

The bacterial flora and the changes of the N-nitrosamine concentration in the operated stomach

Flora bakteryjna i zmiany stężenia N-nitrozoamin w operowanym żołądku

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The studies included 170 patients (103 men and 67 women) aged 23 to 66 years, operated on because of an ulcerous disease. Depending on the method of surgical treatment patients were divided into 5 groups (those after gastric resection with Rydygier's method, after resection with the Billroth II method, after trunk vagotomy with pyloroplasty, after highly selective vagotomy, and after gastro-enterostomy). In all patients a qualitative estimation of the nitrate-reducing bacteria was carried out, as well as a chromatographic test of the selected N-nitrosamine concentration in the gastric juice. It was shown that the changes in the quantity of the nitrate-reducing bacteria and in the N-nitrosamine concentration depended on the type of surgical intervention conducted. The largest mean content of nitrate-reducing bacteria and the highest average N-nitrosamine concentration were confirmed in the gastric juice of patients after gastroenterostomy and after gastric resection with the Billroth II method, and the lowest values - in patients after highly selective vagotomy.

Introduction

In the surgical treatment of the ulcerous disease of the stomach and of the duodenum a wide variety of operative methods is still being used to this day. These operations frequently eliminate, shunt or paralyse the activity of the pylorus. The prolapse of the function of the pylorus disturbs the emptying of the stomach, facilitates the duodeno-gastric reflux, which creates favourable conditions for the growth of bacteria in the operated stomach. The quantitative and qualitative development of the gastric microflora favours the release of oxygenases, enzymes which participate in the activation of procarcinogens, including in the formation of N-nitrosamines [4,6,7,9,12,17,18,20,21]. We may however ask to what extent the nitrate-reducing bacteria influence the increase in the operated stomach of the N-nitrosamine concentration and what is the link with the operative method applied. The fact that in the opinion of many authors, N-nitrosamines participate in the carcinogen-

Badaniami objęto 170 chorych (103 mężczyzn i 67 kobiet) w wieku 23-66 lat operowanych z powodu choroby leżardowej. Uwzględniając sposób leczenia chirurgicznego chorych podzielono na 5 grup (chorzy po resekcji żołądka sposobem Rydygiera, po resekcji sposobem Billroth II, po wago- tomii z pyloroplastyką, po wysoce wybiórczej wago- tomii, po zespoleniu żołądkowo-jelitowym). U wszystkich dokonano oceny jakościowej bakterii redukujących azotany oraz chromatograficznego badania wybranych N-nitrozoamin w soku żołądkowym. Wykazano, że zmiany ilości bakterii redukujących oraz stężenia N-nitrozoamin zależą od rodzaju wykonanego zabiegu operacyjnego. Największą przeciętną zawartość bakterii redukujących i największe średnie stężenie N-nitrozoamin stwierdzono w soku żołądkowym chorych po zespoleniu żołądkowo-jelitowym i po resekcji żołądka metodą Billroth II, a najniższe wartości – u chorych po wysoce wybiórczej wago- tomii.

esis process in the stomach, confers a certain importance to this problem [1,2,8,9].

Material and method

The studies included 170 patients (103 men and 67 women) aged 23 to 66 years operated on because of an ulcerous disease. In a period of 6 months to 33 years preceding the control tests in 38 patients partial gastrectomy was carried out with Rydygier's method, in 36 - resection with the Billroth II method, in 35 - trunk vagotomy with pyloroplasty, in 41 - highly selected vagotomy and in the remaining 20 - gastroenterostomy. 88 patients were operated on because of a gastric ulcer and 82 because of a duodenal ulcer. All groups were comparable in the age, in the basic disease which was the cause of the operation, and in the time that elapsed after the surgical operation.

The control group was made up of 72 patients (45 men and 27 women) aged 21 to 69 years, treated conservatively because of an ulcerous disease (37 cases of gastric ulcers and 35 cases of duodenal ulcers).

All patients were included in a unique model of control consisting of the following:

- endoscopy of the upper segment of the alimentary tract;
- study of the gastric microflora;

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c) chromatographic determination of the N-nitrosamine concentration in the gastric juice.

Identification of the nitrate-reducing bacteria

The material for bacteriological tests comprised samples of the gastric content taken during the gastroscopy. In estimating the microflora of the operated stomach the occurrence of the nitrate-reducing bacteria was considered, mainly of the bacteria of the *Enterobacteriaceae* type, as well as of the Gram-negative non-fermenting rods. The culture and identification of the bacteria were conducted according to the principles presented by Hill, Zaręba and Borowski [9,26], whereas Hoefer's technique was used for the quantitative bacteriological tests [26].

Determination of the N-nitrosamine concentration in the gastric juice

The material for studies included samples of gastric content taken before eating, in the early morning. The subjects analysed were warned not to consume any canned meal or fish products for at least 14 days before the test. The determination of the N-nitrosamines was carried out chromatographically (HPLC) according to the analytical procedure presented by Lin et al., Xu and Reed [13,25]. In the analyses standards from the Sigma (USA) of the following N-nitrosamines were used: N-nitrosodimethanolamine, N-nitrosodimethanolamine, N-nitrosomorpholine, N-nitrosodiethylamine.

For each of the samples tested the concentration of the respective N-nitrosamines was summed and expressed in mmol/l.

Methods of statistical analysis

The results of the studies were statistically elaborated using SAS for IBM PC rel. 6.03. The hypothesis of distribution normality was checked with the W-test given by Shapiro-Wilk (SAS Procedures Guide rel. 6.03). The Wilcoxon test for the estimation of the significance of the differences was used. All decisions were taken at the statistical level $p < 0.05$.

Results

The content of nitrate-reducing bacteria in the stomachs of the patients examined varied from 10^2 ml^{-1} to 10^9 ml^{-1} , including 25 cases of culture being positive, in the further 45 cases the quantity of bacteria amounted to 10^2 ml^{-1} , in 24 – 10^3 ml^{-1} , in 25 – 10^4 ml^{-1} , in 70 – 10^5 ml^{-1} , in 15 – 10^6 ml^{-1} , in 11 – 10^7 ml^{-1} , in 9 – 10^8 ml^{-1} and in 8 cases the content of bacteria amounted to 10^9 ml^{-1} . The average content of nitrifying bacteria after the operation was statistically significantly higher ($p < 0.0001$) than in the control group. It was simultaneously confirmed that the mean quantity of nitrate-reducing bacteria in the operated stomach differed depending on the surgical method applied (table I). The N-nitrosamine concentration in the gastric juice of all the persons examined remained within wide limits, from 0.00012 to 4.8 mmol/l. The average N-nitrosamine concentration in patients operated on was statistically significantly higher ($p < 0.001$) in comparison with the control group. Among the patients operated on the average N-nitrosamine concentration in the gastric juice depended on the surgical method applied, which is presented in table I.

Discussion

Until recently it was thought that the N-nitrosamines occurring in the alimentary canal were exclusively of exogenous origin. This was supported by their frequent presence in the human environment such as soil, water and air. The main source however of

Table I

Content of nitrifying bacteria and N-nitrosamine concentration in the gastric juice of the operated stomach in relation to the type of intervention practised.

Zawartość bakterii redukujących azot i stężenie N-nitrozoamin w soku żołądkowym pacjentów po zabiegach operacyjnych na żołądku w zależności od typu zabiegu operacyjnego.

Patient groups	Quantity of nitrifying bacteria in 1 ml of the gastric juice $\bar{x} \pm \text{SD}$	N-nitrosamine concentration in the gastric juice $\bar{x} \pm \text{SD}$
Group I – patients after resection with the Rydygier method	$1.6 \times 10^5 \pm 4.6 \times 10^4$	0.31 ± 0.05
Group II – patients after resection with the Billroth II method	$2.0 \times 10^7 \pm 2.9 \times 10^6$	0.38 ± 0.08
Group III – patients after vagotomy with pyroloplasty	$2.8 \times 10^4 \pm 1.5 \times 10^3$	0.27 ± 0.06
Group IV – patients after highly selective vagotomy	$4.0 \times 10^3 \pm 2.6 \times 10^2$	0.18 ± 0.12
Group V – patients after gastroenterostomy	$6.3 \times 10^7 \pm 3.1 \times 10^6$	0.46 ± 0.15
Total	$4.63 \times 10^5 \pm 2.9 \times 10^4$ *	0.31 ± 0.05 *
Control group	$3.6 \times 10^3 \pm 1.5 \times 10^2$ **	0.09 ± 0.001 **

\bar{x} – arithmetic mean, SD – standard deviation, * to ** – statistically significant differences

exogenous N-nitrosamines and of their precursors (nitrates and nitrites) in food, are mainly meat and pastry meat products. Only in the eighties was it shown that N-nitrosamines could also form in the stomach with the participation of the microflora occurring locally [2,3,5,10,11,14,15,16]. The majority of gastric bacteria originate from the mouth, the upper respiratory tracts and from food. In the healthy human mainly Gram-negative bacteria occur in the stomach (*Streptococcus*, *Lactobacillus*, fungi - *Candida albicans*) and some anaerobes (*Fusobacterium*, *Veillonella*, *Bacterioides oralis*) in the overall concentration of $25 \times 10^3 \text{ ml}^{-1}$ [9,11,19]. From our studies it follows that the mean quantity of bacteria in the operated stomach is statistically significantly higher than in the control group. The development of the bacterial flora however depends on the method of conducting the intervention. The biggest mean content of nitrate-reducing bacteria was confirmed in the gastric juice of patients after gastroenterostomy and after partial gastrectomy with the Billroth II method, then the lowest average quantity – in patients after highly selected vagotomy. Other authors also paid attention to the possibility of a rich bacterial flora developing in the operated stomach [2,10,11,23]. However tests are rarely undertaken to link the results of bacteriological studies of the gastric juice with the concentration of endogenous N-nitrosamines. From the examinations we conducted it follows that together with the quantitative and qualitative development of the bacterial flora in the operated stomach, the endogenous N-nitrosamine concentration in the gastric juice also increases, exceeding the average concentration in the control group. The results we obtained testify that the N-nitrosamine concentration in the gastric juice of the operated stomach, as the quantity of nitrate-reducing bacteria, points to a link with the type of intervention carried out. If we admit as correct the opinion of many authors [2,22,24] about the important role of the N-nitrosamines in the carcinogenesis in the stomach, then on the basis of the studies we

conducted, we may assert that the patients after gastroenterostomy and after partial gastrectomy with the Billroth II method carried out because of an ulcerous disease are particularly threatened by the development of a pathology of the mucosa of the operated stomach.

Conclusions

1. The surgical treatment of the ulcerous disease favours, though in a differentiated way, the development of the bacterial flora and the increase of the N-nitrosamine concentration in the operated stomach.
2. Patients in whom a gastroenterostomy or a partial gastrectomy with the Billroth II method was carried out because of an ulcerous disease, are subjected to particularly large quantities of nitrate-reducing bacteria and to high N-nitrosamine concentrations in the gastric juice.

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