Chemoprevention by celecoxib in reflux-induced gastric adenocarcinoma in Wistar rats that underwent gastrojejunostomy

Quimiopevenção pelo celecoxibe no adenocarcinoma gástrico induzido por refluxo em ratos Wistar submetidos à gastrojejunostomia

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ABSTRACT

Purpose: To evaluate chemoprevention by celecoxib in cases of reflux-induced gastric adenocarcinoma, in Wistar rats that underwent gastrojejunostomy.

Methods: Sixty male Wistar rats of average age three months underwent surgery and were distributed into three groups: group 1, exploratory laparotomy; group 2, gastrojejunostomy; and group 3, gastrojejunostomy and daily celecoxib administration. After 53 weeks, the animals were sacrificed. Changes in the mucosa of the gastric body of group 1 and in the gastrojejunal anastomosis of groups 2 and 3, observed in histopathological and immunohistochemical examinations, were compared. All statistical analyses were performed using Epi-Info®, version 3.4.3.

Results: Comparison between groups 2 and 3 relative to the presence of adenocarcinoma showed a statistically significant difference (p=0.0023). Analysis of the association between groups 2 and 3 relative to COX-2 expression also showed a statistically significant difference (p=0.0018).

Conclusion: Celecoxib had an inhibiting effect on gastric carcinogenesis induced by enterogastric reflux in an animal model.

Key words: Adenocarcinoma. Stomach. Chemoprevention. Cyclooxygenase inhibitors. Rats.

RESUMO

Objetivo: Avaliar a quimioprevenção pelo celecoxibe no adenocarcinoma gástrico induzido por refluxo, em ratos Wistar, submetidos a gastrojejunostomia.

Métodos: Sessenta ratos machos Wistar, com média de idade de três meses foram operados e distribuídos em 03 grupos: Grupo 1 - Os animais foram submetidos a laparotomia exploradora. Grupo 2 - Os animais foram submetidos a gastrojejunostomia. Grupo 3 - Os animais foram submetidos a gastrojejunostomia e tomaram celecoxib, diariamente. Após um período de 53 semanas, os animais foram sacrificados. As alterações da mucosa do corpo gástrico dos animais do grupo 1 e da anastomose gastrojejunal dos animais dos grupos 2 e 3 foram analisadas no exame histopatológico e imuno-histoquímica e foram comparadas. Todas as análises estatísticas foram realizadas pelo programa Epi Info®, versão 3.4.3.

Resultados: No cotejo entre os animais dos grupos 2 e 3 com relação à presença de adenocarcinoma observou-se uma diferença estatística significante (p=0.0023). A análise de associação entre os grupos 2 e 3 com relação à expressão da COX-2, também evidenciou uma diferença estatística significante (p=0.0018).

Conclusão: O celecoxibe teve efeito inibidor da carcinogênese gástrica, induzida pelo refluxo em ratos.


Introduction

Cyclooxygenase, also known as prostaglandin endoperoxide synthase, is the key enzyme needed for prostaglandin synthesis from arachidonic acid. Two types of cyclooxygenase have been identified: COX-1 and COX-2. In many situations, COX-1 enzyme is produced constitutively in the gastric mucosa, while there is high production of COX-2 at inflammation sites and in neoplastic tissue.

Over the last few years, celecoxib (a specific COX-2 inhibitor) has been used to reduce the number of polyps in a rat model for adenomatous polyposis and in cases of duodenal adenomatous polyposis in humans. This drug is believed to act primarily by inhibiting the cyclooxygenase enzyme, with the advantages of having fewer gastric and renal side effects and not inhibiting platelet function.
Chemoprevention is taken to mean the use of drugs or natural agents with the purpose of preventing, inhibiting or reversing the processes of carcinogenesis.

Some unanswered questions motivated us to evaluate chemoprevention by celecoxib in cases of reflux-induced gastric adenocarcinoma, in Wistar rats that were subjected to the carcinogenic model of gastrojejunostomy.

Methods

This study was approved by the Research Ethics Committees of the Postgraduate Surgery Programs of UNIFESP and UNCISAL.

Sixty male Wistar rats, of average age three months and average weight 300g and acclimatized to the conditions of the vivarium of the Foundation and University of Health Sciences of Alagoas (UNCISAL), underwent surgery with intra-abdominal anesthesia of ketamine hydrochloride (50 mg/ml), at a dosage of 0.2 ml/100g of the animal’s weight. The animals were randomly divided into three groups: group 1, consisting of 10 animals that underwent explorative laparotomy; group 2, consisting of 25 animals that underwent gastrojejunostomy; and group 3, consisting of 25 animals that underwent gastrojejunostomy and received celecoxib orally (10 mg/kg/day). After a 53-week observation period, the animals were sacrificed and their stomachs were removed for macro and microscopic analysis.

Anatomopathological examination

1 – First stage (hematoxylin-eosin staining). All the surgical specimens were fixed in buffered formalin and the routine used in the pathology laboratory of Santa Casa de Misericórdia of Maceió, Alagoas, was followed. The mucosa of the gastric body of the group 1 animals and mucosa of the gastrojejunal anastomoses of the group 2 and 3 animals were evaluated and compared. The microscopic mucosal abnormalities found were chronic gastritis, foveolar hyperplasia, intestinal metaplasia, dysplasia and adenocarcinoma. Chronic gastritis was defined by the presence of mononuclear cells in the submucosa and lamina propria of the stomach. Foveolar hyperplasia was defined as stretching and twisting of the gastric foveolae. Intestinal metaplasia was recognized morphologically by the presence of goblet cells. The definition and classification of dysplasia obeyed the criteria proposed by Ming et al.

Adenocarcinoma was defined by the presence of atypical gastric glandules that invaded the submucosa, muscularis propria or serosa.

For the purposes of analyzing the occurrences of chronic gastritis, the foveolar hyperplasia and intestinal metaplasia were grouped and named inflammatory-metaplastic abnormalities.

2 – Second stage (immunohistochemistry). Representative blocks were selected from each case for immunohistochemical examination by means of the two-stage polymer technique, using the polyclonal antibody anti-COX-2/H62 (Santa Cruz Biotechnology Inc, USA), in accordance with Shi et al. The negative control was processed without the primary antibody and the positive control was obtained from cases of gastric adenocarcinoma that were already known to express COX-2.

Immunohistochemical evaluation

A Leitz optical microscope was used, with magnifications of 40x and 400x. The analysis was performed by two pathologists without previous knowledge of the study groups. COX-2 expression was deemed to be present when fine granulation of brown color was observed in the cytoplasm of the tumor cells.

Statistical methods

All the statistical analyses (frequencies of adenocarcinoma, dysplasia, inflammatory-metaplastic lesions and analyses of associations between groups 2 and 3) were performed using the Epi-Info® software, version 3.4.3. For the nullity hypothesis, p was set to be less than 5% or 0.05. An asterisk (*) was placed on the cases with statistical significance.

Results

Inflammatory-metaplastic lesions were observed in 12 cases (48%) among the group 2 animals and in 24 cases (96%) among the group 3 animals. Chronic gastritis, foveolar hyperplasia and intestinal metaplasia were included. No microscopic abnormalities of the gastric mucosa were observed in the group 1 animals.

A slight degree of dysplasia was observed in three cases (12%) among the group 2 animals. There was no dysplasia among the group 1 and 3 animals.

Adenocarcinoma was observed in 10 cases (40%) among the group 2 animals and in one case (4%) in group 3. All the tumors presented vegetative growth, of well-differentiated type, and had developed at the level of the gastrojejunal anastomosis (Figures 1 and 2).
Chemoprevention by celecoxib in reflux-induced gastric adenocarcinoma in Wistar rats that underwent gastrojejunostomy

Fisher’s exact test, \( p = 0.0023^* \); Odds Ratio: 16 (1.85 – 137.97); RR: 10 (1.38 – 72.39)

In this experiment, COX-2 expression was observed in four out of the 36 cases (11.1%) of inflammatory-metaplastic lesions, in one out of the three cases (33.3%) of dysplasia and in nine out of the eleven cases (81.8%) of adenocarcinoma (Figures 3 and 4).

Comparison between groups 2 and 3 in relation to the presence of adenocarcinoma showed a statistically significant difference in adenocarcinoma development: Fisher’s exact test, \( p = 0.0023^* \); Odds Ratio: 16 (1.85 – 137.97); RR: 10 (1.38 – 72.39) (Table 1).

**TABLE 1** – Analysis of the association between groups 2 and 3 in relation to the presence of gastric adenocarcinoma

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Yes Frequency</th>
<th>Yes %</th>
<th>No Frequency</th>
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<td>24</td>
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<td>25</td>
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<tr>
<td>TOTAL</td>
<td>11</td>
<td>22</td>
<td>39</td>
<td>78</td>
<td>50</td>
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Fisher’s exact test, \( p = 0.0023^* \); Odds Ratio: 16 (1.85 – 137.97); RR: 10 (1.38 – 72.39)
Analysis of the association between groups 2 and 3 in relation to COX-2 expression showed a statistically significant difference: Fisher’s exact test, \( p=0.0018^* \); Odds Ratio: 10.10 (2.05 – 54.95); RR: 6.00 (1.49 – 24.10) (Table 2).

<table>
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<tr>
<th>GROUP</th>
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<th>%</th>
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<th>%</th>
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Analysis of the association between COX-2 expression and gastric adenocarcinoma in groups 2 and 3 showed a statistically significant difference between the adenocarcinoma that expressed COX-2 and the adenocarcinoma that did not express it: Fisher’s exact test, \( p=0.000034^* \); Odds Ratio: 27.29 (4.99 – 230.94); RR: 11.57 (2.84 – 47.03) (Table 3).

<table>
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<tr>
<th>COX-2</th>
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<tr>
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Fisher’s exact test, \( p=0.000034^* \); Odds Ratio: 27.29 (4.99 – 230.94); RR: 11.57 (2.84 – 47.03)
shown in only three macrophages out of 22 rats examined\textsuperscript{(20)}. In the present study, it was observed in one out of the three cases of dysplasia (33.3\%) among the group 2 animals. With regard to adenocarcinoma, expression was observed in nine out of eleven cases (81.8\%), in the tumor cell cytoplasm. These results, relating to COX-2 expression in gastric adenocarcinoma in rats, have never been published previously in the literature. In clinical studies, COX-2 was expressed in 51 to 76\% (mean of 73\%) of gastric tumors according to Northern blot or RT-PCR, in 67 to 83\% (mean of 73\%) according to Western blot and in 43 to 100\% (mean of 62\%) according to immunohistochemistry\textsuperscript{(21)}. The great variability in the expression of this protein in literature can be explained by the use of many types of antibodies, the differences in the methods used for quantifying reaction positivity and the way in which the specimens were fixed. Because this is an immunological reaction, antigen recovery depends on the state of conservation of the paraffin blocks and the adequacy of specimen fixing in buffered formalin. Use of inadequate solutions of formalin may block the protein binding sites on the antibody, thereby not allowing antigen recovery.

Comparing groups 2 and 3 in relation to the presence of adenocarcinoma, a statistically significant difference between the animals in these groups was observed with regard to cancer development, such that the group 3 animals were protected from having cancer through the use of celecoxib (p=0.0023). It was also observed, in analyzing the association between groups 2 and 3 in relation to COX-2 expression, that the group 2 animals had statistically significantly greater COX-2 expression than seen in the group 3 animals (p=0.0018). Analysis of the association between COX-2 expression and adenocarcinoma in groups 2 and 3, a statistically significant difference was observed between the adenocarcinomas that expressed COX-2 and those that did not express it (p=0.000034). Some other studies have proved that the specific COX-2 inhibitor (celecoxib) and sulindac have a chemopreventive effect in animal models that have been inoculated with human gastric cancer cells (xenografts)\textsuperscript{(22,23)}. In another study, celecoxib and indomethacin were also effective in inhibiting the growth of lineages of human gastric cancer cells (AGS and MKN), through inducing apoptosis and stopping the cell cycle and not by suppressing COX-2 and prostaglandin E\textsubscript{2}\textsuperscript{,24}. In a model for gastric carcinogenesis using N-methyl-N’-nitro-N-nitrosoguanidine (MNNG), celecoxib and indomethacin were observed to provide chemoprevention by a mechanism independent of COX-2\textsuperscript{(25)}. A complementary study comparing the kinetic cell abnormalities in the glandular stomach of rats subjected to nonresecting procedures: an experimental long-term study. World J Gastroenterol. 2004;28:127-42.

In our experiment, celecoxib was observed to be effective for chemoprevention against carcinogenesis induced by enterogastric reflux. The mechanisms responsible for this chemopreventive action are not well known. However, it is known that the main mechanism for the action of this drug as an anti-inflammatory and analgesic agent would be through inhibition of cyclooxygenase-2, thereby blocking the synthesis of prostaglandins from arachidonic acid. There is evidence that chemopreventive mechanisms may function through inhibition of angiogenesis either via a COX-dependent route or not. Other mechanisms could arise through induction of tumor cell apoptosis or inhibition of cell proliferation. We believe that the chemoprevention mechanism of this drug may result from the sum of the effects already mentioned.

Conclusion

Celecoxib had an inhibiting effect on reflux-induced gastric carcinogenesis in rats.

References


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