GASTRIC CARCINOGENESIS MODEL USING FINNEY PYLOROPLASTY: EXPERIMENTAL STUDY IN RATS

Modelo de carcinogênese gástrica utilizando piloroplastia de Finney: estudo experimental em ratos

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ABSTRACT – Background. The duodenogastric reflux has been implicated as a potential carcinogen for the stomach and esophagus and is one of the factors that may explain the development of gastric stump cancer. Experimental models of carcinogenesis in the stomach stump or in the duodenogastric anastomosis are well defined. Aim - To develop an experimental model of gastric carcinogenesis through the Finney pyloroplasty, evaluate the influence of ingestion of sodium nitrite in this model, analyze the concentrations of bile acids and the pH of the stomach. Methods - A hundred and ten Wistar rats were operated and divided into four groups: Group I (15 rats) underwent laparotomy (Sham group); Group II (15 rats) underwent laparotomy (Sham) and ingestion of sodium nitrite in drinking water; Group III (40 rats) submitted to the Finney pyloroplasty and Group IV (40 rats) submitted to the Finney pyloroplasty and ingestion of sodium nitrite in drinking water. After 50 weeks of surgery, the rats were sacrificed and samples collected for analysis of gastric pH, dosing of bile acids and histological analysis. Results - The immediate postoperative mortality was 9%, and during the experiment, 10 rats died. The control group (I) did not show gastric lesions; the control group with sodium nitrite (II) developed papillomas in the pre-stomach in 16.6%; the operated groups with pyloroplasty had adenomas in 10.3% in Group III and 14.2% in Group IV, and adenocarcinoma in 55.1% in Group III and 14.2% in Group IV. The implementation of glands into the submucosa and muscle in the area of anastomosis (mucosa deployment) was not sufficient criterion for deciding on the malignancy of the lesions, requiring the simultaneous presence of atypical cells. The concentration of bile acids in gastric juice was higher in Groups III and IV. The measurement of gastric pH was not different in both groups. Conclusion - 1) The Finney pyloroplasty is suitable experimental model of gastric carcinogenesis; 2) it induced duodenogastric reflux; 3) the duodenogastric reflux served as a carcinogen for the stomach; 4) there was no relationship between pH and the development of gastric carcinoma; 5) sodium nitrite did not act as a carcinogen for the stomach of rats.

RESUMO – Racional - O refluxo duodenogástrico tem sido implicado como potencial carcinógeno para o estômago e esôfago e é um dos fatores que podem explicar o desenvolvimento de câncer no coto gástrico. Modelos experimentais de carcinogênese no estômago ressecado ou nas gastrojunoanastomoses estão bem definidos. Objetivos - Desenvolver um modelo experimental de carcinogênese gástrica através de piloroplastia à Finney, avaliar a influência da ingestão de nitrito de sódio nesse modelo, analisar as concentrações de ácidos biliares e o valor do pH gástrico. Métodos - Foram operados 110 ratos Wistar divididos em quatro grupos: Grupo I (15 ratos) submetidos à laparotomia (grupo Sham); Grupo II (15 ratos) submetidos à laparotomia (Sham) e à ingestão de nitrito de sódio na água de beber; Grupo III (40 ratos) submetidos à piloroplastia à Finney; Grupo IV (40 ratos) submetidos à piloroplastia à Finney e à ingestão de nitrito de sódio na água de beber. Após 50 semanas da operação, os ratos foram sacrificados, coletadas amostras de suco gástrico para análise do pH, dosagem dos ácidos biliares, e realizada análise histológica. Resultados - A mortalidade pós-operatória imediata foi de 9% e, ao longo do experimento, 10 ratos morreram. O grupo controle (I) não apresentou lesões gástricas; o grupo controle com nitrito de sódio (II) desenvolveu papilomas no pré-estômago em 16,6%; os grupos operados com piloroplastia apresentaram adenomas em 10,3% no Grupo III e 14,2% no Grupo IV, e adenocarcinoma em 55,1% no Grupo III e 14,2% no Grupo IV. A implantação de glândulas para dentro da submucosa e muscular, na zona de anastomose (implantação mucosa), não foi critério suficiente para decidir sobre a malignidade das lesões, sendo necessária a presença simultânea de atipias celulares. A concentração de ácidos biliares do suco gástrico foi maior nos Grupos III e IV. A medida do pH gástrico não foi diferente nos grupos estudados. Conclusão - 1) A piloroplastia à Finney é modelo experimental adequado de carcinogênese gástrica; 2) ela induziu refluxo duodenogástrico; 3) o refluxo duodenogástrico atuou como carcinógeno para o estômago; 4) não houve relação entre o pH gástrico e o desenvolvimento de carcinoma; 5) o nitrito de sódio não atuou como carcinógeno para o estômago dos ratos.
Gastric cancer is one of the most important health problems worldwide. It is a disease with high mortality rate with five year survival rate, less than 20%.

A hypothesis that has received considerable attention is that N-nitrous compounds are involved in gastric carcinogenesis\(^8,23\). This hypothesis is supported by experimental studies in animals and epidemiological studies, in which were found positive associations with the consumption of preserved food, smoked and salted foods\(^19\) and negative associations with diets rich in fruits and vegetables that are rich in antioxidants, such as carotenoids and vitamin C.\(^13\) In an experimental study has been shown that ascorbic acid inhibits the formation of nitrous compounds, reducing the induction of tumors\(^22\), and epidemiological study proved that ascorbate-rich diet reduces the risk of gastric cancer\(^20\).

The endogenous formation of N-nitrous compounds in the stomach may occur when both an amine or amide and a nitrosating agent such as nitrite, nitrate or nitrogen oxides, are present and is blocked by antioxidants\(^5\).

Clinical studies in patients at high risk of gastric cancer have found data supporting the hypothesis of endogenous nitrosation. Caygill et al.\(^7\) by measuring the pH, the concentration of nitrate-reducing bacteria and nitrate and nitrite in gastric juice in patients with stomach surgery pernicious anemia and controls, found results that confirm the hypothesis that metabolites of nitrite, nitrous compounds probably, are related to excess risk of cancer in patients with decreased gastric acidity.

In 1922, Balfour\(^1\) described the first time an adenocarcinoma of the gastric stump after operation for peptic ulcer. Other authors have described this cancer\(^15,16\), which is defined as carcinoma that occurs in the stomach remaining after gastric resection performed for benign disease. At least five years after the operation is required to avoid misdiagnosis. Using this definition, the prevalence of gastric stump carcinoma, according to the literature, ranges from 0.8% to 8.9% of patients undergoing gastric resection\(^20\). Two meta-analysis showed significant risk of gastric cancer, reconstruction after total gastrectomy with Billroth I or Billroth II that becomes apparent 15 years after the operation.

Duodenogastric reflux has been described as the cause of the clinical syndrome called reflux gastritis or gastritis alkaline\(^8\). Most of it, is due to previous gastric operation with removal or damage to the pyloric sphincter; is well established that such interventions lead to excessive duodenal reflux and may damage the gastric mucosa\(^5,14\). Duodenogastric reflux is, in a sense, normal in post-prandial event, and therefore its pathogenicity depends on volume and duration of exposure to gastric contents.

Experimental studies in rats show that duodenogastric reflux produces glandular stomach adenocarcinoma, squamous cell carcinoma in the pre-stomach, and promotes the development of Barrett’s esophagus, esophageal adenocarcinoma and squamous cell carcinoma of the esophagus\(^10,12,18,25,34,35,37\).

Carcinoma of the stomach is a rare spontaneous cancer in animals. Only rare and isolated cases have been described\(^6\). The first model to develop gastric adenocarcinoma in rats used the carcinogen N,N'-2,7-fluorenylenebisacetamide (2,7-FAA)\(^26\). Sugimura e Fujimura\(^13\) reported in 1967 that N-methyl-N’-nitro-Nitosoguanidines (MNNG) is effective in producing gastric adenocarcinoma and this has been the most widely used carcinogen induction of tumors for histological studies of the development factors and modulators of carcinogenesis.

The objectives of this study were to: 1) develop an experimental model of gastric carcinogenesis in rats operated with the Finney pyloroplasty; 2) evaluate the influence of ingestion of sodium nitrite in drinking water; 3) analyze the concentrations of bile acids and pH value in rat with Finney pyloroplasty or laparotomy and submitted or not the ingestion of sodium nitrite.

**METHODS**

**Animals and experimentation environment**

The sample consisted of 110 rats (Rattus norvegicus) of male Wistar supplied by Central Animal Laboratory, Federal University of Pelotas. With six weeks of life the animals were transferred to the vivarium of the Division of Animal Production and Experimentation of the State Foundation of Production and Health Research to adapt for two weeks. The animals were kept in plastic boxes. There were five rats per box. The cages were cleaned three times a week. All animals were kept in a vivarium daily for periods of 12 hours of daylight, under continuous flow of air and at room temperature. They were weighed every four weeks during the experiment. All items pertinent to the experiment were followed in accordance with the International Guiding Principles for Biomedical Research Involving Animals and the researchers sought to treat animals avoiding or minimizing the discomforts, risk or pain as ethical imperatives. All data were recorded and stored in a database in Excel, specifically designed for this purpose.

**Food and water**

The animals were fed from birth to 21 days of...
life, with breastfeeding. After this period, with water and food for rodents "ad libitum". The ration was changed twice a week. The drinking water came from the supply network of Porto Alegre and was changed every two days. The sodium nitrite was diluted in drinking water at a dose of 3 g/l as the only option offered drinking water to animals in Groups II and IV, from the 1st. week of life until the end of the experiment. The sodium nitrite solution was stored in a refrigerator between 8º and 11º and changed twice a week.

**Experimental design**

The experiment consisted of surgery (laparotomy or pyloroplasty) in association or not with the use of sodium nitrite in drinking water. The 110 rats were divided into groups formed as follows: Group I - underwent laparotomy with manipulation of handles (Sham group) received only water throughout the experiment; Group II - underwent laparotomy with manipulation of handles (Sham) received sodium nitrite diluted in drinking water from three weeks after the operation and throughout the experiment; Group III - subject to the Finney type pyloroplasty, received only water throughout the experiment; Group IV - submitted to the Finney type pyloroplasty, received sodium nitrite diluted in drinking water throughout the experiment.

**Surgical procedure and handling of samples**

The operations were performed in all groups, when the rats reached eight weeks old, weighing between 250 g and 350 g. After fasting for 12 hours for food and water, they were anesthetized by intramuscular injection of zolazepam hydrochloride and tiletamine hydrochloride at a dose of 50 mg/kg. After completion of shaving, the animals were placed on a plate and performed surgical antisepsis with iodine alcohol. The procedure began with a midline incision and opening of the abdominal cavity.

In the Sham group, after the opening of the cavity, was performed the manipulation of the abdominal viscera and closing the incision with nylon sutures.

In pyloroplasty groups, after the opening of the cavity, was proceeded the visualization of the caudal stomach, providing the perfect identification of terminal esophagus, pre-stomach, glandular stomach and the entire duodenum. It was done a continuous seroserosa suture joining the anterior wall of the pre-pyloric antrum (greater curvature) with the first and second portions of the duodenum. Next, an incision was made parallel to the suture lines in gastric and duodenal wall at the upper end of the seroserosa suture, coming down to the end of the incisions and then continuing up joining the right border of the duodenum to the left edge of the gastric wall - called Finney pyloroplasty (Figure 1). All sutures in the stomach and duodenum were performed with thread No. 7-0 cardiovascular Prolene.

Postoperatively, the animals were not receiving food and water for 12 to 24 hours.

In 50 weeks after surgery they were sacrificed by inhalation of ethylether in a fume cupboard, painlessly. The elapsed time between death and autopsy of the animal never exceeded five minutes. A midline incision was made from the xiphoid to the pubis, proceeded to the ligation of the duodenum, just after the anastomosis, and the collection of gastric juice for analysis. The incision was enlarged cranially including the chondrosternal plastron. An en-bloc resection was performed including stomach, duodenum, jejunum and proximal thoracic esophagus till the cervical region. The specimen was opened through an incision along the greater gastric curvature, washed with water, lying on cardboard and fixed with pins. After photographic identification, all specimens were placed one by one, in bottles with 10% formalin for later macroscopic description.

After ligation of the duodenum, so that there was no reflux due to manipulation, intubation is performed with nelaton tube no. 12 and instillation of 5 ml of saline into the stomach. Gastric content was aspirated and placed into two bottles labeled and stored in a freezer at -70° C for later analysis of

![FIGURE 1 – Pyloroplasty performed in Groups III and IV](Image)
pH and concentration of bile acids.

The analysis of pH was performed at the Laboratory of Digestive Physiology of Biosciences Institute of UFRGS, using a pH meter B374 Micronal brand.

The dosage of bile acids was performed at the Laboratory for Research on HCPA through Bele Acids kit supplied by the laboratory using Sigma enzymatic colorimetric method and by the enzyme 3-hydroxysteroid dehydrogenase-alpha according to the technique described by Fausa e Skalhegg.

Macroscopic examination was performed with the aid of a magnifying glass. The number, size, position and characteristics of each lesion were recorded. For description was used the anatomical terminology proposed by Robert.

After macroscopic observation, the stomach was sectioned in a direction tangential to its main axis and embedded in paraffin sections of the esophagus (1/4), pre-stomach (2/4), glandular stomach (anastomosis - 3/4) and duodenum (4/4). Histological sections of four micrometers thick, cut into Aotec microtome with disposable razors, were stained with hematoxylin-eosin.

The histological sections were examined in all common optical microscope by a pathologist who was unaware of the same type of treatment performed on the specimen under study. Histopathological changes were classified as: 1) normal stomach: pre-stomach with squamous epithelium, stomach glandular mucosa with consists of two components foveolar epithelium covering the surface layer of columnar cells with uniformly distributed and pyloric glandular tissue with glands, depending on the area studied; 2) chronic gastritis: mild - small number of mononuclear inflammatory cells (lymphocytes and plasma cells) in the mucosa and/or lamina propria; moderate - greater number of inflammatory cells; marked - many inflammatory cells spread through the mucosa, 3) ulcer: morphological pattern of inflammatory reaction characterized by a local defect or excavation, with loss of substance, affecting the entire thickness of the epithelium, necrosis in the background; 4) squamous epithelial hyperplasia: thickening of the epithelium at the expense of increasing the number of cells; 5) glandular hyperplasia: proliferation of glandular mucosa without atypia or destruction of tissue architecture; 6) implementation of mucosa: development of benign glands below the submucosa or muscularis in the area of anastomosis by invagination or implantation, no evidence of atypia; 7) foreign body granuloma: macrophages aggregate around foreign particulate matter (suture) forming granulomas; 8) squamous papilloma: a benign neoplasm of the squamous epithelium, usually exophytic pattern with epithelial hyperplasia and keratinization; 9) adenoma: benign glandular epithelium neoplasia, usually exophytic growth pattern with an increased number of glands well organized and minimal cellular atypia, with no sign of invasion of the basal layer; 10) dysplasia: mild (low grade) - amendment of the basal cell layers, reaching up to one third of the thickness of the epithelium, with pleomorphic nuclei mitoses occurring in the lower third of the epithelium, out of the basal layer; sharp (high grade) dysplastic changes affect the entire thickness of the epithelium, which is composed of cells with markedly abnormal nuclear polarity lost; 11) adenocarcinoma: malignant neoplasm of glandular epithelium, with relatively preserved glandular structure, nuclear polymorphism, increased mitotic activity and atypical mitoses with atypical cells and basement membrane invasion.

After completion of tabulation, the data were classified into six stages, in ascending order, as always the greatest severity of the diagnosis of each piece under study: 1) stage 1: normal esophagus and stomach, mild and moderate gastritis; 2) stage 2: epithelial hyperplasia, glandular hyperplasia, severe gastritis and ulcers; 3) stage 3: foreign body granuloma and deployment mucosa; 4) stage 4: papilloma and adenoma; 5) stage 5: dysplasia; 6) stage 6: adenocarcinoma.

Statistical Analysis

Were used the following tests: Chi-square to compare proportions between more than two groups, with Yates correction for comparisons between groups 2-2, Kruskal-Wallis test to compare continuous data and ordinal variables between more than two groups, Mann-Whitney to compare continuous data and ordinal variables between individual groups. The significance level was 5%. The data variable concentration of bile acids and pH which present skewed distribution were expressed as medians and dispersion measure used was the interquartile range (25th percentile vs EQA and percentile 75) The database was converted for analysis in the package SPSS version 8.0 for Windows.

RESULTS

Development of the experiment

Were operated a total of 110 rats, 10 of these (9%) died in the immediate postoperative period. The cause of death was gastric dilatation of unknown cause in three rats, pre-drilling of the stomach in two and unknown in five. Ten animals died during the development of experiment, one in Group I, three in Group II, three in Group III and three Group IV. Was identified in four lung infection, probably fungal and in other cases it was not possible to determine the cause of death. The
remaining ninety rats survived to 50 weeks and are the object of this study.

The variations in weight during the experiment did not differ between groups.

The macroscopic changes found in the esophagus, stomach and duodenum of rats are described in Table 1. Only one rat showed macroscopic alterations in the esophagus, an ulcer and esophageal middle third. In this case there was total obstruction of transit due to reflux of gastric tumor and contents into the esophagus dilated it. Stomach tumors were defined as when they were greater than or equal to 1.5 cm in diameter and when they had pearly aspect suggestive of malignancy, all tumors were partially (11) or totally (1) obstructive.

**TABLE 1 - Macroscopy in different groups**

<table>
<thead>
<tr>
<th>Gr n°</th>
<th>Experiment</th>
<th>n</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<tbody>
<tr>
<td>I</td>
<td>Laparotomy</td>
<td>14</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>II</td>
<td>Laparotomy + sodium nitrite</td>
<td>12</td>
<td>10 (83.4%)</td>
<td>0</td>
<td>0</td>
<td>2 (16.6%)</td>
<td>0</td>
<td>0</td>
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<tr>
<td>III</td>
<td>Pyloroplasty</td>
<td>29</td>
<td>10 (34.4%)</td>
<td>0</td>
<td>0</td>
<td>3 (10.3%)</td>
<td>0</td>
<td>16 (55.1%)</td>
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<tr>
<td>IV</td>
<td>Sodium nitrite + pyloroplasty</td>
<td>35</td>
<td>12 (34.2%)</td>
<td>2 (5.7%)</td>
<td>10 (28.5%)</td>
<td>5 (14.2%)</td>
<td>1 (2.8%)</td>
<td>5 (14.2%)</td>
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Microscopic changes are described in Table 2. There are described the findings of the esophagus because, except for the case of esophageal ulcer which also corresponded to the ulcer microscopy, all were considered normal. In the control group (I), all stomachs were considered histologically normal (stage 1). In the control group with sodium nitrite (II), 83.4% of stomachs were considered normal (stage 1) and 16.6% developed papillomas in the pre-stomach (stage 4). There was no statistically significant difference between these two groups. It might be noted that there was development of adenocarcinoma (stage 6) in the stomach of rats with pyloroplasty (Groups III and IV).

In the group submitted to pyloroplasty alone (Group III) in 10 rats (34.4%) histological findings were considered normal (stage 1), three rats (10.3%) developed adenomas (stage 4) and 16 (55, 1%) adenocarcinoma. Comparing the Group III to Group I and Group II, was found a significant difference (p = 0.0001).

In the group undergoing pyloroplasty and received NaNO2 in the drinking water (Group IV) in 12 rats (34.2%) the histological finding was considered normal, in two (5.7%) were found reactive inflammatory changes such as ulcers, severe gastritis, glandular and epithelial hyperplasia (stage 2), 10 (28.5%) had mucosal implantation and granuloma, foreign body in the area of anastomosis (stage 3), five (14.2%) developed adenomas (stage 4) in one (2.8%) was found high-grade dysplasia (stage 5), five (14.2%) developed gastric cancer (stage 6). The difference was significant between Group I and Group IV, p = 0.0001, and Group III, p = 0.01. Comparing the group III and group IV, the difference was also significant, p = 0.03. There was no intestinal metaplasia. All intestinal-type adenocarcinomas were well or moderately differentiated.

Were not recognized metastases in animals that developed adenocarcinoma, and tumors of the small intestine, liver or colon.

**Concentration of bile acids and pH**

The pH value and concentration of bile acids in gastric juice are shown in Figures 2 and 3, respectively. There was no statistically significant difference in pH value between the groups, the median was 2.9 (Group I), 2.6 (Group II), 3.2 (Group III) and 2.6 (Group IV). The concentration of bile acids in gastric juice was significantly higher in Group IV (median 255 mol/l) compared to Group I (22 mol/l).

**TABLE 2 - Histological findings in the stomach in different groups, in stages**

Gr nº | Experiment | n  | 1 | 2 | 3 | 4 | 5 | 6 |
<table>
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**FIGURE 2 – Box-plot graph comparing the pH value in different groups.** The median is represented by the horizontal center line of the box (in bold) and lower quartiles (P25) and top (P75) by the lines above and below that line the box. The minimum and maximum values are represented by vertical lines coming out of the box (whiskers) and outliers are indicated by e *e. There was no significant difference between groups.
There is evidence that the operated stomach cancer and non operated stomach cancer has similar carcinogenic process, based on clinical, histopathological and p53 gene alterations common to both\(^3\). Studies that evaluated genetics, molecular biology and infection in carcinomas of the stomach operated or not, found differences in relation to infection by H. pylori and Epstein-Barr virus, suggesting that there may be some different etiological factors\(^2,3,6\). The study of carcinogenesis in the operated stomach and its etiological factors can lead to clarification of the mechanisms of development and progression of gastric cancer.

Reviewing the literature, it can be found that different techniques have been used in the surgical induction of gastric cancers. Techniques with gastric resection and reconstruction of gastrointestinal transit providing duodenogastric reflux of various intensities and techniques where the stomach is resected, but there is reflux of duodenal contents, have varying incidences of adenocarcinoma in up to 71%. In this study, a pyloroplasty was performed by the technique described by Finney, which consists of an anastomosis between the stomach and first portion of the duodenum, providing ample communication and, theoretically, increased degree of reflux. Was not found in the literature any reference that had used this technique in the surgical induction of gastric tumors. In gastroenteroanastomosis with adenocarcinoma in rats with 32, 48 and 56 weeks. Analyzing the lability index increased cell proliferation observed in the gastric mucosa adjacent to the anastomosis compared to the area near the gastrotomy scar and suggest that duodenogastric reflux is associated...
with a 100% increase in this index. All tumors were found in this study of the anastomosis or close to it. This finding coincides with reports in the literature that the induced lesions appear in the anastomotic area, which seems to make clear relation to the operation. However, only gastrostomy does not induce proliferative lesions or cancer and no author found this type of injury in rats where it was made with Billroth II reconstruction loop in Roux-en-Y, suggesting that the operation is not sufficient stimulus, and required the presence of duodenal juice for the development of proliferative lesions.

The histological criteria for diagnosis of lesions in the scar area of the anastomosis must be well defined. The anatomical features of the architecture of gastric and duodenal wall are destroyed by the surgical section, is in the process destroyed the muscle layer of the mucosa, one of the references to the criteria for epithelial invasion. Some authors have considered only the criteria for submucosal invasion or muscle as adenocarcinomas to define excessive glandular proliferation. Others consider absolutely necessary strict standards, based on cytologic criteria to differentiate intramucosal dystopias caused by surgical trauma of genuine carcinomas. In this study, the atypical cells without glandular proliferation in the area of anastomosis was not used to decide on the malignancy of the lesions. Kobayasi et al. define the invagination of glandular mucosa into the submucosa, subserosa or muscular area of the anastomosis as “implanted mucosa.” Where this invagination of glands below the muscle pattern was concentric fibrosis around the mucosa with scar tissue and invariably foreign body granuloma, these cases were classified as stage 3.

Similar pattern in the area of anastomosis was described by Schlake e Nomura analyzing the stomach of rats subjected to BI resection. They also said that rat’s stomach carcinoma develops through two different ways: adenocarcinoma in an adenomatous polyp; the growth of oriented serous adenocarcinoma, whose precursor is proliferation of intramural adenocarcinoma. All adenocarcinomas found in this experiment had polyloid features with exophytic growth, infiltrating the gastric wall, but none came to the serosa. In Group IV, only in five rats was found adenocarcinoma, but considering the process, five adenomas and one case of high-grade dysplasia could be classified as premalignant lesions.

The measurement of pH and concentration of bile acids was used to assess whether the model in practice could produce duodenogastric reflux. The total concentration of bile acids in gastric contents in Group IV was significantly higher than in Groups I and II (p = 0.002, p = 0.01) confirming the occurrence of duodenogastric reflux. In Group III although the concentration of bile acids was high, there was no statistically significant difference to the control groups (I and II). In Group III, 37.9% of the rats had fully or partially obstructing tumors, which may have decreased the reflux and the time of collection of gastric juice. Comparing the concentrations of bile acids in gastric contents of rats in Group III where tumors were obstructive or not or even with other abnormalities, were found higher concentrations. With serial measurements of bile acids during the development of the experiment, probably would be found high values at early stages, when it had not yet developed tumors. The median pH value ranged from 2.9 to 3.2 and there was no significant difference between groups. While in Group III, where there was a higher incidence of carcinoma, had the highest median (3.2). There was no difference between gastric pH in rats that developed adenocarcinoma and those who did not, which is consistent with the literature. Although the proliferation of nitrite-reducing bacteria can be quantified, this study did not aimed to do this evaluation.

The induction of tumors by sodium nitrite (NaNO₂) could be caused by in vivo nitrosation of amines and amides. The nitrosation of amines is proportional to the concentration of nitrite and, therefore, the concentration may be critical in the evaluation of carcinogenicity. The lethal dose for 50% of the rats is 180 mg/kg body weight and methemoglobinemia is used as an index of toxicity. Here was used the maximum concentration of NaNO₂, tolerated by rats, 3 g/l and there was no methemoglobinemia.

The papers on experimental carcinogenesis using nitrites, present controversial results. In this model, sodium nitrite in drinking water did not induced adenocarcinoma. Andreollo et al. found 5.6% of carcinoma in rats operated with gastrectomy or gastrojejunostomy in BII resection associated with truncal vagotomy followed by eight months receiving potassium nitrite in drinking water. In an extensive review of nitrites, nitrates and nitrosous compounds, Gangolli et al. concluded that there is no evidence in experimental studies that nitrite is carcinogenic to animals.

When compared Group III and Group IV, which differ only in the experimental design due to the ingestion of sodium nitrite in drinking water, there was a significant difference in histological findings. The group receiving sodium nitrite (IV) had lower incidence of adenocarcinoma (stage 6) and most of pre-malignant dysplasia as adenomas (stage 4 and 5). It seems that nitrite did not potentiate carcinogenesis induced by duodenogastric reflux or the follow-up time was not sufficient for development of adenocarcinoma in Group IV. In the gastric microenvironment in rats with pH below 5, nitrite disappears quickly and destruction is
accelerated by the presence of food. On the other hand, is necessary that nitrosating nitrite must be acidified to form HNO2. In addition, the mouse is able to synthesize L-ascorbic acid, which is effective in blocking the intragastric nitrosation. Perhaps, in this experiment, nitrite was eliminated before it could interact with bile acids. Urinary concentration of nitrite and nitrosous compounds in gastric juice were not measured, but it seems that intragastric nitrosation did not occur in this model.

Duodenogastric reflux induced by the Finney pyloroplasty had a carcinogenic effect in the glandular stomach of rats. The composition of the refluxed contents may be the key to explain this effect. The pancreatic enzymes may damage the gastric mucosal barrier, leaving the cells exposed to potential carcinogens. Prior to secretion in the biliary tract, 98% of bile acids are conjugated with taurine and glycine, which increases the solubility. The soluble bile acids can enter cells of the gastric mucosa and damage the epithelial barrier. There is evidence of molecular damage caused by bile by breaking DNA, increasing cell proliferation, and occurs more rapidly in more acidic pH medium, ie, the intracellular accumulation is greater between the acidic luminal and cytoplasmic neutral medium, ie, the intracellular accumulation is greater and occurs more rapidly in more acidic pH.

There is evidence of molecular damage caused by bile by breaking DNA, increasing cell proliferation, and tissue damage by decreasing the activity of the carbonic anhydrase enzyme, which is a cellular protective enzyme present in high concentration in normal gastric mucosa.

**CONCLUSIONS**

The Finney pyloroplasty, without external carcinogen, is a suitable experimental model of gastric carcinogenesis in rats and it causes duodenogastric reflux; this reflux acted as a carcinogen for the stomach. There was no relationship between pH and the development of gastric adenocarcinoma. The sodium nitrite did not act as a carcinogen for the stomach.

**REFERENCES**


