be similar to the damage found in humans; furthermore, this damage is typically associated with the clinical symptomatology of gastritis. Macroscopic tissue lesions were not observed in the renal system; therefore, we included the evaluation of serum creatinine levels, which is a key marker of renal function.\textsuperscript{24}

To better understand the normal conditions of the gastric mucosa, we analyzed the data from the CMC group. For this comparison, we used naïve animals that were not submitted to gavage or any other procedure but rather were simply housed in the vivarium (data not shown). The CMC group showed a gastric lesion area of approximately 12%, and naïve animals showed gastric damage of approximately 10% (p > 0.05).

Interestingly, while the use of etoricoxib alone did not induce gastric lesions, its use in rats receiving LDA resulted in the highest percentage of gastric lesioning. The safety of etoricoxib use alone may be the result of its selective inhibition of COX-2, which consequently preserves the protective COX-1 function in the gastric mucosa. However, prior inhibition of COX-1 (due to LDA administration) combined with the use of a selective COX-2 inhibitor leads to gastric lesions.\textsuperscript{2,25,26,27} Indeed, clinical studies have reported the loss of this "protective effect" of selective COX-2 inhibitors when COX-1 is inhibited by another NSAID.\textsuperscript{25,26,27} The current predicted biological mechanism behind this phenomenon highlights that COX-2 is associated with the healing process, and its inhibition in patients previously subjected to a drug therapy that inhibits COX-1 delayed the normal healing of the mucosa and thus created a higher risk of gastric lesions.\textsuperscript{2}

We determined that the isolated use of ibuprofen provoked a significant increase in gastric damage when compared with the CMC group, and the combination of ibuprofen and LDA resulted in an exacerbation of this damage. This effect was likely due a synergic effect of these drugs on the inhibition of COX-1.\textsuperscript{28} In support of our findings, a clinical study conducted with 1,009 patients over two weeks observed a 1.35 relative risk of gastric bleeding in NSAID users, which rose to 3.59 when NSAID use was combined with LDA.\textsuperscript{29} Additionally, another clinical
study reported the risk of hemorrhage for users of LDA alone, NSAIDs alone, and the combination of LDA and NSAIDs as 3.9, 5.3, and 12.7, respectively. Thus, we affirm that the concomitant use of LDA and NSAIDs tends to considerably increase the presence of gastric lesions.

Our study also evaluated the effect of LDA on the gastric mucosa. Previous clinical trials suggested this therapy was a risk factor of gastric lesions due to the inhibition of COX-1. Although we observed increased gastric damage in the LDA group when compared to the CMC group, this difference was not statistically significant. A previous study suggested that the probability of an LDA-induced lesion is directly proportional to the dose administered. Indeed, the probability of developing an injury was approximately doubled in users taking 300 mg/day when compared with users taking 75 mg/day. In a clinical study performed with daily doses of 100, 100–200, or more than 200 mg, patients who took 100 mg/day presented a lower risk of gastric complications when compared with the other doses. In another trial, long-term users of 75 and 100 mg/day presented a lower incidence of ulcers when compared with doses of 101 to 325 mg/day. The lack of significance observed in our present study may be associated with the selected LDA dose of 6.75 mg/kg/day. This dose is equivalent to 75 mg/day in humans, which is the lowest clinical dose used for cardioprotection.

We also evaluated the effects of the selected drugs on renal tissue and function. None of the groups showed significant lesions or functional changes. Due to the inhibition of the COX enzyme and, consequently, prostaglandins (PGs), the use of COX-2 selective or non-selective inhibitors may alter the glomerular filtration rate and the regulation of sodium and water. However, these authors asserted that PGs are typically not the main players in the maintenance of homeostasis in physiological situations but rather only exert their function in instances of previous renal disease.

Moreover, when using NSAIDs for a short period of time, the possible hemodynamic alterations in the renal system are reversible; however, long-term and high-dose treatments exacerbate the risk of toxicity. Thus, although LDA was used for a prolonged period of time, its detrimental effects were reduced by using a lower dose in our study. In contrast, when using ibuprofen and etoricoxib at conventional clinical doses, the risks of renal toxicity were reduced with a short treatment duration.

Our research is limited because the use of animal models prohibits the extrapolation of our findings to clinical practice. Additional studies assessing a range of LDA doses are required because the cardioprotective dose of LDA varies from 75 to 325 mg.

**Conclusion**

Our results suggest that even the acute use of an NSAID (regardless of COX-2 selectivity) can induce gastric damage when combined with the long-term use of LDA in an animal model. Thus, additional studies, including clinical assessments, are needed to clarify this interaction, and clinicians should be mindful of prescribing NSAIDs to patients on an LDA regimen.

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**References**


