THE ROLE OF DUODENO-GASTRIC REFLUX IN FORMATION OF PRECANCEROGENIC GASTRIC LESIONS - AN EXPERIMENTAL STUDY

UVOGA DUODENOGASTRIČNOG REFLUKSA U NASTANKU PREKANCEROZNIH LEZIJA ŽELUCA – EKSPERIMENTALNA STUDIJA

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Summary

Introduction. Duodenogastric reflux, commonly encountered as an aftermath of gastroenteroanastomosis, with or without gastric resection (Billroth I, Billroth II), vagotomy and pyloroplasty surgery, is known to cause inflammatory-dystrophic-metaplastic lesions of gastric mucosa. Our objective was to determine the effects of surgery-induced duodenogastric reflux on the development of precancerogenic lesions or carcinoma in correlation with the reflux duration. Material and Methods. The experiment was performed on three groups of Wistar rats with 1) Billroth II-induced reflux surgery, 2) resection of the Roux-en-Y type reconstruction, and 3) control group with no resection. The aim of the experiment was to study the effects of duodenogastric reflux on the rat gastric mucosa in correlation with two different types of gastroenteroanastomosis 8, 16 and 24 weeks after the surgery. Results. In Billroth II group, hyperplastic changes were observed as early as in week 16. Statistically significant results were recorded in week 24, with 6.7% of metaplastic alterations, including dysplasia of all three degrees, dominantly severe dysplasia in 66.67%, early carcinoma in 20% and gastric carcinoma in 6.67%. In the Roux-en-Y group, gastric mucosa remained predominantly normal (60%), with somewhat increased frequency of gastritis and dysplasia in week 24. In the control group, the finding of normal gastric mucosa was constant. Conclusion. The experiment confirms that direct contact of duodenal juice with gastric mucosa associated with Billroth II resection causes precancerogenic lesions. Development of adenocarcinoma caused solely by duodenogastric reflux, excluding a carcinogenic agent is possible 20 weeks after the experiment – earlier than suggested by previous researchers.

Key words: Duodenogastric Reflux; Precancerous Conditions; Gastric Mucosa; Gastritis; Stomach Neoplasms; Gastric Juice; Surgical Procedures, Operative; Adenocarcinoma; Rats, Wistar

Sažetak

Introduction

Since the first reports of duodenogastric reflux (DGR) 200 years ago, doctors have been intrigued with its potential role in the development of upper gastrointestinal tract disorders. Postresection syndromes include the long-recognized Dumping syndrome, efferent limb syndrome, "small stomach" syndrome, poor absorption syndrome and, lately, a particular nosological entity recognized as postsurgical reflux gastritis and slow stomach discharge [1]. Duodenogastric reflux, enterogastric reflux (EGR), bile reflux or alkaline reflux post-resection gastritis are all synonyms for a phenomenon that can be described as the "reflux of duodenal content through non-competent pyloric valvula, from duodenum to stomach, i.e., from duodenum and small intestines, through anastomosis, to stomach". Duodenogastric reflux is commonly encountered as an aftermath of gastroenteroanastomosis with or without gastric resection (Billroth I, Billroth II), vagotomy and pyloroplasty surgery, gastroduodenostomy, but also in non-operated patients. Billroth II (B II) resection type gastroenteroanastomosis and pylorus removal in Billroth I (B I) resection create conditions for constant duodenobiliary-pancreatic juice reflux to the stomach, which cause inflammatory-dystrophic-metaplastic lesions of gastric mucosa consequently damaging its physiological functions and creating conditions for the development of some other diseases [2].

Considering these findings, our objective was to determine the effects of DGR following various types of gastroenteroanastomosis on the development of precarcinogenic lesions, or carcinoma, in correlation with the duration of such condition.

Material and Methods

The experiment was conducted at the Institute for Experimental Medicine of the Medical School, University of Niš, Serbia. The experiment included 90 experimental rats – male, Wistar species, their average weight being 225 grams (ranging from 130 to 320 grams), and the average age 8.5 weeks, obtained from the Medical School Niš Varium. The experimental study design involved two experimental groups and one control group. Each group was comprised of 30 animals. Three subgroups of 5, 10 and 15 animals were formed within each of the three groups, depending on when they were sacrificed (week 8, 16 and 24 after the surgery, respectively).

In order to trigger chronic duodenal reflux, the first experimental group was subjected to B II surgery; the second one included the secretory stomach resection with Roux-en-Y reconstruction, while the third one, i.e. the control group, was not subjected to any surgery. Ketamine hydrochloride was used as an anesthetic (Ketamidor 10%, Richter Pharma ag, Wels, Austria), administered preoperatively in the doses of 0.1 ml per 100g/weight intraperitoneally. Anastomoses were performed by extramucosal stitches with adequately thick, monofilament sutures (Prolen, Ethicon®-7-0, 8-0). Following the surgical procedures, the animals were placed separately into adequately prepared cages. Each animal started to receive 5%-glucose, tap water and physiological solution in the ratio 1:1 twelve hours after the intervention; whereas soft food was induced 48 hours after the intervention. After 4 days, the animals were moved back to group cages for the rest of the experimental period. The control group received food and tap water only throughout the experiment.

The animals were sacrificed by administration of diethyl ether overdose. The stomach was then resected along the small pyloric curve from the pylorus to the cardia, and rinsed with the physiological solution. The materials were processed pathohistologically at the Institute for Pathology of the Clinical Centre, Niš. Fixed stomachs were cut into stripes (the average width being 2 mm) from the predefined regions and wherever pathological changes could be observed macroscopically. 45 µm (micrometre) paraffin sections were stained by the following methods: 1) Classic Hematoxylin and eosin stain method (HE), 2) Histochemical methods: a) Alcian Blue – Periodic acid Schiff (AB-PAS), Ph 2.5 - for mucosal (intestinal and pyloric metaplasia, dysplasia and carcinoma verification, b) Van Gieson – for collagen fiber, i.e., atrophic gastritis and scirrhous carcinoma variants (desmoplastic reaction).

Due to a large number of precarcinogenic lesions, pathohistological types and subtypes, and considering evident discrepancies between the Japanese pathologists’ classification on one side and European and American pathologists on the other side, we decided to apply the lesion classification defined by V. Katić [3].

Statistical Processing

The obtained results were analysed and statistically processed by a descriptive statistics method and by applying the relevant statistical significance tests, illustrated in tables or graphically. The database was created in Microsoft Office Excel 2007, and SPSS programme, version 12.0 (Statistical Package for Social Sciences) was used for statistical processing. All statistical tests were considered passed if the probability of null hypothesis was equal to or smaller than 5%. Data were processed by: Pearson’s χ²-test (chisquare test), Fisher-Freeman-Halton test (Fisher’s exact test), Single-factor analysis of variance (ANOVA), including Brown-Forsythe correction, Tukey HSD test.

Abbreviations

DGR – duodenogastric reflux
EGR – enterogastric reflux
B I – Billroth I
B II – Billroth II
Results

In correlation of experimental groups with time, hyperplastic changes were proved in 80% of the cases, and gastritis in 20% of the cases in B II group. At week 16, the observed changes included hyperplasia in 30%, gastritis in 20% and dysplastic changes of dominantly mild type in 20% of the cases. At week 24, all three degrees of dysplasia were found, whereas severe dysplasia was the most dominant (66.67%), but the incidence of early carcinoma (20%) and carcinoma (6.67%) was surprisingly high considering the short duration of the experiment (Table 1). χ²-test has shown a statistically significant correlation between the pathohistological finding in the Billroth resection groups in all three time intervals (p<0.001).

The additional analyses showed statistically significant differences between the group observed for 24 weeks and the two other groups, p=0.001, p=0.011. However, the 8- and 16-week groups did not show any statistically significant difference, p=0.336. In Roux-en-Y group, the findings were similar in all three observed periods – normal gastric mucosa was proved in 60% of the animals; gastritis and hyperplasia in 20% each, resulting most probably from the surgical intervention (Table 1). Pearson’s test did not show any statistically significant correlation between the pathohistological findings in the subgroups with Roux-en-Y reconstruction, in any of the three observed periods, p=0.983. In the control group, the independence test did not show any significant statistical data among pathohistological findings in the non-operated group in any of the observed periods – a constant pathohistological finding was expected.

In the experimental groups, the test showed a statistically significant correlation between the observed periods and the pathohistological findings: p=0.01; p=0.001; p<0.001 for 8, 16 and 24 weeks, respectively. In terms of correlation of experimental groups with the type of operation, in B II group, where duodenogastric reflux was expected, 80% of hyperplastic changes and 20% of gastritis were found after 8 weeks. On the other hand, in Roux-en-Y group normal gastric mucosa was found in 60%, while hyperplasia and parastomal gastritis were found in 20% each after 8 weeks. Finally, no pathohistological changes of the gastric mucosa were observed in the control group 8 weeks after the experiment had been initiated (Table 2). Additional analyses among 8-week groups showed a statistically significant correlation between the pathohistological findings in the B II and control group: p=0.008. No statistically significant correlation was found between the B II and control groups on one side and Roux-en-Y on the other side p=0.167, p=0.444. Sixteen weeks after DGR influence, a shift from precancerous lesions to more severe forms was recorded in B II group, resulting in 30% of hyperplasia, 20% gastritis, 20% metaplasia and as much as 30% of mild or moderate dysplasia cases.

In Roux-en-Y group, no significant changes could be recorded after 8 weeks, i.e. the findings included normal mucosa in 50%, hyperplasia in 30% and gastritis in 20% of the cases as well as at the stomal ridge. As expected, no pathohistological changes were recorded in the control group (Table 2) 16 weeks after the experiment, the additional analyses suggest a statistically significant correlation of pathohistological findings among all groups p=0.033, p<0.001, p=0.033.

Twenty-four weeks after inducing DGR into the gastric stump mucosa by B II intervention, significant differences were observed in the pathohistological finding. The finding of metaplasia was present in 6.7%, dysplasia in 66.7%, early gastric carcinoma in 20% and gastric adenocarcinoma in 6.7%. In Roux-en-Y group mucosa was also normal in 60%; hyperplasia was found in 20% and gastritis in 20%. All animals in the control group had a normal pathohistological finding (Table 2). In the groups observed for 24 weeks, the additional analyses indicated a statistically significant correlation of the pathohistological findings among all three groups: p<0.001, p=0.001, p=0.017. The animals did not develop carcinoma in any of these regions 10 weeks after the beginning of the experiment. A high incidence of mild atypical hyperplasia (62.5%) was observed around anastomosis A. Erosions of superficial mucosa and penetration of glandular cysts into submucosa was also observed. A half (50%) of mild hyperplasia cases associated with anastomosis A and B were accompanied by ulcer.

In week 20, adenocarcinoma, in association with anastomosis A, was microscopically identified in 18.8% of the rats. Adenocarcinoma was diagnosed in 12.5% of the rats in association with anastomosis B. The 40-week group showed a significantly higher incidence of carcinoma (34.4%) associated with anastomosis A compared to anastomosis B (6.3%) (P<0.05).

Discussion

Duodenogastric reflux is one of several identified risk factors for gastric carcinogenesis [4]. Other risk factors include Helicobacter pylori infection and smoking [5,6], which also increase DGR [7,8]. Increased levels of gastric acid have been intensely studied in relation to atrophic gastritis, intestinal metaplasia and dysplasia [9,10]. The experiments performed on rats confirm that DGR causes gastric carcinoma even without any additional exogenous carcinogens [11]. However, increased gastric levels of N-nitrous compounds in patients with gastric resection suggest that DGR contributes to nitrite transformation into potentially carcinogenic substances [12]. The effects of DGR on gastric carcinogenesis have been studied predom-
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stantly on animal stomach corpus, with resected an-
trum [13-15]. Reports on DGR role in antrum car-
cinogenesis are, therefore, rare [11,16]. It is not entire-
ly clear how these alterations of mucosa, induced by
DGR, lead to carcinoma. In humans infected by Heli-
cobacter pylori, DGR causes reactive gastritis and in-
creases intestinal metaplasia [9]. In experiments with
animals, DGR induces mucosal corpus changes prior
to carcinoma development or around the carcinoma-
affected site. These changes include a loss of specific
cells, hyperplasia of mucosal elements, adenocystic
glandular proliferation, erosions and ulcer [17,18]. In
addition, DGR enhances cell proliferation, extends
cell cycle time and S-phase in corpus mucosa [19].

Among the numerous factors (e.g., hypochlo-
rhysria, excessive growth of bacteria, hypogas-
trinemia and duodenogastric reflux), which con-
tribute to the development of gastric stump carci-
noma, long-term exposure of gastric mucosa to
bile and pancreatic juice is singled out as a strong
factor since stump carcinomas are frequently en-
countered on anastomosis. B II anastomosis is af-
fected by greater reflux than that of B I [20-22].
Based on the previous research, as well as the as-
sumption that B II is a reflux surgery, we decided
to focus solely on the role of duodenogastric re-
flux in that group (along the 8, 16 and 24 weeks’
timeline) without adding any carcinogens. Such
results confirm a significant role of duodenogastric
reflux in the formation of precarcinogenic
gastric lesions. We have further studied the pro-
cess of carcinogenesis induced by DGR.

Biliary acids affect the gastric mucosa resulting
in backward diffusion of H ions through the mucosa,
which makes it easier for potential carcinogens to
penetrate through the mucosal barrier towards the
mucosal stem cells, where their effects are exerted
[23,24]. In normal conditions, gastric content is ster-
ile; however, duodenal juice reflux and decreased
acid secretion can lead to bacteria colonization in the
stomach. There is a solid correlation between the
pathohistological findings between DGR quantity,
gastric juice pH and the duration of contamination
[25]. Higher pH values, nitrite levels and the number
of bacteria with nitrate-reductase were found in pa-
tients after B II, compared to B I.

Takamiya et al. have reported on the results of
an experimental study on rats, where the rats, which
were subjected to BII gastrectomy, developed gas-
tric stump carcinoma without any additional expo-
sure to carcinogens [26]. This result was also sup-
ported by a couple of other studies and has been
widely acknowledged. Such tumors, usually adeno-
carcinoma, occur at the gastrojejunostomal sites
and are accompanied by pathohistological findings,
including pseudo pyloric metaplasia, adenocystic
changes and adenoma [19,27-29]. Atrophic gastritis,
intestinal metaplasia, cystic glandular dilatation
and foveolar hyperplasia are frequently encoun-
tered in rats with BII intervention around stoma.
Such pathohistological findings were also recorded in rats with DGR [19,29]. There is a close correlation between postgastrectomic duodenal reflux, foveolar hyperplasia and the cell kinetics parameters [9,30]. Foveolar hyperplasia represents the reactive regeneration of gastric mucosa as a response to duodenal contact effects and includes an increased cell proliferation with the vertical shifting (cell shifting from the bottom towards the surface of crypts) and expansion of proliferating zone has been recorded following partial gastrectomy [31-34]. Such observation is in accordance with the results of cell kinetics monitoring in experimental carcinogenesis after B I and B II gastrectomy [35].

The results indicate that the direct contact of duodenal juice with gastric mucosa induces precarcinogenic lesions in correlation with the duration of exposure to DGR in such a way that the incidence of hyperplasia and ulcerous changes decreases as the experiment progresses, to be followed by an increased percentage of irreversible lesions and carcinoma around the anastomotic lesions. It is assumed that gastric stump carcinogenesis is also associated with the surgery-induced altered environment since several reports suggest the role of changed pH [21] or bacterial flora in gastric juice [36,37]. Several different types and degrees of gastritis were associated with BII anastomosis. Some authors indicate a correlation between sub chronic ulcers, co-existent with adenocarcinoma [38]. Our findings do not support a direct histological correlation between these types of lesions.

From surgical perspective, the presented findings can be associated with the choice of surgical technique - procedure.

**Conclusion**

Long-term exposure of gastric mucosa to biliary and pancreatic juices is singled out as a powerful factor for gastric carcinoma development, which explains a high incidence of gastric stump carcinoma at anastomosis. Duodenogastric reflux is in direct correlation with the evidence of precarcinogenic lesions and duration of mucosal exposure to it. The direct contact of duodenal juice with gastric mucosa induces the formation of precarcinogenic lesions. The incidence of reversible changes is reduced in time, while the incidence of irreversible changes and carcinoma is increased. The incidence of Duodenogastric reflux-induced adenocarcinoma is possible as early as 20 weeks after the experiment, which is significantly earlier than reported by other authors. These findings should be checked on humans.

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**Table 2. Comparative analysis of pathohistological finding with regard to surgery type**

<table>
<thead>
<tr>
<th>Time (weeks)</th>
<th>Type of surgery</th>
<th>Pathohistological finding</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vreme (nedelja)</td>
<td>Vrsta hirurgije</td>
<td>Patohistološki nalaz</td>
<td>Ukupno</td>
</tr>
<tr>
<td>8</td>
<td>Billroth II</td>
<td>4 1</td>
<td>5 100%</td>
</tr>
<tr>
<td></td>
<td>Roux-en-Y</td>
<td>3 1</td>
<td>5 100%</td>
</tr>
<tr>
<td></td>
<td>Control gr.</td>
<td>5</td>
<td>5 100%</td>
</tr>
<tr>
<td>16</td>
<td>Billroth II</td>
<td>3 2 2 3</td>
<td>10 100%</td>
</tr>
<tr>
<td></td>
<td>Roux-en-Y</td>
<td>5 3 2 3</td>
<td>10 100%</td>
</tr>
<tr>
<td></td>
<td>Control gr.</td>
<td>10</td>
<td>10 100%</td>
</tr>
<tr>
<td>24</td>
<td>Billroth II</td>
<td>9 3 3 6.7% 66.7% 20% 6.7%</td>
<td>15 100%</td>
</tr>
<tr>
<td></td>
<td>Roux-en-Y</td>
<td></td>
<td>15 100%</td>
</tr>
<tr>
<td></td>
<td>Control gr.</td>
<td></td>
<td>15 100%</td>
</tr>
</tbody>
</table>

**Legend:** Pathohistological findings: 0 - Normal mucosa, 1 – Hyperplasia, 2 – Gastritis, 3 – Metaplasia, 4 – Dysplasia, 5 – Early carcinoma, 6 – Carcinoma

**Legend:** Patohistološki nalaz: 0 - Normalna mukoza, 1 – Hiperplazija, 2 – Gastritis, 3 – Metaplazija, 4 – Displazija, 5 – Rani karcinom, 6 – Karcinom
References


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