MORPHOLOGICAL CHANGES IN THE STOMACH MUCOSA OF RATS CAUSED BY ENDOGENOUS BILE ACIDS

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“Bile reflux” is a common term to denote a process of placing duodenal contents in the stomach and/or lower oesophagus. It is most often associated with functional or organic failure of the pylorus and is a not uncommon postoperative condition after pyloric section, resection or by-passing. Gastrotoxicity of the replaced small intestinal mixture leading to lesions in gastric mucosal barrier, is caused by an increased ability to reabsorb hydrogen ions along with migration of blood proteins and electrolytes towards lumen of the stomach. Consequently, histamine secretion becomes increased, leading to inflammatory and haemorrhagic changes or ulcer niches.

The aim of the study was to demonstrate histological and microscopic changes in the gastric mucosa following reflux and to determine if long-term exposure to refluxed duodenal contents will produce tumorous changes in the organs tested.

Material and methods. The study consisted of 25 mature female Wistar rats weighing 180-200 g. Bile reflux to the stomach was produced experimentally by surgical drainage. Final evaluation was performed after 55 weeks.

Results. Findings were as follows: gastric changes were noted in basal and parietal cells, no tumorous foci were found in histological samples. Slight morphological changes can be caused by short periods of gastric mucosa exposure to the gastrotoxic small intestinal mixture.

Conclusions. Endogenous bile acids cause morphological changes in the stomach mucosa of rats. In particular, these changes affect the ultrastructure of basal and parietal cells. No neoplastic foci were found in the examined organs.

Key words: bile reflux, operations, changes in gastric mucosa

“Bile reflux” is a process by which backflow of duodenal contents enter into the stomach. As a result of duodenal content reflux, the stomach mucosa is exposed to alkaline mucus with a pH ranging from 8 to 9.5. This high pH is produced by Bruner’s duodenal glands such that urogastrone (a peptide that inhibits gastric secretion), bile, pancreatic enzymes (mainly proteolytic enzymes such as trypsogen, chymotripsine, procarboxypeptidase) and lipolytic enzymes may function properly.

Insufficiency of the stomach and gut barrier can be caused by operative procedures that involve cutting, excising or shunting of the pylorus. These procedures can lead to inflammatory lesions of the mucosa, atrophic and inflammatory changes, and cellular metaplasia (1-5). Some sources even consider these changes to be premalignant lesions (6, 7).

One of the first researchers who focused his attention on the importance of bile reflux to the stomach was Beaumont in the year 1833.
(8). At the end of 19th century, bile reflux was described as post-surgical complications by Wolffer and Billroth (8). The decades following brought research by Braitwait (8), Fisher and Cohen (9). In the 1870s, Alexander Williams and Toye connected an occurrence of clinical symptoms with lack of proper functioning of the pylorus. In 1962, Du Plessis (8) was the first to describe that syndrome as “Alkalin reflux gastritis” (ARG). Intensification of pathological changes in the stomach mucosa depends on the type of procedure that was undertaken. BII-type draining procedures result in disadvantageous circumstances that can cause occurrence of clinical symptoms of ARG. Because there was a limited number of cases that could help examine the morphological aspects of changes in the upper part of the alimentary tract after undergoing some type of procedure, we were urged to undertake histological and ultrastructural research of the stomach mucosa of Wistar rats which underwent the same procedures that caused bile reflux in humans.

Aims of the research:
1. To reveal morphological changes in the stomach mucosa of rats after procedures that could cause duodenal content reflux.
2. To determine if long-term exposure of duodenal contents cause malignant lesions in the researched organ.

MATERIAL AND METHODS

Twenty-five adult rats were used in the research; they were Wistar strain females with a body weight of 180-200 g. Rats were taken from the Central Animal House of the Medical University of Silesia in Katowice. Animals were divided into 3 groups – a control group (5 rats) in which only laparotomy was conducted, a group in which gastrotomy was conducted with no other surgical intervention (10 rats), and a group of 10 animals in which gastroenterostomy was performed between the greater curvature of the stomach and side of the first intestinal loop, at a the length of 10 mm. The gastroenterostomy was made with a series of single, one-layer stitches using 6-0 silk suture. All animals that underwent opening of the alimentary tract lumen were given a 5% solution of glucose with multielectrolytes for drinking; no solid food was administered to them. Morphological assessments of changes in the stomach mucosa were made using light and electron microscopy 55 weeks after the procedure; in 23 cases, the animals survived the procedure and perioperative period. Animals were then killed via ether narcosis by systemic perfusion of a glutaraldehyde solution. After lavaging the stomach with a physiological saline solution, tissue samples of 1 mm thickness were taken from the gastroenterostomy region (in the researched group) and from the gastrotomy region and the body of the stomach (in the control group). After initial and secondary fixation, epone blocks were cut on an OUM-3 ultramicrotome to obtain semi-thin sections (of 1 um thickness); these were mounted on plain glass slides and stained with toluidine blue for assessment via a Rechner company Uni-War light microscope (1000x magnification). After appropriate trimming of the epone blocks, the tissue was sliced in ultrathin sections, mounted on a copper grid, and contrasted with a saturated solution of uranyl acetate and lead citrate solution.

Specimens were photographed and analysed with a JEOL company JEM 100CX electron microscope.

RESULTS

In the control group and in the group of animals that underwent gastrotomy, no morphological changes in the mucosa were found upon both light and electron microscopic examination.

During the light microscopic examination, animals that underwent gastroenterostomy showed irregular structure of tubular glands with local atrophy of the cell membranes. Electronograms with magnification of 7500x (fig. 1, 2) showed a concentration of transport vesicles (p) in the cytoplasm of parietal cells (O) with simultaneous reduction of the endoplasmic tubule system (k). Mitochondria (m) show symptoms of edema with atrophy of the mitochondrial crests. In principal cells (G), the rough endoplasmatic reticulum (rER) locally suffered from serious dilatation. In other cases, structural atrophy occured.

DISCUSSION

In patients who did not undergo any procedures, “bile reflux” can be asymptomatic and is by some sources treated as a physiological phenomenon (8, 9, 10).
Harmful effects of duodenal contents on the stomach mucosa arouse controversy in cases of patients who underwent stomach surgery and in cases of mucosa barrier disorder. Reflux of duodenal contents into the stomach caused so called “spurious proliferation” in the gastric foveola area (pseudohyperplasia fovealis) as a result of chronic inflammation of the stomach (11). As some sources demonstrate (4, 8, 12), the type of procedure conducted as well as exposure time may influence morphological changes of the stomach mucosa while in contact with bile. It was proved (2, 7, 11) that a BII-type distal gastroenterostomy increases the risk of stomach cancer, while the smallest changes occur after Roux-en-Y gastric bypass surgery, considered by some as an anti-reflux procedure. Inflammatory changes (2, 11, 14) are most common in areas adjacent to those influenced by surgical procedures. Such areas may also become malignant after long-term influence of refluxed bile (6, 13). Obtained results do not show such intensity of changes as suggested by a number of sources (14, 15, 16). It may be due to a relatively short observation time, which is a consequence of the short lifespan of examined animals.

Taking into consideration the fact that morphological changes adjacent to surgically treated areas are a considerably well-known issue, more time was devoted to research a response of the stomach mucosa remote from the surgically-treated areas. Changes visible in a light microscopic examination are minimal, yet more attention should be devoted to results of electron microscope examination which show symptoms of edema and widening of the tubule system of the rough endoplasmatic reticulum in parietal and principal cells. This may be a result of higher activity of redox systems and increased protein synthesis. However, these changes are reversible as no vital signs of catabolic processes were found which would result in increased lysosome, cytolysosome or phagosome count.

CONCLUSIONS

1. Endogenous bile acids cause morphological changes in the stomach mucosa of rats. In particular, these changes affect the ultrastructure of basal and parietal cells.
2. No neoplastic foci were found in the examined organs.

REFERENCES


**COMMENTARY**

A long-time ago, surgeons noticed that deprivation of the pylorus following surgery lead towards the development of symptoms and significant stomach mucous membrane changes. Thus, entero-enterostomy after BII operations or Traverso’s method, instead of Whipple’s procedure were undertaken. Clinical observations were and are a stimulant of experimental tests; the presented study is such an example. The study demonstrated the depth of gastric mucous membrane changes due to biliary reflux. However, the short observation period should not bring to light oncological suspicions because after 55 weeks, one should not expect neoplastic transformation in the stomach. Gastric stump carcinomas after BII resections are usually observed tens of years after the operation. In most cases, one should try to limit the possibility of duodenal content reflux to the stomach. Unpleasant symptoms, such as bitter taste in the mouth, might pose a significant problem for many patients. In conclusion, one should try to avoid operations which eliminate or bypass the pylorus due to an increased oncological danger, as well as a decreased quality of life after the operation.

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