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Histology of gastric biopsies from peptic ulcer patients before and after short-term treatment with omeprazole or H_2-receptor antagonists

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Summary. Sixty patients with duodenal or prepyloric ulcers were given omeprazole (30 or 40 mg o.m.; average period of treatment: 2.9 weeks) or histamine H_2-receptor antagonists (cimetidine 400 mg b.i.d. or ranitidine 150 mg b.i.d.; average period of treatment: 3.5 weeks) for a single period ranging between 2 and 6 weeks. At the end of the treatment period fasting plasma gastrin levels were moderately increased in both groups in comparison with the pretreatment values. Endoscopic biopsies were taken from the oxyntic mucosa at the beginning and at the end of the treatment period. Light microscopy of the biopsies was aimed particularly at determining the number of endocrine cells. In addition, the mucosal thickness and the volume densities of the parietal cells, the lamina propria and the gland lumina were measured. There were no significant differences in the endocrine or parietal cell populations, between biopsies taken from the patients before and after treatment with omeprazole or histamine H_2-receptor antagonists. The mucosal thickness and the densities of the lamina propria and of the gland lumina remained unaffected by the treatment.

Key words: Gastric mucosa – Gastrin – H_2 receptor antagonists – Morphometry – Omeprazole

Introduction

Treatment of peptic ulcer patients relies to a large extent on neutralization or reduction of secretion of gastric acid. In theory, such treatments could directly influence the structure of the gastric mucosa. In addition, elevation of the antral pH might result in hypergastrinaemia, and as a consequence trophic changes such as an increase in mucosal thickness and/or an hyperplasia of endocrine cells might occur in the oxyntic mucosa (Solcia et al. 1986).

The present study was designed to investigate whether the histology of the oxyntic mucosa changed during short-term treatment of peptic ulcer patients with omeprazole or with histamine H_2-receptor antagonists. Particular emphasis was placed on the study of the endocrine cells.

Materials and methods

Sixty patients with endoscopically verified duodenal or prepyloric ulcers were included in the study. Demographic data on the patients are summarized in Table 1.

The patients were randomly assigned to either of the following treatment groups: omeprazole 30 mg (22 patients) or 40 mg (6 patients) in the morning, cimetidine (400 mg in the morning and in the evening, 24 patients) or ranitidine (150 mg in the morning and in the evening, 8 patients). The drugs were given for 2, 4 or 6 weeks, depending on the endoscopic assessment of healing which was carried out after 2 weeks of medication and, if the ulcers were not healed, again at 4 and 6 weeks. Since healing was faster in the patients treated with omeprazole than in those given H_2-receptor antagonists, the average treatment period was shorter in the former group than in the latter: 2.9 weeks vs 3.5 weeks.

Table 1. Demographic data on the patients

	Treatment	
	Omeprazole	H_2-receptor antagonists
	$n=28$	$n=32$
Male: Female	17:11	18:14
Mean age, males (years)	50	52
range	31–84	30–80
Mean age, females	61	63
range	46–76	43–80
Duodenal ulcer: prepyloric ulcer	23:5	23:9

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Endoscopic biopsies were obtained before the start of the treatment and when healing was endoscopically confirmed. At each endoscopy a minimum of six biopsies were taken from the oxyntic mucosa, about 10 cm distal to the cardia along the greater curvature. Three of the biopsies were fixed without delay at room temperature in a modified Karnovsky solution, consisting of 3% formaldehyde, 4% glutaraldehyde and 0.05% picric acid in 0.16 M potassium phosphate buffer, final pH 7.2–7.4. After rinsing, postfixation in 1% osmium tetroxide and enbloc staining with uranyl acetate, the tissue samples were dehydrated in ethanol and embedded in Polybed, which is an epoxy resin.

The three remaining biopsies were fixed in Bouin's solution. Following dehydration in ethanol these biopsies were embedded in paraffin, cut into 6-μm-thick sections, and stained with antibodies directed against chromogranin-A. The antiserum (no. 137, generously provided by Dr. T. O'Connor, V.A. Medical Center, La Jolla, Calif., USA) was raised against adrenal medullary chromogranin-A, and was used at a dilution of 1:160 (indirect immunofluorescence) or 1:640 (immunoperoxidase). Chromogranin antisera are believed to demonstrate all endocrine cells in the human gastric mucosa (Simonsson et al. 1988). Parallel sections were silver stained according to the Grimelius or the Sevier-Munger methods. The Grimelius method stains all endocrine cells of the oxyntic mucosa except the D (somatostatin) cells. The Sevier-Munger method is known to demonstrate 5-hydroxytryptamine-containing enterochromaffin (EC) cells and enterochromaffin-like (ECL) cells selectively (Simonsson et al. 1988; Solcia et al. 1975).

Nine sections (three from each biopsy) were stained according to each of the three methods. Cells displaying a nucleus were counted per field of view in sections cut roughly perpendicular to the mucosal surface and comprising the entire epithelial layer. At the magnification used ($\times 16$ objective lens) the entire mucosa is visible within one field of view. A square grid was inserted into the eye-piece ($\times 12$) and all stained cells within or at the edge of the frame were counted; the frame covered 0.6×0.6 mm. This grid did not always cover the most superficial layers of the mucosa, but the regions of glands containing endocrine cells were always covered.

The resin-embedded tissues were cut into sections 1 μm thick, roughly perpendicular to the mucosal surface, and stained with toluidine blue. The sections were oriented so as to include the surface of the mucosa and a portion of the muscularis mucosae. In some cases, however, the biopsies were quite shallow and contained neither the muscularis mucosae nor the bottom of the glands.

The histological examination of the plastic sections was carried out in a light microscope and the endocrine cells were recognized by their size, shape, localization and tinctorial properties. The examination comprised a qualitative and, whenever possible, a quantitative evaluation. One section from each biopsy was examined. The qualitative evaluation was aimed primarily at detecting any deviation from the normal of the endocrine cells, such as hyperplasia (diffuse or focal), micronodules or carcinoids. In addition, other signs of pathological processes were looked for, such as mucosal atrophy- or hypertrophy, intestinal metaplasia, gastritis, dysplasia and malignancy.

The quantitative evaluation of the plastic sections was carried out only on sections that comprised the entire thickness of the epithelial layer and, as a rule, also a portion of the muscularis mucosae. A randomly chosen band, about 110 μm wide, from the bottom of the glands to the mucosal surface, was analysed by morphometry. For this purpose, the microscope was used with a $\times 100$ oil-immersion objective lens, and the eye-piece was equipped with a 121-point square grid. Using the point-counting method the volume densities of the parietal cells, the endocrine cells, the gland lumina and the lamina propria were determined. The total mucosal volume and the epithelial cell volume, respectively, served as reference volumes; the muscularis mucosae was excluded from these volumes. The volume density of a structure is defined as the proportion of a tissue volume which is occupied by that structure. The total number of test points per band averaged 1000. For further description of the morphometric procedures, see Weibel (1979) and Helander (1976).

The thickness of the epithelial layer was measured, using a $\times 6.3$ or $\times 10$ objective lens and a micrometer inserted into the eye-piece.

Blood samples for determining the fasting plasma gastrin levels were taken from patients in the morning. This was done before the start and at the end of the treatment. Analyses of gastrin contents were carried out with a radio-immunoassay method (Diagnostic Products, Los Angeles, Calif., USA); the detection limit was about 8 pg/ml, and the upper normal limit about 90 pg/ml.

Within the treatment groups comparisons of baseline values with end-of-treatment values were based on the *t*-test for paired observations. Between treatment groups comparisons of the changes during treatment were based on the unpaired *t*-test. The tests were two-sided with a significance level of 5%.

Results

Qualitative evaluation of the biopsies from the patients revealed an increased amount of inflammatory cells in the lamina propria of most of the sections. In the great majority of cases, these cells were observed in the superficial part of the mucosa. In a few cases, there were additional indications of gastritis, such as elongated gastric pits and occasional dilated gland lumina. The number of inflammatory cells before and after treatment appeared to be similar, and there were no striking differences between the two treatment groups.

There were no indications of intestinal metaplasia, dysplasia or malignancy. All the techniques which were used to demonstrate endocrine cells revealed scattered cells predominantly in the basal half of the mucosa (Fig. 1). The cells were round or irregular in shape (Fig. 2) and sometimes displayed cytoplasmic processes of varying length. The number of endocrine cells appeared normal, and there was no diffuse or focal hyperplasia, no micro-nodules and no carcinoids.

The results from the quantitative evaluations are listed in Table 2; only patients for whom data are available from both the start and the end of the treatment period are included. There were no significant differences between patients before and after treatment. In particular, the frequency of endocrine cells in the oxyntic mucosa did not change significantly. This result was consistent with all three histochemical staining methods for endocrine cells, as well as with the plastic sections. It applies equally to patients treated with H_2-receptor antagonists and to those given omeprazole. The density of endocrine cells was higher in the female patients than in the males: in sections obtained from pre-entry biopsies from male patients the stained cells averaged 13/field of view (range = 5–35 cells/field of view; $n = 33$ patients; Sevier-Munger stain). The corresponding figure for the female patients was 32 cells/field of view (range = 5–76; $n = 22$). The difference is highly significant ($P < 0.001$). It is impossible to judge to what extent this difference depends on the selection of patients and to what extent it depends on age and sex (the female patients were on an average 11 years older than the male patients; see Table 1).

Fasting plasma gastrin levels increased significantly during treatment both with omeprazole and with H_2-

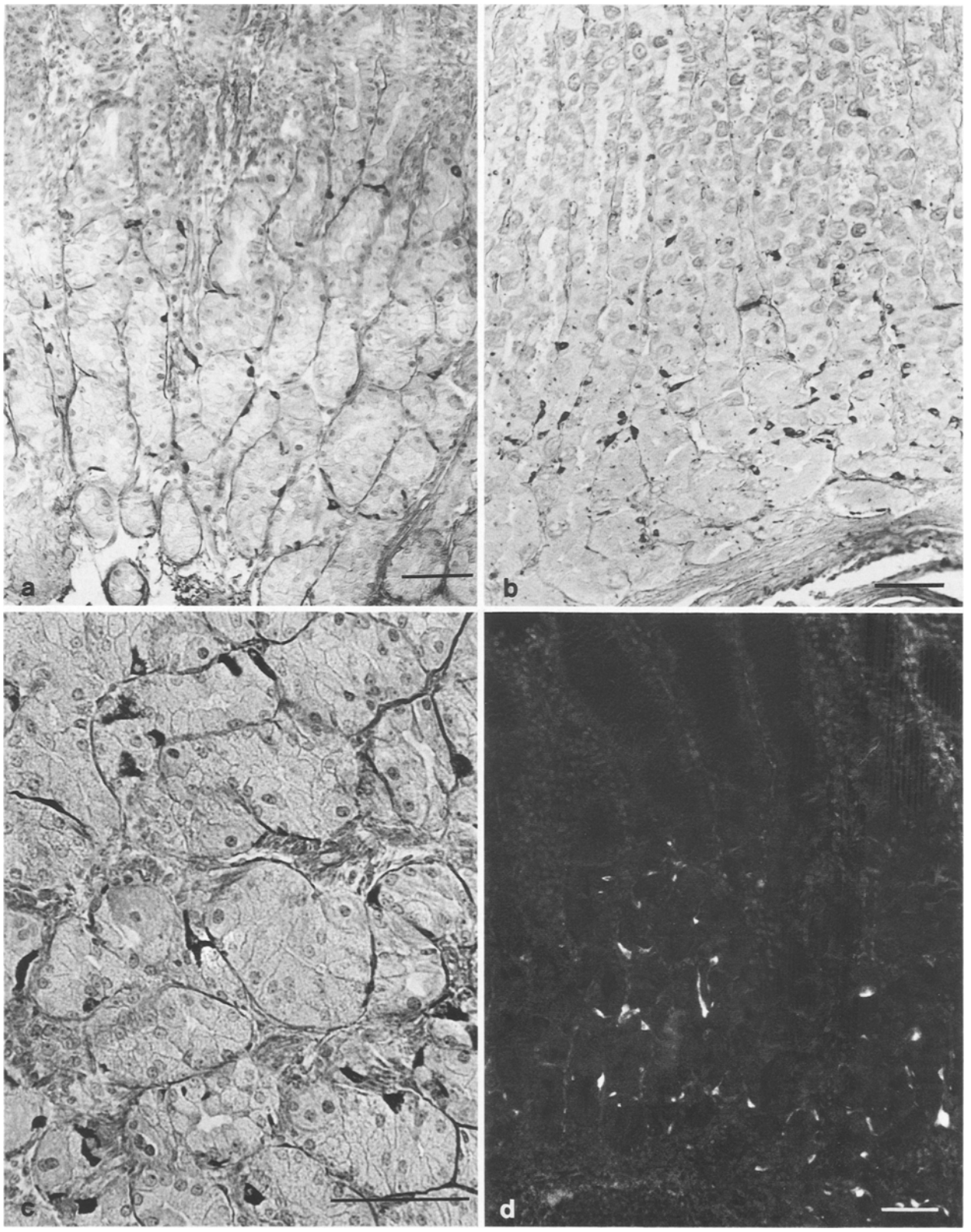

Fig. 1a–d. Endocrine cells in paraffin sections from biopsies visualized by the various staining techniques: Sevier-Munger silver stain (**a** ×125; **c** ×275), Grimelius silver stain (**b** ×125), and antibodies against chromogranin-A (**d** ×110). Most endocrine cells are found in the basal portion of the oxyntic mucosa, but a few of them are also observed in more superficial mucosal layers. **c** illustrates the polymorphism of the various endocrine cells, some of which display long, slender cytoplasmic processes. *Bar*, 100 μm

Table 2. Morphometrical data on oxyntic mucosa in biopsies from peptic ulcer patients (means ± SEM) (numbers of patients in parentheses)

Structure	Units	At entry of omeprazole treatment	At end of omeprazole treatment	At entry of H_2-antagonist treatment	At end of H_2-antagonist treatment
Lamina propria volume density	% of mucosal volume	38.5 ± 3.2 (9)	39.0 ± 1.6 (9)	41.1 ± 2.5 (16)	41.2 ± 2.3 (16)
Gland lumen volume density	% of mucosal volume	2.0 ± 0.3 (9)	1.4 ± 0.2 (9)	1.5 ± 0.2 (16)	2.0 ± 0.4 (16)
Parietal cell volume density	% of mucosal volume	12.0 ± 1.2 (9)	14.8 ± 0.8 (9)	11.6 ± 0.9 (16)	10.5 ± 0.9 (16)
Parietal cell volume density	% of epithelial volume	19.5 ± 1.8 (9)	25.3 ± 1.9 (9)	19.7 ± 1.0 (16)	17.9 ± 1.1 (16)
Parietal cell nucleus	% of cell volume	6.9 ± 0.4 (9)	8.2 ± 0.5 (9)[a]	8.3 ± 0.6 (16)	9.4 ± 0.7 (16)
Parietal cells	No. per mm^2	626 ± 52 (9)	610 ± 33 (9)	602 ± 52 (16)	547 ± 40 (16)
Endocrine cell densities					
Chromogranin stain	No./field of view	30 ± 2.5 (27)	30 ± 2.0 (27)	32 ± 3.5 (31)	30 ± 2.1 (31)
Grimelius stain	No./field of view	27 ± 3.6 (27)	26 ± 2.1 (27)	23 ± 1.9 (29)	27 ± 1.9 (29)
Sevier-Munger stain	No./field of view	20 ± 3.3 (26)	17 ± 1.8 (26)	22 ± 2.7 (29)	19 ± 2.5 (29)
Plastic sections	No./mm^2	62 ± 6 (9)	53 ± 7 (9)	62 ± 6 (16)	63 ± 8 (16)
Plastic sections	% of mucosal volume	0.26 ± 0.03 (9)	0.24 ± 0.04 (9)	0.26 ± 0.02 (16)	0.25 ± 0.04 (16)
Plastic sections	% of epithelial volume	0.44 ± 0.00 (9)	0.41 ± 0.07 (9)	0.44 ± 0.03 (16)	0.41 ± 0.06 (16)
Mucosal thickness paraffin sections	mm	0.80 ± 0.02 (23)	0.81 ± 0.01 (23)	0.80 ± 0.01 (28)	0.81 ± 0.01 (28)
Mucosal thickness plastic sections	mm	0.85 ± 0.04 (9)	0.85 ± 0.03 (9)	0.83 ± 0.02 (16)	0.84 ± 0.03 (16)

[a] Significantly larger than at entry ($P < 0.05$)

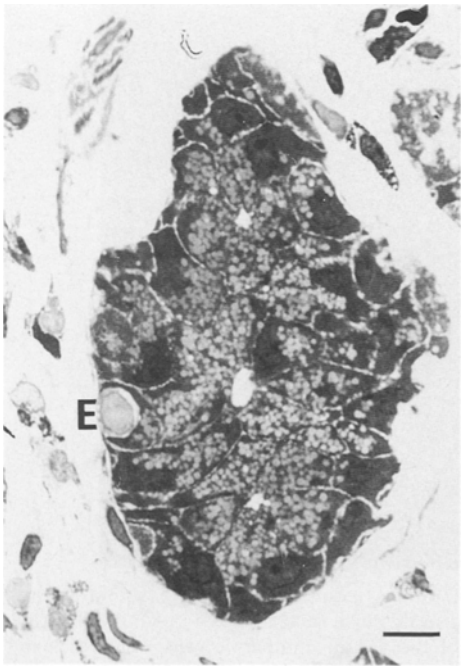

Fig. 2. Toluidine-blue-stained plastic section from oxyntic mucosa in peptic ulcer patient. Endocrine cells (*E*) are recognized by the small size, the relatively large nucleus, the almost lucid cytoplasm, and the peripheral position in the glands. *Bar*, 10 μm. × 800

Table 3. Fasting plasma gastrin levels (pg/ml, median and range)

	Omeprazole $n = 26$	H_2-receptor antagonists $n = 29$
Before treatment	35 (< 10–268)	30 (< 10–347)
At end of treatment	79 (< 10–739)	54 (12–348)
Increased during treatment	19	25
Unchanged during treatment	1	1
Decreased during treatment	6	3

receptor antagonists, but there was no statistically significant difference between these two groups (Table 3).

Discussion

When comparing the results from the two groups of patients it should be borne in mind that omeprazole was given for a shorter period of time than the H_2-receptor antagonists: the mean treatment periods were 2.9 and 3.5 weeks, respectively.

The biopsies from the patients did not reveal any significant changes in the endocrine cell population, visualized by four different staining methods, during the treatment with omeprazole or histamine H_2-receptor antagonists. Moreover, the endocrine cell population did

not differ significantly from that seen in historical controls, comprising healthy, male individuals, 19–35 years old (Helander et al. 1986). In these subjects the parietal cell density was significantly higher, and the lamina propria density significantly lower than in the ulcer patients studied in the present investigation. However, the differences in demographic composition of the groups preclude any more detailed comparison of these data.

There was a highly significant difference in endocrine cell densities, measured in sections stained according to the Sevier-Munger method, between male and female patients. This difference might be a true sex difference, but it might also reflect the difference in age between the patient groups: the mean age of the female patients was about 10 years higher than that of the male patients. Green et al. (1989) recently observed a similar sex difference in the calculated ECL cell density in biopsies from 46 patients with a normal endoscopic appearance. The female patients over 55 years of age displayed 32% more ECL cells/visual field than the males of similar age. Among the younger patients the males had 15% more ECL cells than the females.

Both omeprazole and histamine H_2-receptor antagonists caused increased levels of fasting plasma gastrin, but due to the very large variation between the patients within the two groups it is not possible to decide whether there was any true difference between these two groups. The increase in gastrin levels is undoubtedly related to the reduction of acid secretion as has been demonstrated, for example, by Lanzon-Miller et al. (1987). In previous investigations of ulcer patients, the blood gastrin levels rapidly returned to normal upon discontinuation of treatment (Festen et al. 1984; Karvonen et al. 1986). In a study where patients with reflux oesophagitis were given 40 mg omeprazole once daily for 12 weeks and then 20 mg for another 40 weeks, the median fasting gastrin levels rose from 38 to 55 pmol/l during the first 12 weeks. No further change was observed during the continued treatment (Dent et al. 1989).

The absence of histological changes in gastric biopsies from patients given short-term treatment with acid inhibitors does not preclude the development of such changes during long-term treatment. Data in this field are sparse, but in gastric biopsies from the patients mentioned above, who were treated with omeprazole for 1 year, there were no pathological changes in the argyrophil cell population of the oxyntic mucosa (Dent et al. 1989). Similar results have been obtained in other long-term studies where omeprazole has been given for 1 year or more (Lamberts et al. 1988; Solcia et al. 1989).

It is concluded that a short-term treatment of peptic ulcer patients with omeprazole or histamine H_2-receptor antagonists does not influence the general histology of the oxyntic mucosa, including the frequency of endocrine cells.

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