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Histamine gastric ulceration and its prevention by degraded carrageenan: the effect of aminoguanidine sulphate

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Pretreatment of the guinea-pig with intravenous aminoguanidine sulphate, a drug believed to inhibit histaminase, changes the gastric ulcer response following a dose of histamine acid phosphate (2·5 mg/kg) which elicits a submaximal ulcer response, to that of a dose (5 mg/kg) which normally elicits a maximal gastric ulcer response. A reduction in the volume and acidity of secretion also occurs. Intraduodenal administration of degraded carrageenan prevents this action of aminoguanidine. Conversely, in the presence of aminoguanidine, carrageenan fails to protect against histamine ulceration.

HISTAMINE-induced gastric ulceration can be diminished by intraduodenal degraded carrageenan in the pylorus-ligated guinea-pig (Anderson & Soman, 1963) suggesting a humoral effect of carrageenan. Since it has been shown (Giertz, Hahn, Schmutzler & Seseke, 1964) that the naturally occurring sulphated polysaccharide, heparin, has a histaminase-liberating action in the guinea-pig, we have examined whether histaminase is involved in the ulcer-preventing action of carrageenan. We report results which show that aminoguanidine, a histaminase inhibitor (Schuler, 1952), potentiates histamine gastric ulceration in the guinea-pig and abolishes the ulcer-preventing action of degraded carrageenan.

Experimental

MATERIALS AND METHODS

Seven weight-matched groups of adult male guinea-pigs of a strain susceptible to histamine-induced gastric ulceration (P strain used by Anderson & Soman, 1965, and obtained from Ponchilla Farms, Mapperly, Nottingham) were maintained on diet 18 and fasted 24 hr (water *ad lib.*).

All animals were subjected to identical treatment and operative technique; where a drug was omitted in any group, normal saline (same volume, same route) was administered instead.

Aminoguanidine sulphate. 2 mg/kg intravenously (1 ml/kg in saline) was given at 24 hr and 0 hr before the experiment. In the absence of knowledge of the absorption of aminoguanidine sulphate after administration by other parenteral routes, the intravenous route was used to ensure total availability.

Degraded carrageenan. Ebimar (Glaxo-Evans, Paris) was used (200 mg/ml) (Anderson & Duncan, 1965) in saline; 1 ml solution was given intraduodenally 0·5 hr before, and repeated immediately after, histamine injection.

Gastric ulcer production. The animals were anaesthetised with sodium pentobarbitone, 30 mg/kg i.p., and the gastroduodenal junction ligated.

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After pyloric constriction, histamine acid phosphate (2·5 or 5 mg/kg s.c. in saline, 1 ml/kg) was administered, and degraded carrageenan was injected into the duodenum. The animals were killed 1 hr after the administration of histamine; ulceration was scored on a 4+ scale (Anderson & Soman, 1965) and averaged for each group. In groups 1 and 2 the number of animals showing ulceration was also recorded. Secretion volumes were measured, and free and total acidities titrated using Topfer's reagent and phenolphthalein respectively.

The drugs administered to the various groups are in Table 1.

TABLE 1. EFFECT OF AMINOGUANIDINE SULPHATE ON HISTAMINE ULCERATION IN THE GUINEA-PIG, AND ITS ABOLITION OF THE ANTI-ULCER ACTION OF DEGRADED CARRAGEENAN

Group	No. of animals	Medication	Gastric ulceration	Volume of gastric secretion (ml/kg)	Acidity of gastric secretion (m-equiv./litre)	
					Free	Total
1	12	—	0·13 ± 0·07	14·9 ± 0·9	65 ± 1·4	72 ± 1·3
				P < 0·05	P < 0·001	P < 0·001
2	12	A	0·33 ± 0·09	17·3 ± 0·7	83 ± 2·2	91 ± 2·1
3	16	H_1	2·3 ± 0·35	30·5 ± 1·7	94 ± 4·1	103 ± 3·8
			P < 0·01		P < 0·005	P < 0·005
4	15	A + H_1	3·5 ± 0·32	27·5 ± 1·2	78 ± 2·8	87 ± 2·8
5	8	A + C + H_1	1·9 ± 0·7	38·5 ± 5·2	96 ± 6·3	104 ± 6·2
6	11	H_2	3·63 ± 0·28	26·5 ± 0·8	76 ± 4·1	84 ± 3·6
			P < 0·4			
7	12	A + C + H_2	3·25 ± 0·3	29·7 ± 0·9	78 ± 4·5	86 ± 4·4

Values are averages for the group ± standard error of the mean.
A = aminoguanidine sulphate 2 mg/kg i.v., twice.
H_1 and H_2 = histamine acid phosphate 2·5 and 5·0 mg/kg s.c., respectively.
C = degraded carrageenan 1 ml of 20% solution, intraduodenally, twice.

Results

The results are in Table 1.

The differences in gastric ulceration scores and in the numbers of animals which showed ulceration in groups 1 and 2, were not significant.

Additional experiments were made with two groups of an ulcer-resistant strain, one of 7 and one of 8 guinea-pigs (T strain used by Anderson & Soman, 1965, and obtained from Tuck, Rayleigh, Essex). Animals were treated with aminoguanidine (2 mg/kg i.v., twice) 24 hr before and at the same time as they were given histamine acid phosphate (2·5 mg/kg s.c.). In one group this produced significant ulceration (2·1 ± 0·6); in the other group, degraded carrageenan (2 ml of 20% solution) in addition, failed to reduce this ulceration (1·9 ± 0·7) and to change the volume and acidity of secretion.

Discussion

Aminoguanidine sulphate is believed to inhibit histaminase-type activity (Schuler, 1952; Blaschko, Friedman, Harves & Nilsson, 1959) and Dr. W. Schmutzler tells us that at 2 mg/kg i.v. in the guinea-pig, deactivates plasma histaminase, including that liberated by intravenous sulphated polysaccharide. In the present experiments, reduction of histaminase activity by aminoguanidine could be deduced from the results of group 2

where, although neither the increase in average ulceration nor the number of animals showing ulceration is significantly greater than in group 1, there is a trend towards increased ulceration. Although not significant in itself, this trend should be considered with the simultaneous significant increase in volume and acidity of the gastric secretion. This combined response can be elicited by 0·5–1 mg/kg histamine acid phosphate s.c. in the absence of aminoguanidine. The effect of aminoguanidine may be due either to increased endogenous histamine following inhibition of histaminase, or to the toxicity of aminoguanidine itself, or to both.

The maximum average responses in this type of experiment are 3·0–4·0 (ulcer score), 30–40 ml/kg and 80 m-equiv./litre upwards (volume and acidity of gastric secretion, respectively). Histamine acid phosphate, 2·5 mg/kg s.c., gives a maximum acid response but a sub-maximal ulcer score; 5·0 mg/kg gives a maximum ulcer score but a sub-maximal acid response (Anderson & Soman, 1965). Groups 3 and 6 in the present experiments confirm these findings. When aminoguanidine is given to animals receiving 2·5 mg/kg histamine (group 4) there is a significant increase in gastric ulceration, with decrease in acidity. This change corresponds to the condition obtained by a histamine acid phosphate dose of 5 mg/kg (group 6). These results support the view that pre-treatment with aminoguanidine makes more histamine available *in vivo* and also support the apparent trend to increased ulceration in groups 1 and 2.

The addition of carrageenan (group 5) to the histamine (2·5 mg/kg) and aminoguanidine treatment, restored the response (ulceration and acid) obtained with histamine-aminoguanidine (group 4) to that of group 3 (histamine alone); it appears therefore that dose for dose the histaminase-inhibiting activity of aminoguanidine can be equated to the effect of carrageenan. Dr. Schmutzler further told us that he has found that intravenous degraded carrageenan has a histaminase-liberating effect. The antagonism between aminoguanidine and carrageenan seen in the present experiments is therefore consistent with Schmutzler's observation, and suggests that the effects seen in the stomach after carrageenan given intraduodenally could be due to the histaminase-liberating effect of the carrageenan. Although this implies some uptake of degraded carrageenan by the duodenal mucosa, it does not indicate the origin of histaminase if indeed this is involved.

The results of group 6 show that with a dose of 5 mg/kg of histamine there is a reduction in the volume and acidity of secretion, with an increase in ulceration. Addition of aminoguanidine to this dose of histamine acid phosphate (5 mg/kg) resulted in the death of a number of animals in the group and the results are not included. This was not unexpected since the decrease in acid secretion and the increase in ulceration seen with 5 mg/kg histamine acid phosphate alone is a manifestation of acute histamine toxicity (Anderson & Soman, 1965); addition of aminoguanidine is equivalent to increasing the histamine dosage still further. However, group 7 shows that addition of carrageenan to such a combination maintains all the components of the

response at the level obtained when the 5 mg/kg dose of histamine is given alone (group 6). Conversely, the ulcer preventing action of carrageenan is abolished by aminoguanidine. It is interesting to note that at both doses of histamine, the same doses of aminoguanidine and carrageenan appear to cancel each other out.

It would appear, therefore, that there could be a relation between histaminase and the antisecretory and anti-ulcer activities which have been described (Anderson & Watt, 1959; Bonfils & Lambling, 1960; Anderson & Soman, 1963) for degraded carrageenan.

Two questions remain. Firstly, if we assume uptake of degraded carrageenan by the duodenum, do degraded carrageenan and aminoguanidine interact with mutual inactivation; and secondly, do aminoguanidine and histamine have synergistic toxicity? No evidence for interaction between aminoguanidine and degraded carrageenan could be found *in vitro*; some evidence for synergistic toxicity can be inferred for aminoguanidine and histamine (Schmutzler, 1965).

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