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The effect of 90 days treatment with Omeprazole on 24 hour plasma gastrin profiles in female Wistar rats

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Omeprazole (OM) is a novel irreversible proton pump inhibitor which can virtually abolish acid secretion in the stomach and has great utility in the treatment of acid related disorders [1]. In a 2-year carcinogenicity study conducted as part of the safety evaluation of OM, the contract laboratory performing the study reported the occurrence of carcinoids and enterochromaffin-like (ECL) cell hyperplasia in the oxyntic mucosa of Sprague–Dawley rats [2]. Following the discovery of carcinoids, the clinical programme was suspended and research to clarify the mechanism of carcinoid development was initiated. A further carcinogenicity study was conducted at lower doses in female Sprague–Dawley rats only, in an attempt to produce a "no-effect" dose. This was not successful and resulted in a low incidence of carcinoids even at the lowest dose of 5 μmol/kg (1.73 mg/kg) [3]. Since then, much interest and research has identified and begun to define a clear association between ECL cell hyperplasia, carcinoids and the trophic hormone gastrin [4–6]. It is now widely thought that gastrin, which is secreted in response to high pH and the presence of food in the stomach, is hypersecreted during periods of profound inhibition of acid secretion and during this time high concentrations of plasma gastrin may be sustained [5]. Gastrin has a general trophic effect on the oxyntic mucosa and stimulates ECL cells to divide and proliferate [4, 7, 8]. Therefore, prolonged administration of high doses of the proton pump inhibitor OM will result in profound long term inhibition of acid secretion and sustained hypergastrinaemia, leading to ECL cell hyperplasia and because of the sustained proliferative stimulus, eventually the appearance of carcinoids. If this hypothesis is correct, other potent long acting inhibitors of acid secretion should also induce carcinoids. This is indeed the case and carcinoids have been found in rat carcinogenicity studies of three long-acting H_2-receptor antagonists, namely SK&F 93479 in Wistar rats [9], loxitdine and tiotidine in Sprague–Dawley rats [10, 11], whereas shorter-acting agents such as cimetidine, ranitidine, famotidine and nizatidine were not carcinoid-inducing in carcinogenicity studies.

The case for the causative role of gastrin in the induction of gastric carcinoids is quite strong, but there has been little attempt to define a quantitative relationship between gastrin, the hyperplastic response and the induction of carcinoids. This area of knowledge is a vital component in understanding the precise role of gastrin in gastric mucosal growth and may be of great value if potent inhibitors of acid secretion are to be developed as therapeutic agents and if toxicologists are to continue to evaluate their carcinogenic potential in rats. The present studies were designed to investigate the effect of OM on 24 hr gastrin profiles in female rats taken at intervals over a 13-week dosing period to allow a detailed examination of the total exposure of animals to the gastrin stimulus.

Materials and methods

Female Wistar (Charles River) rats (250–280 g) were group housed in plastic cages on soft Greenwood granules. Food (PRD pellets, Labsure Ltd) and water were provided *ad lib*. The air temperature was maintained at 21 ± 2°, humidity was maintained at 40–60% and fluorescent lighting provided between 0600 and 1800 hr GMT. Each animal was dosed daily by oral gavage with either vehicle, 1, 5, 15 or 100 mg/kg Omeprazole (99.4% pure) suspended in 0.5% gum tragacanth for 90 days. Dosing was carried out between 0800 and 1000 hr each day. Timed blood samples were taken from a lateral tail vein on days 1, 15, 30, 61, and 90. The samples were taken at 3, 6, 9, 12, 16, 20 and 24 hr post-dose on each bleed day. Plasma samples were assayed in duplicate for gastrin by radioimmunoassay using a commercially available kit (Becton Dickinson). Duplicate results did not vary by more than 10%. Gastrin values were calculated as mean ± SE and statistical analysis was performed using Wilcoxon's rank sum test.

Results

Clinical observations and necropsy. There were no clinically observable treatment related effects and at necropsy there were no macroscopic abnormalities in any tissue.

Plasma gastrin. Plasma gastrin concentrations in the control group were fairly constant over 24 hr even at peak feeding times. The mean values were between approximately 70 and 125 pg/mL. Control values were distributed normally and did not change significantly during the 90 day experiment (Table 1).

Mean plasma gastrin concentrations in rats dosed at 1 mg/kg were not significantly elevated at any time during the experiment (Fig. 1A).

In rats dosed with 5 mg/kg OM plasma gastrin concentrations were not significantly elevated above control values on day 1. On day 15, mean peak plasma gastrin concentrations were elevated to 184 ± 72 and 186 ± 46 pg/mL at 3 and 6 hr post-dose, respectively, although there is no statistical difference from control mean values. By 9 hr, mean gastrin concentrations had returned to control levels. Essentially the same picture was presented on day 30 for these animals, except that the values from the treated animals at 3 and 6 hr were statistically different ($P < 0.05$) from concurrent controls. On day 61, plasma gastrin had

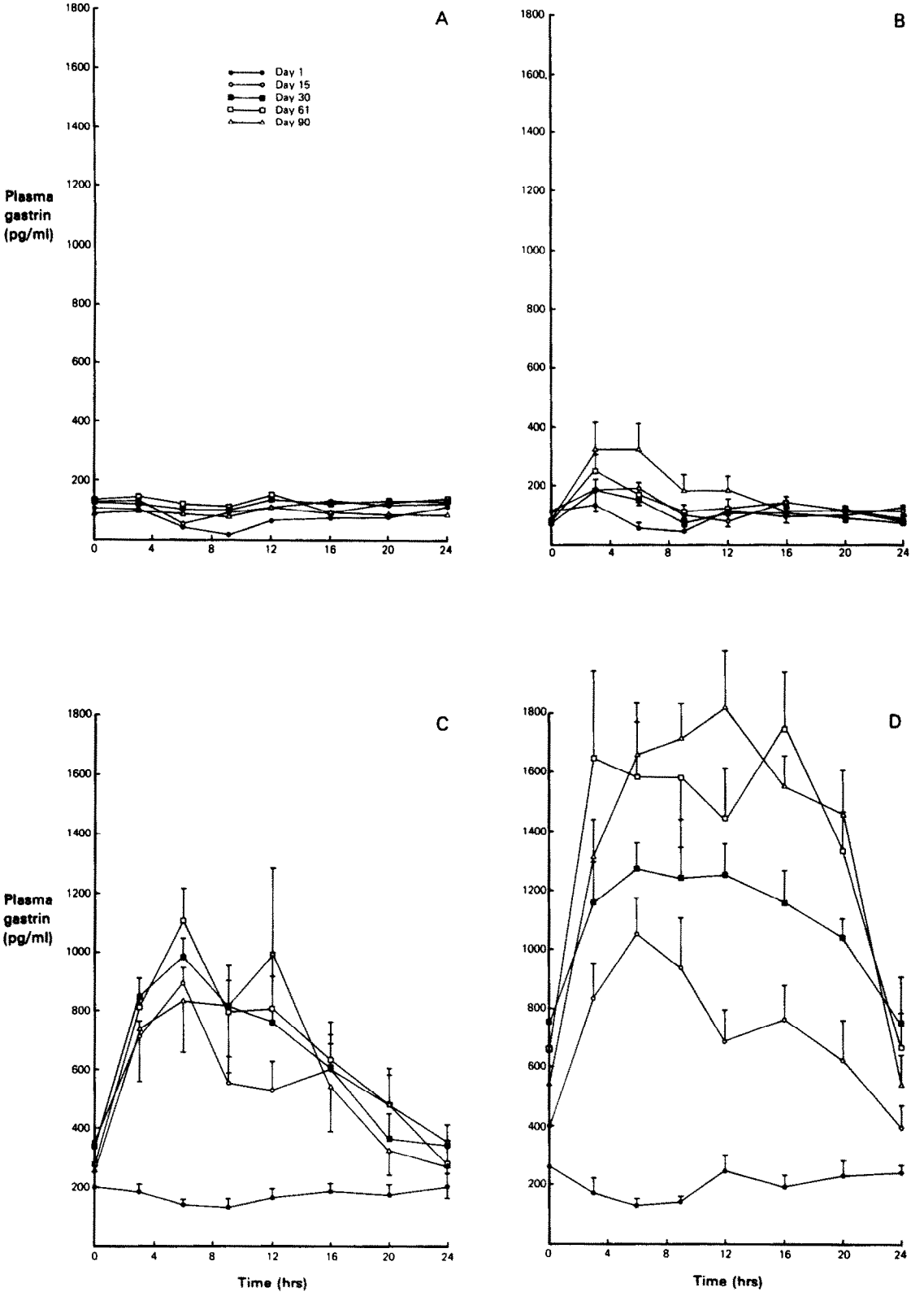

Fig. 1. Circulating plasma gastrin concentrations in rats treated with OM for 90 days. Graph: A, 1 mg/kg OM; B, 5 mg/kg OM; C, 15 mg/kg OM; D, 100 mg/kg OM. Animals were dosed at $t = 0$. In graphs A and B results are expressed as mean ± SE where N = 5. Statistical differences are not highlighted. In graphs C and D, N = 4 from day 30 onwards; premature deaths were due to dosing accidents.

Table 1. Circadian variation of plasma gastrin concentrations in control female rats

	Time (hours post-dose)						
	3	6	9	12	16	20	24
Day 1	94 ± 5	88 ± 16	30 ± 4*	79 ± 8	87 ± 8	69 ± 6	123 ± 18
Day 90	95 ± 10	87 ± 16	77 ± 10	95 ± 9	73 ± 10	81 ± 14	89 ± 17

Animals were dosed with vehicle as described in Materials and methods.
Results are expressed as mean ± SE where N = 5.
* These values were unusually low. No other data approached the low values obtained here. In view of this, it is suggested that this is an aberrant result and should be treated with suspicion.

increased to 254 ± 57 pg/mL at 3 hr but had returned to control values by 9 hr post-dose. On day 90 mean plasma gastrin concentrations were increased above control values until 16 hr post-dose. The maximum plasma gastrin recorded was 319 ± 94 at 3 hr post-dose (Fig. 1B).

Plasma gastrin concentrations in rats dosed at 15 and 100 mg/kg OM were consistently raised above control values at each time point throughout the study. On each successive measurement day, the extent of hypergastrinaemia increased such that on day 90, mean peak plasma gastrin concentrations were increased to 996 ± 285 and 1816 ± 197 pg/mL for animals dosed with 15 and 100 mg/kg OM, respectively (measured at 12 hr post-dose) (Figs 1C and D).

Finally, the individual plasma gastrin concentrations from animals within a treatment group showed a significant spread and made the use of parametric statistics inappropriate. For example, in Fig. 2, day 90 plasma gastrin concentrations in animals 2 and 5 were highly elevated in response to 5 mg/kg OM whereas plasma gastrin concentrations in animal 3 were not elevated above control values at any time.

Discussion

The present study demonstrates a direct relationship between the dose of OM and the circulating concentration of plasma gastrin in Wistar rats. These data support the findings of Ryberg *et al.* [12] and of Carlsson *et al.* [5] who showed that daily oral doses of 40, 125 and 400 μmol/kg (13.8, 43.1 and 138 mg/kg) OM to female Sprague–Dawley rats for 1 week caused a dose related increase in plasma gastrin at 2 and 24 hr post-dose. The results also demonstrate that low doses of OM will stimulate gastrin release but it may take some time to elicit a clear response, e.g. up to 61 days at 5 mg/kg OM. Even high doses of OM appeared to take time to develop a full response and thus it may not be prudent to use short term tests to predict the potential of antisecretory agents to cause hypergastrinaemia, as was suggested by Katz *et al.* [13]. Katz *et al.* [13] used a 5 day dosing regimen and bled the rats on day 5 after overnight food deprivation. It is likely that only high doses of a potent long acting inhibitor such as OM would cause a sustained hypergastrinaemia after 5 daily doses; in addition, food deprivation would diminish the

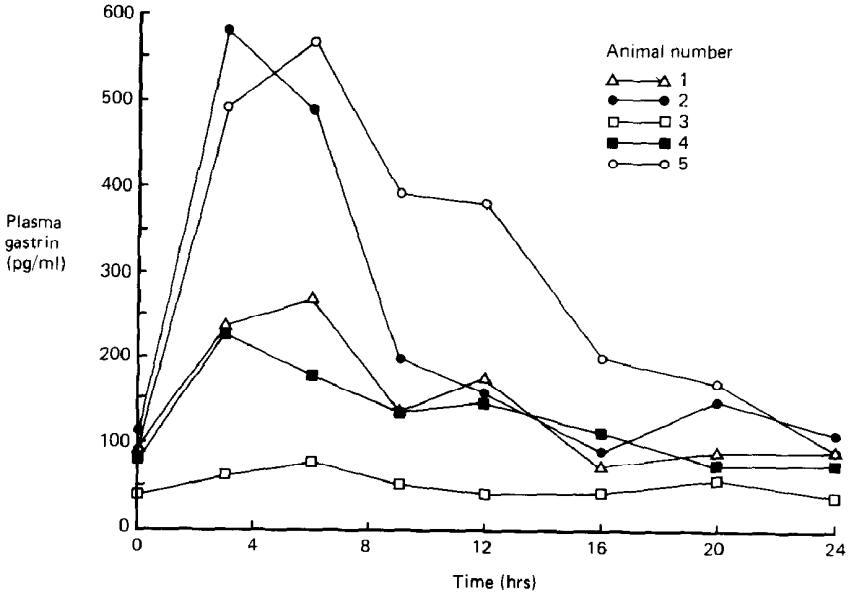

Fig. 2. Circulating plasma gastrin concentrations in individual animals treated with OM (5 mg/kg) for 90 days. Animals were dosed at $t = 0$. All results are means of duplicate samples.

gastrin response [5] such that it would be quite difficult to detect the effects of lower doses of OM, even those which have already been shown to induce carcinoids.

The peak plasma gastrin concentrations measured in rats dosed with 15 and 100 mg/kg OM were generally found between 3 and 12 hr post-dose. During this time there appeared to be a high gastrin plateau, especially from day 30 onwards. Thus, there was a period of at least 9 hr each day in which the stimulatory effects of gastrin were maximal. During the remaining 15 hr gastrin levels were decreasing but still elevated above control values. Animals dosed with 15 or 100 mg/kg OM were therefore under continuous gastrin drive and from the experiences of Ekman *et al.* [2] would have likely gone on to develop ECL cell hyperplasia and perhaps gastric carcinoids. However, the results from the group dosed with 5 mg/kg OM showed that for at least 8 hr of the day, circulating gastrin was at control values. This dose was approximately 3 times greater than the lowest dose in the second OM carcinogenicity study in which a non-carcinoid inducing dose could not be established. The question to be answered is then clear; what systemic exposure to gastrin is required to cause a hyperplastic response and eventual tumour induction? In attempting to answer the question a number of factors must be taken into consideration. Firstly, the present studies were conducted in Wistar rats which may secrete gastrin in response to OM differently from the Sprague–Dawley rats used in the OM carcinogenicity study and therefore the data must be carefully considered before making a direct comparison between present and previous studies. However, the gastrin concentrations shown graphically at 2 hr post-dose after 10 weeks dosing with 10 μmol/kg (3.5 mg/kg) OM [4] were not dissimilar from the values obtained in the present studies at 3 hr post-dose after 60 or 90 days dosing with 5 mg/kg OM. Similarly, the response to 40 μmol/kg (13.8 mg/kg) OM for 3 months by the Sprague–Dawley rats (approx. 1000 pg/mL at 2 hr post-dose) is similar to the response by Wistar rats after 90 days at 15 mg/kg OM (733 ± 173 pg/mL at 3 hr post-dose) reported here. Therefore the data suggest that Sprague–Dawley and Wistar rats respond quite similarly to OM in terms of gastrin secretion. Secondly, there may be differences in the way that different strains of rat respond to equivalent concentrations of gastrin, such that some strains may be more sensitive to the hyperplastic effects of gastrin than others. Thirdly, within each dose group there was marked inter-invididual variation in gastrin concentrations upon OM treatment. Figure 2 represents the individual plasma gastrin concentrations upon 5 mg/kg OM treatment on day 90. It is clear that some individual animals respond very strongly to OM in terms of gastrin secretion while others hardly respond at all. However, it is not known whether those stronger responders within the group would be more likely to become hyperplastic than weakly responsive individuals, so to understand more clearly the trophic effects of gastrin we need to correlate the individual gastrin profiles with the corresponding histopathological findings in the oxyntic mucosa of each animal. Finally, it is not unreasonable to assume that 5 mg/kg OM has not elicited a full gastrin response from the rats after 90 days and that it may take longer to produce sustained hypergastrinaemia at lower doses.

In conclusion, the data presented here describe the 24 hr profile of circulating gastrin in female rats in response to 90 days treatment with OM. A dose of OM reportedly high enough to induce gastric carcinoids in female rats did not cause sustained hypergastrinaemia but did cause a transient hypergastrinaemia soon after dosing. There are no published data on the effects of long-term treatment with OM on circulating gastrin levels in female rats and so the initial question of what systemic exposure to gastrin is required for carcinoid induction is difficult to answer. What these results do suggest, however, is that it may not be necessary to have sustained hypergastrinaemia for the full lifetime of the rat to stimulate the hyperplastic response leading to carcinoid induction. In saying this, it is not our intention to imply that any hypergastrinaemia, no matter how transient would necessarily be carcinoid-inducing; indeed, the H_2-receptor antagonist SK&F 93479 caused significant but transient hypergastrinaemia after dosing at 200 mg/kg to Wistar rats yet, in a 2-year rat carcinogenicity study, no carcinoids were seen at that dose [14]. In humans, there is good evidence that sustained hypergastrinaemia for many years, such as that associated with fundic atrophic gastritis [15], can cause ECL cell hyperplasia and gastric carcinoids and there is some concern that long-term treatment with omeprazole may raise serum gastrin sufficiently to induce gastric carcinoids. The current evidence suggests this is unlikely to occur since omeprazole treatment of gastric ulcer or reflux oesophagitis patients will cause only a moderate hypergastrinaemia which can be controlled by lowering the dose or suspending treatment intermittently [16].

On a more practical level, in experiments designed to predict the potential of antisecretory compounds to stimulate ECL cell hyperplasia it is clear that peak plasma gastrin levels are important. Measurements taken up to 9 hr post-dose are likely to detect any increase in plasma gastrin in animals not under continuous gastrin drive. In addition, a 24 hr "trough" measurement would be of value to determine if animals are continuously hypergastrinaemic.

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