CLINICAL RESEARCH

Intragastric bacterial activity and nitrosation before, during, and after treatment with omeprazole

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Abstract

Ten healthy volunteers were studied before, during, and after treatment with omeprazole 30 mg daily for two weeks. On the 14th night mean nocturnal (2100-0700) intragastric acidity was significantly decreased by 75% ($p < 0.001$). At 0700, 22 hours after the last dose of omeprazole, there were significant increases in the bacterial count and the nitrite and N-nitrosamine concentrations in the gastric juice ($p < 0.001$). Three days later these changes had resolved.

Short term treatment of healthy volunteers with omeprazole is associated with a short lived increase in the gastric bacterial flora, with endogenous production of *N*-nitroso compounds.

Introduction

Omeprazole, an H^+, K^+-ATPase inhibitor, causes a profound and long lasting decrease in secretion of gastric acid.[1-5] Omeprazole 30 mg daily for one week causes a 97% decrease in 24 hour intragastric acidity in patients with duodenal ulcer, with median pH rising from 1·4 to 5·3.[2] A rise in intragastric pH has often been associated with an increased number of bacteria in gastric juice.[6-12] Oral bacteria in saliva survive in gastric juice with a pH between 4·0 and 5·0, and bacterial proliferation may be expected when the pH is higher.[13] Intragastric bacteria may reduce dietary nitrate to nitrite and also facilitate intragastric formation of *N*-nitroso compounds.[14-15] This broad class of compounds containing the *N*-nitroso group is carcinogenic in a variety of organs and in many animal species.[15]

The object of the present study was to investigate the bacterial flora, and the extent of *N*-nitrosation, of gastric contents before, during, and after treatment with omeprazole.

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Subjects and methods

We studied 10 healthy male volunteers; their mean age was 22·6 years (range 19-26) and their mean weight 78·5 kg (range 67-90 kg), and all were non-smokers. Before the study secretion of gastric acid was measured: the subjects had a mean peak acid output of 26 mmol (mEq)/h (range 20-52 mmol/h) after administration of pentagastrin 6 μg/kg intramuscularly. Antibiotic treatment and antiseptic mouth washes were forbidden throughout the study.

Each volunteer was studied six times: before treatment, on the night of the 14th daily dose of omeprazole, and three, six, nine, and 15 days after stopping treatment. Omeprazole was taken orally as a 30 mg enteric coated capsule before breakfast. To encourage compliance with the drug regimen each subject used a Casio F85 alarm chronograph watch.

On each study day the subjects ate an identical dinner at 1730. Thereafter, they took nothing by mouth except 50 ml still Malvern Water (Schweppes Ltd, St Albans) to facilitate the passing of a nasogastric tube at 2030. Aliquots of gastric juice were aspirated hourly from 2100 until 0700. The gastric juice was analysed as follows.

Acidity—The pH of the gastric aspirates was measured immediately on the bench with a combined glass electrode (Radiometer, Copenhagen) calibrated with buffers of pH 1·09, 4·01, and 7·00.

Microbiology—The samples of gastric juice obtained at 0700 were aspirated using a new, sterile, plastic bladder syringe for each subject. Aliquots of the juice were immediately cultured aerobically and anaerobically by one of us (MP) using standard methods. A surface viable count was performed,[16] and the ability of each bacterial isolate to reduce nitrate to nitrite was assessed.[17]

Nitrate and nitrite concentrations—Gastric juice (5 g) was added to a bottle containing a pellet of sodium hydroxide. The samples were stored immediately at −20°C and later analysed in one batch for nitrate and nitrite concentrations.[18]

N-nitroso compounds—All glassware used in the study was cleaned with acetone (Analar, BDH). Aliquots of gastric juice were stabilised by adding sulphamic acid (50 mg/10 ml juice), then immediately

extracted into ethyl acetate treated with sulphamic acid. The extracts were stored at −20°C and later analysed in one batch. The concentration of total *N*-nitrosamines was measured with a chemoluminescence analyser.[19]

The study was approved by the ethical committee of the Royal Free Hospital, and all subjects provided informed written consent. The statistical significance of observed differences was tested by two way analysis of variance.

Results

During the night after the 14th dose of omeprazole 30 mg there was a consistent decrease in hourly intragastric acidity compared with before treatment (fig 1). Nocturnal hydrogen ion activity fell from a mean (SEM) of 44·1 (9·4) mmol(mEq)/l before treatment to 11·0 (4·5) mmol/l, a 75% decrease ($p<0·001$). In the subsequent studies mean nocturnal hydrogen ion activity was not significantly different from that before treatment, except on the 15th night after omeprazole was stopped, when the acidity was raised (+36%; $p<0·01$; table).

At 0700, 22 hours after the 14th dose of omeprazole, there were significant increases in the total bacterial count in the gastric juice ($p<0·001$), in the nitrite concentration ($p<0·001$), and in the total *N*-nitrosamine concentration ($p<0·001$); there was a non-significant fall in the concentration of nitrate (fig 2). These changes had all resolved three days after omeprazole was stopped (fig 2, table).

When all the data in this study were considered the intragastric bacterial count and nitrite and *N*-nitrosamine concentrations were all significantly and positively related to intragastric pH, with a significant negative correlation between nitrate concentration and intragastric pH (fig 3).

Nine bacterial genera were identified in the gastric contents—namely, *Corynebacterium*, *Staphylococcus*, *Neisseria* (commensal types), *Lactobacillus*, *Veillonella*, *Streptococcus* (α haemolytic, and non-haemolytic), *Bacillus*, *Bacteroides*, and *Acinetobacter*. All these organisms were found in gastric juice before the start of treatment; no new species were isolated during or after treatment with omeprazole. Neither *Enterobacteriaceae* nor *Strep faecalis* were isolated in any gastric aspirate.

The study was well tolerated by all the subjects. There were no clinically important abnormalities in the routine haematological or biochemical safety studies performed before and after 14 days of treatment with omeprazole.

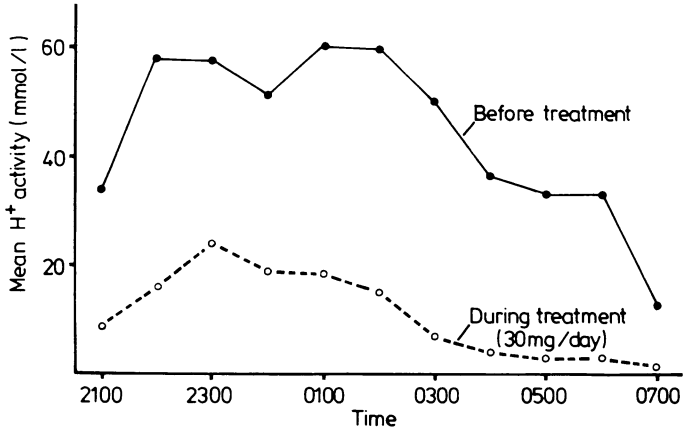

FIG 1—Mean hourly intragastric hydrogen ion (H^+) activity from 2100 to 0700 before and during treatment with omeprazole 30 mg every morning for two weeks in 10 healthy volunteers.

Conversion: *SI to traditional units*—H^+ activity: 1 mmol/l = 1 mEq/l.

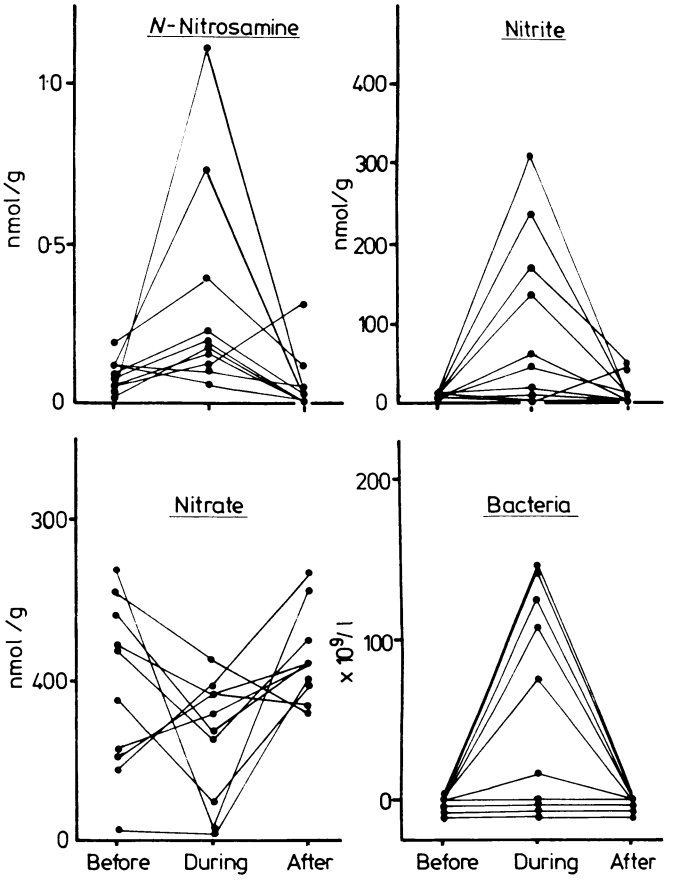

FIG 2—*N*-nitrosamine, nitrite, and nitrate concentrations and total bacterial count in gastric juice at 0700 in 10 healthy volunteers before and during (day 14) treatment with omeprazole 30 mg every morning, and three days after end of treatment.

Conversion: *SI to traditional units*—Nitrite: 1 nmol/g ≈ 46 ng/g. Nitrate: 1 nmol/g = 62 ng/g.

Discussion

Omeprazole 30 mg/day has a considerably more potent antisecretory effect than conventional doses of either cimetidine or ranitidine. When compared with placebo in patients with duodenal ulcer cimetidine 200 mg thrice daily with 400 mg at night caused the median 24 hour intragastric pH to rise from 1·4 to 1·7, and ranitidine 150 mg twice daily caused a rise from 1·4 to 2·4.[2] In a different group of patients with duodenal ulcer omeprazole 30 mg every morning increased 24 hour intragastric median pH from 1·4 before treatment to 5·3.[2] Omeprazole differs from conventional H_2 antagonists not only in the

Mean (SEM) intragastric acidity and bacterial activity at 0700 before, during, and after treatment with omeprazole 30 mg/day in 10 subjects

	Before treatment (day 0)	During treatment (day 14)	Days after end of treatment			
			+3	+6	+9	+15
Nocturnal (2100-0700) hydrogen ion activity (mmol/l)	44·1 (2·8)	11·0 (1·4)**	36·2 (3·0)	49·2 (3·0)	47·7 (2·5)	60·0 (2·7)*
pH at 0700†	1·89	3·09**	1·97	1·72	1·84	1·42
No of bacterial genera/subject	4·5	4·3	1·1	1·9	0·5	0·7
(% nitrate reducing)	(62)	(91)	(91)	(100)	(100)	(100)
(No of subjects with bacteria)	(7)	(7)	(3)	(4)	(2)	(2)
Bacterial count (× 10⁸/l)	0·3	58·6**	3·3	7·7	0·001	0·001
Nitrate (nmol/g)	381 (68·7)	254 (49·9)	454 (35·4)	466 (52·5)	413 (50·2)	532 (54·3)
Nitrite (nmol/g)	7·0 (1·2)	99·5 (34·9)**	15·2 (6·1)	14·2 (3·6)	18·3 (10·5)	7·5 (2·2)
N-nitrosamine (nmol/g)	0·09 (0·02)	0·33 (0·11)**	0·05 (0·03)	0·10 (0·03)	0·09 (0·03)	0·05 (0·02)

*$p<0·01$, **$p<0·001$ compared with before treatment; all other differences non-significant ($p>0·05$).
†Mean hydrogen ion activity expressed as pH.
Conversion: SI to traditional units—Hydrogen ion activity: 1 mmol/l = 1 mEq/l. Nitrate: 1 nmol/g ≈ 62 ng/g. Nitrite: 1 nmol/g ≈ 46 ng/g.

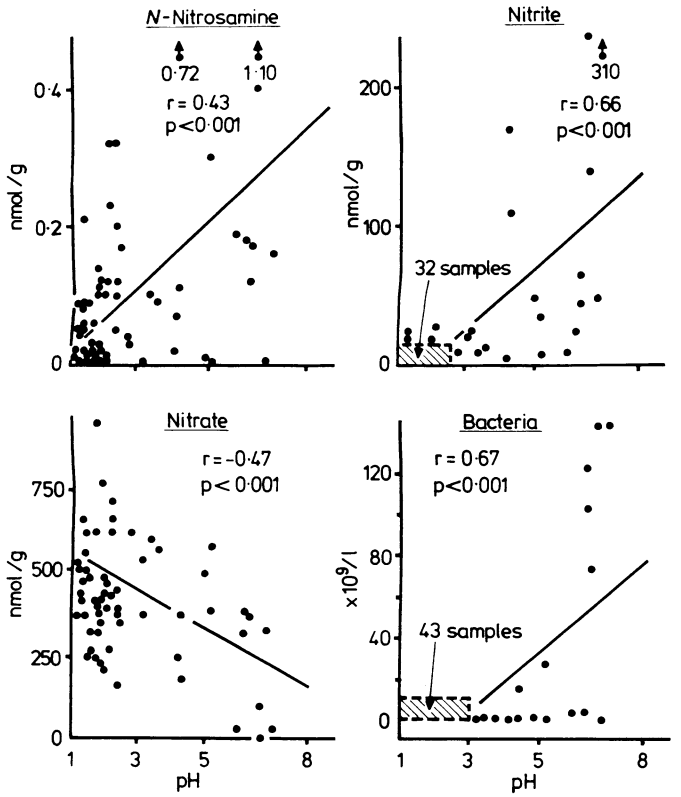

FIG 3—*N*-nitrosamine, nitrite, and nitrate concentrations and total bacterial count related to pH in samples of gastric juice at 0700 in 10 healthy volunteers before and during (day 14) treatment with omeprazole for two weeks, and three, six, nine, and 15 days after treatment.

Conversion: SI to traditional units—Nitrite: 1 nmol/g ≈ 46 ng/g. Nitrate: 1 nmol/g = 62 ng/g.

strength of its antisecretory activity but also in the duration of its action. Omeprazole 30 mg every morning caused virtual anacidity from 1200 to 2400 in patients with duodenal ulcer,[2] quite unlike the intermittent acidity seen at different times of the day in patients receiving full doses of either cimetidine or ranitidine.[20]

The substantial, and sustained, increase in 24 hour intragastric pH caused by omeprazole makes bacterial contamination of gastric contents during treatment with the drug highly likely.[21] This was confirmed by the present study, which showed a significant rise in the number of bacteria in the gastric juice during the night of the 14th day of treatment with omeprazole. The present study may underestimate the changes that occur in patients: healthy subjects may be less susceptible to omeprazole's prolonged antisecretory effect than patients with duodenal ulcer. Omeprazole 30 mg every morning for seven days in nine patients with duodenal ulcer caused a 92% decrease in nocturnal acidity (2100-0700),[2] whereas in this study the same dose for 14 days in healthy subjects caused only a 75% decrease in nocturnal acidity.

During this study concentrations of nitrite and total *N*-nitrosamine in the gastric juice rose significantly; there was a non-significant fall in the concentration of nitrate in the gastric juice. Similar results have been observed in mid-morning aspirates obtained from patients who had not only fasted overnight but had also taken a dose of cimetidine in the morning.[10 12] When the action of cimetidine was investigated in 24 hour studies of healthy volunteers and patients with duodenal ulcer no significant differences in intragastric bacterial count or in nitrite or *N*-nitroso compound concentrations were reported.[11 22] In the latter studies, however, the quantitative analysis of *N*-nitroso compounds was carried out by a simplified procedure that does not necessarily discriminate *N*-nitroso compounds from other related compounds also derived from nitrite or nitrate.[23]

Within three days of treatment with omeprazole being stopped gastric acidity had returned to pretreatment values and the changes in bacterial count and nitrite and *N*-nitrosamine concentrations had been reversed. In patients with duodenal ulcer, however, intragastric acidity may take longer than three days to return to pretreatment values: 24 hour intragastric acidity in such patients was significantly lowered by 26% seven days after two weeks of treatment with omeprazole 30-60 mg/day.[5] It is not clear why the intragastric bacterial population decreased progressively in the days after the end of treatment, although the lowest counts were observed when intragastric acidity was highest.

Full dose treatment with omeprazole is unlikely to be recommended for the long term management of patients with uncomplicated peptic ulceration. The risks of transient bacterial contamination of the stomach are difficult to quantify[15]: complications of many years of unremitting anacidity are rare in the stomachs of patients with pernicious anaemia. For the minority of patients who may require long term treatment with full dose omeprazole simultaneous prophylactic treatment with ascorbic acid or α tocopherol might limit the formation of *N*-nitroso compounds.[15]

For many patients duodenal ulceration is a chronic illness that requires a strategy for long term management. The results of this study suggest that such patients could receive intermittent short courses of full dose omeprazole at times of symptomatic relapse without risking long term intragastric bacterial colonisation.

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References

1 Lind-T, Cederberg C, Ekenved G, Haglund U, Olbe L. Effect of omeprazole—a gastric proton pump inhibitor—on pentagastrin stimulated acid secretion in man. *Gut* 1983;**24**:270-6.
2 Walt RP, Gomes MdeFA, Wood EC, Logan LH, Pounder RE. Effect of daily oral omeprazole on 24 hour intragastric acidity. *Br Med J* 1983;**287**:12-4.
3 Konturek SJ, Kweicien N, Obtulowicz W, Kopp B, Oleksy J. Action of omeprazole (a benzimidazole derivative) on secretory responses to sham feeding and pentagastrin upon gastrin and pancreatic polypeptide in duodenal ulcer patients. *Gut* 1983;**25**:14-8.
4 Londong W, Londong V, Cederberg C, Steffen H. Dose-response study of omeprazole on meal-stimulated gastric acid secretion and gastrin release. *Gastroenterology* 1983;**85**:1373-8.
5 Sharma BK, Walt RP, Pounder RE, Gomes MdeFA, Wood EC, Logan LH. Optimal dose of oral omeprazole for maximal 24 hour decrease of intragastric acidity. *Gut* 1984;**25**:957-64.
6 Gray JDA, Shiner M. Influence of gastric pH on gastric and jejunal flora. *Gut* 1967;**8**:574-81.
7 Giannela RA, Broitman SA, Zamcheck N. Gastric barrier to ingested microorganisms in man: studies in vivo and in vitro. *Gut* 1972;**13**:251-6.
8 Ruddell WSJ, Bone ES, Hill MJ, Walters CL. Pathogenesis of gastric cancer in pernicious anaemia. *Lancet* 1978;i:521-3.
9 Reed PI, Haines K, Smith PLR, House FR, Walters CL. Gastric juice N-nitrosamines in health and gastroduodenal disease. *Lancet* 1981;ii:550-2.
10 Reed PI, Smith PLR, Haines K, House FR, Walters CL. The effect of cimetidine on gastric N-nitrosamine concentration. *Lancet* 1981;ii:553-6.
11 Milton-Thompson GJ, Ahmet Z, Lightfoot NF, *et al.* Intragastric acidity, bacteria, nitrite, and N-nitroso compounds before, during, and after cimetidine treatment. *Lancet* 1982;i:1091-5.
12 Stockbrugger RW, Cotton PB, Eugenides N, Bartholomew BA, Hill MJ, Walters CL. Intragastric nitrites, nitrosamines, and bacterial overgrowth during cimetidine treatment. *Gut* 1982;**23**:1048-54.
13 Bartholomew BA, Hill MJ, Hudson MJ, Ruddell WSJ, Walters CL. Gastric bacteria, nitrate, nitrite and nitrosamines in patients with pernicious anaemia and in patients treated with cimetidine. In: Walker EA, Gricinte L, Castegnaro M, Burzsonyi M, eds. *N-nitroso compounds: analysis, formation and occurrence.* Lyons: IARC, 1980:595-608. (IARC scientific publication No 31.)
14 Anonymous. Cimetidine and gastric cancer. *Drug Ther Bull* 1983;**21**:65-7.
15 Tannenbaum SR. N-nitroso compounds: a perspective on human exposure. *Lancet* 1983;i:629-32.
16 Miles AA, Misra SS. The estimation of the bactericidal power of the blood. *J Hyg (Camb)* 1938;**38**:732-49.
17 Cowan ST, Steel KJ. *Manual for the identification of medical bacteria.* London: Cambridge University Press, 1974:176-7.
18 Cox RD. Determination of nitrate and nitrite at the parts per billion level by chemiluminescence. *Anal Chem* 1980;**52**:332-5.
19 Walters CL, Downes MJ, Edwards MW, Smith PLR. Determination of a non-volatile N-nitrosamine on a food matrix. *Analyst* 1978;**103**:1127-36.
20 Walt RP, Male PJ, Rawlings J, Hunt R, Milton-Thompson GJ, Misiewicz JJ. Comparison of the effects of ranitidine, cimetidine and placebo on the 24 hour intragastric acidity and nocturnal acid secretion in patients with duodenal ulcer. *Gut* 1981;**22**:49-54.
21 Anonymous. Bacteria in the stomach. *Lancet* 1981;ii:906-7.
22 Barnard J, Darkin DW, Howard OM, *et al.* N-nitroso compounds in cimetidine-treated duodenal ulcer patients. *Gut* 1983;**24**:A974.
23 Smith PLR, Walters CL, Reed PI. Importance of selectivity in the determination of N-nitroso compounds as a group. *Analyst* 1983;**108**:896-8.

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