AN EFFECT INDUCED BY CIMETIDINE ON CRYPT CELL PROLIFERATION IN THE RAT SMALL INTESTINE

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SYNOPSIS

Using a stathmokinetic technique, to study the rate of crypt cell proliferation, it was found that in rats treated with cimetidine, there was a statistically significant increase in mitotic rate in the crypts of both jejunum and ileum as compared with that in saline injected controls. The significance of these findings is discussed.

INTRODUCTION

Because it seemed unlikely that gastric pH changes could affect the pH of the colonic contents, the finding of hyperplasia of both gastric and colonic mucosa in dogs following gastric resection (Zufarov et al. (1973 and 1974)) appeared to suggest that the fall of gastric secretion in this case was probably not important in producing this effect.

However, truncal vagotomy, also known to lead to greatly decreased gastric acid secretion, has been observed by Ballinger et al. (1964) and Liavag and Vaage (1972) to result in atrophy of the small intestinal mucosa of the dog and rat, followed by a compensatory hyperplasia 5 weeks post-operatively, thus possibly suggesting a trophic role for the vagus nerve.

It was hoped therefore, that if a pharmocological agent was administered which would decrease the secretion of gastric acid by a direct action on the gastric mucosa and not via the vagus nerves, that this might possible elucidate to some extent the role of gastric acid secretion in the control of crypt cell proliferation in the small intestinal crypts of the rat.

Such a substance is cimetidine (Pounder et al. (1977) (Henn et al. (1975). So it was decided to observe the effect of this agent, delivered in a dosage known to result in a prolonged fall in gastric acid secretion, on the rate of crypt cell proliferation in the rate small intestine.

MATERIAL AND METHODS

METHODS

Male Sprague-Dawley albino rats weighing between 400-560g maintained at approximately 22°C and darkness between

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2200 hours and 0800 hours were used in this experiment.

Cimetidine, supplied as Tagamet (S.K.F. labs.) -200 mgm/2 ml ampoules was injected intraperitoneally into 5 rats in a dosage of approximately 50 mg/kg every 2 hours commencing at 0953 hours, and continuing until 1610 hours on the same day.

This relatively high dose of cimetidine was used to ensure that there was a prolonged resulting decrease in gastric acid secretion, as demonstrated by Maslinski and Sewing (1977).

A control group of 5 rats were injected intraperitoneally every 2 hours with a similar amount of normal saline commencing at 1010 hours, and finishing at 1620 hours.

Rats of both the test group and controls were killed at hourly intervals between 1 and 4 hours after an intraperitoneal dose of 0.45 — 0.5 mg of colchicine (as recommended by Tannock (1967)). The first group of rats,(no colchicine administered), were killed at 1315 hours. Specimens 2 cms in length were taken from a region approximately 5 cms distal to the duodenojejunal flexure and 5 cms proximal to the ileo-caecal region. These were fixed in Bouins solution, paraffin sections were made and stained with haematoxylin eosin stain. Counts of metaphases only were made on each tissue specimen in 40 randomly selected crypts sectioned longitudinally so that their glandular lumen could be identified throughout the lower one-fourth (as described by Tutton (1975)).

The metaphase count on sections from rats to which no colchicine had been administered was included so that a more complete picture of the resting mitotic state of the tissue could be obtained.

It should be emphasized that phases of mitosis other than metaphase were not counted in sections from rats not treated with colchicine. Using the least squares method, graphs of mitotic index versus duration of colchicine treatment were constructed for each experimental group of tissues having mitoses collected for period of 1-4 hours. The mitotic index from each rat represented a point on the regression line. An estimate of the regression coefficient was made for each graph. The mitotic rate thus derived was expressed as mitoses/cell/hour.

The 'F' test was applied to estimate the statistical significance of apparent differences between the mitotic rate of the 2 groups. To confirm the efficacy of intraperitoneal cimetidine in reducing gastric acid secretion in rats as described by Maslinski and Sewing (1977) the gastric content of 5 rats receiving intraperitoneal cimetidine was removed, suspended in normal saline and the pH estimated using a digital pH meter. The pH measurements thus obtained were compared with the pH of the gastric contents in a control group of 10 similar rats obtained at a similar time during the day.

RESULTS

In animals treated with cimetidine, the mitotic rate in the jejunum was 0.0926 ± 0.0031 mitoses per cell per hour as compared with the rate in saline treated controls 0.0731 ± 0.0040 mitoses per cell per hour (see Table 1). Comparison of these values by the 'F' test showed a statistically significant difference, ($P < 0.05$).

The mitotic rate in the distal ileum in cimetidine treated animals was found to be 0.0886 ± 0.0031 mitoses per cell per hour as compared with 0.0723 ± 0.0044 mitoses per hour in the distal ileum of saline treated controls (see Table II).

Comparison of these values by the 'F' test showed statistically significant difference ($P < 0.05$). It was concluded that the mitotic rate in the crypts of Lieberkuhn of the rat small intestine, jejunum and ileum, was significantly increased in the rats treated with cimetidine as compared with a control group treated with normal saline.

The pH of the gastric contents of a control group of 10 rats was found to be 3.51 ± 0.13. The readings for pH of the gastric contents of rats treated with cimetidine in the dosage 50 mg/kg were found to be consistently and significantly raised above this level during the four hours of the trial (see Fig. 1). Thus it seems reasonable to assume that in this case cimetidine has had a significant effect on diminishing gastric acid secretion.

EXPERIMENTAL TREATMENT	MITOTIC RATE (MITOSES/CELL/HOUR.) (± S.E.)
CIMETIDINE TREATED RATS.	0.0926 ± 0.0031
SALINE TREATED RATS	0.0731 ± 0.0040

Table 1

Comparison of the mitotic rates in the cyrpts of Lieberkuhn of the jejunum of cimetidine treated and saline treated animals expressed as mitoses/cell/hour.

EXPERIMENTAL TREATMENT	MITOTIC RATE (MITOSES/CELL/HOUR) (± S.E.)
CIMETIDINE TREATED RATS	0.0886 ± 0.0031
SALINE TREATED RATS	0.0723 ± 0.0044

Table 2
Comparison of the mitotic rates in the crypts of Lieberkuhn of the ileum of cimetidine treated and saline treated animals, expressed as mitoses/cell/hour.

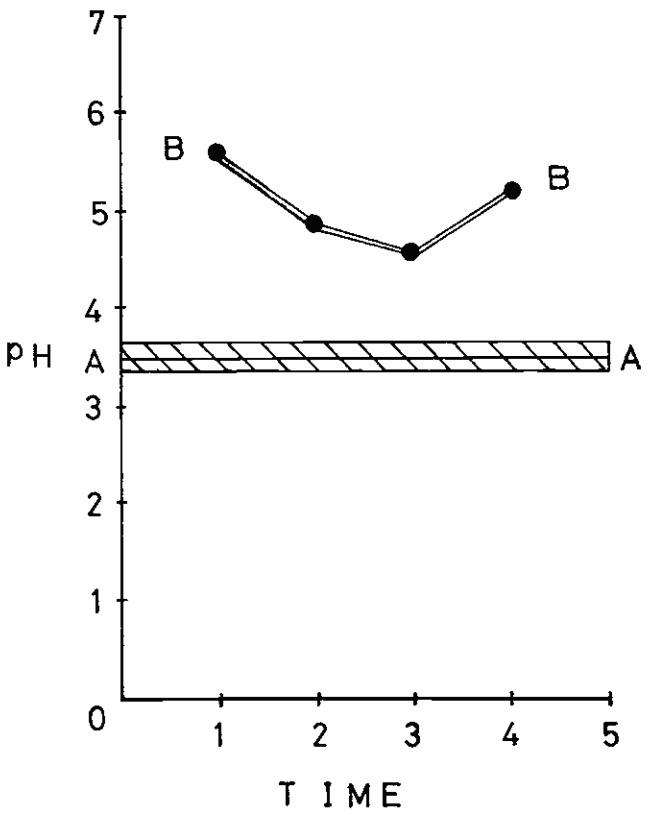

Figure 1 A graphical comparison of the pH values of gastric contents of rats treated with cimetidine for 4 hours at a dosage of 50 mg/kg (B) and a control group (A).

DISCUSSION

Thus it would appear that lowering of the gastric acid secretion in the rat by administration of cimetidine in a high dosage is associated with a significant increase in the crypt cell proliferation rate in both the jejunum and ileum. Admittedly the increase is not a great one but it is statistically significant.

It seems unlikely that the experimental result could be due to the effects of the intraperitoneal route of administration as opposed to the oral route, since a control group of rats was treated intraperitoneally with saline.

It should be pointed out that no attempt was made in this experiment to apply the findings to the human situation. Firstly, the mode of administration in humans is orally and not intraperitoneally, secondly the dose is much higher than that used therapeutically in man. In this experiment, for instance, the equivalent of 600 mg/kg over 24 hours was given compared with the recommended therapeutic dose approximately 20 mg/kg over 24 hours in man.

Incidentally, there were no fatalities as a result of the administration of cimetidine in this case. The aim in this case was to produce a prolonged fall in gastric acid secretion and observe its effect on the small intestine and this was achieved.

It is obvious that, since changes in the pH of the gastric contents cannot be equated with changes in the pH of the contents of the small intestine (Gibaldi (1977)), no statement can be made from these results as to the role of possible changes in the pH of the small intestinal content in the effect produced by cimetidine in this case. However, it seems unlikely, on this basis, that this effect of cimetidine was mediated alone by a change in the pH of the intestinal content.

Supporting this contention that pH changes alone are not of great significance in the control of crypt cell proliferation in the small intestine of the rat are the following findings. Clarke et al. (1976) found the rate of crypt cell proliferation to be increased in a loop of jejunum with low pH contents in contrast to Altmann (1971) who found the same effect in a jejunal loop exposed only to high pH pancreatic secretions.

There seems to be little indication that the small but definite hyperplastic effect on the crypts observed in this case is probably due to the anti-histaminic effect of cimetidine, since Tutton, (1977), has shown that if histamine has any effect it is to produce hyperplasia of the crypts.

We are thus left with a curious and unexplained effect of cimetidine in this case. Other factors may be operative e.g., changes in serum gastrin levels or prolactin levels. Gastrin is known to have a trophic effect on the small intestine (Johnson et al. 1975).

Incidentally, whilst serum gastrin level changes accompany changes in gastric pH in many cases, Maslinski and Sewing (1977) using the same dosage of cimetidine in rats, concluded that the effect of cimetidine in that case was not likely to have been mediated by gastrin. This of course

does not exclude the action of gastrin in this case but it probably makes it less likely. Furthermore, the effects of cimetidine on the blood supply to the villi have not as yet been elucidated. In this connection, Touloukian and Spencer (1972) and Lundgren et al. (1966) have stressed the possible importance of variations in the blood supply to the villi in the control of crypt cell proliferation.

It is suggested that further studies on the effect of cimetidine on the proliferative mechanism of the gastric mucosa might be of value. Whilst one must be guarded in interpreting these results in the light of the human situation, because of species differences and the dosage levels used, it would be interesting to speculate to what extent the ulcer healing capacities of cimetidine are due to a primary effect on the gastric mucosa as well as an effect mediated by decreased gastric secretion.

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