

Inhibitory Effect of Duodenal Factors Against Ulceration of Stomach in Rats*

S.S. Hong, H.Y. Kim, H.W. Lee** and Y.B. Lee

*Departments of Pharmacology and Pathology, Yonsei University,
College of Medicine, Seoul, Korea*

ABSTRACT

The protective effects of specific duodenal mucosal extracts, as well as of intraduodenal olive oil, against ulceration in the ulcerogenic preparation used by Shay et al. were studied. Both the intraduodenal oils and intravenous administration of the specific mucosal extracts were effective in preventing the occurrence of the gastric lesions produced by pyloric ligation for 20 hours in fasting rats. The anti-ulcer efficacy was most marked by olive oil, Vitrum secretin or Boots pancreozymin and was least with mineral oil or Boots secretin. The acidity and pepsin content of gastric juice determined 5 hours after pyloric ligation in fasting rats was significantly decreased by olive oil and by the specific extract except the Boots secretin. These results suggested that the specific duodenal mucosal extracts are probably enterogastrone liberated by fat in the duodenum. It is not explained why intraduodenal mineral oil has some effect in preventing the ulcerative lesions. The mineral oil

induced a suppression of pepsin content along with an increase of acid content in the juice. Intraduodenal acid was also slightly effective and alkali was ineffective in prevention of gastric lesions. However in both groups the acid and pepsin contents were not low. Pretreatment with atropine, hexamethonium or acetazolamide was moderately effective in preventing the ulceration. Acetazolamide showed a marked increase in pepsin and a decrease in acid content of gastric juice.

INTRODUCTION

As early as 1886 Ewald and Boas noted that the addition of olive oil to a gruel test meal in human subjects, in comparison with gruel alone, depressed the acid concentration of the gastric contents and delayed the emptying of the meal from the stomach. The phenomena of the inhibitory action of fat was further studied by Farrell and Ivy(1926)who demonstrated that the feeding of olive oil to a dog with an autotransplanted fundic pouch caused inhibition of the motor activity. This led Feng et al.(1929) to seek evidence that fat inhibited gastric secretion in a similar manner. Subsequent studies on extracts of duodenal mucus have failed to

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**Present address: Department of Biochemistry, Temple University, Philadelphia, Pennsylvania, U. S. A.

define the substance involved, although the concept is accepted that enterogastrone is a duodenal hormone inhibiting both gastric motility and acid secretion (Magee, 1962).

Recently specific extracts of duodenal mucosa containing secretin, cholecystokinin and pancreaticozym activity have been prepared (Jorpes and Mutt, 1959). The secretin preparation was found to inhibit gastric acid secretion by the studies of Greenlee et al. (1957) and others (Jordan and Peterson, 1962; Wormsley and Grossman, 1964). The extracts containing cholecystokinin-pancreozymin activity were also found to inhibit gastric acid secretion (Gillespie and Grossman, 1964; Jordan and De la Rosa, 1964) and its motility (Johnson and Magee, 1965) and was claimed to exceed secretin preparation.

Sandweiss (1943) reported a principle extractable from the small intestinal mucosa which exerts an anti-ulcer effect in Mann-Williamson dogs and named it anthelone. The effect was not due to its gastric acid depressant activity. However, its hormone status is uncertain.

The present study was performed to observe the effect on ulceration of the rat stomach in the ulcerogenic preparation of two extracts of duodenal mucosa, one containing secretin and the other cholecystokinin-pancreozymin, as well as the effect of oils or other agents in the duodenum.

MATERIALS AND METHODS

Albino rats of both sexes weighing 150 to 200 g were used. The animals were starved for forty-eight hours and then pyloric ligatures were placed for 5 or 20 hours by the procedures used by Latzel (1913) and Shay et al. (1945). During the starvation period, water was permitted ad libitum but

coprophagy was avoided. Fifty-seven rats were used in the first series of experiments. All rats were given pyloric ligature for 20 hours after 48 hour starvation. The first group consisted of untreated controls. The second to the fifth group received intraduodenal instillation of oil, mineral oil, 0.1 N HCl and 0.1 N NaOH respectively, in doses of 2 ml, after pyloric ligation. Fifty-four rats were used in the second series of experiments. All rats were processed as in the first series of experiments except for intravenous administration of the specific duodenal extracts instead of intraduodenal treatment. The first group was a control and the second to fifth groups received secretin prepared by Vitrum (Sweden), secretin by Boots (England), Cecekin by Vitrum and pancreaticozym by Boots respectively in doses of 2 units each into the tail vein. Fifty-three rats were used in the third series of the experiments. All rats were processed as in the second series of experiments and received agents eliciting gastric depression. The first group was of untreated controls. The second and third groups received intramuscular administration of atropine and hexamethonium in doses of 1 mg/kg. The fourth group received acetazolamide in doses of 5mg/kg into the tail vein. The fifth group was given procaine into the duodenum in doses of 2 ml of 1% solution.

Another design of experiments similar to the above series was performed for analysis of gastric juice. Each group consisted of 6 animals and pyloric ligature was placed for 5 hours after 48 hour starvation. The treatment to each group was the same as in the previous experiment.

Upon the completion of an experimental period the rats were killed under ether anesthesia by exsanguination from the femo-

ral artery. The stomach was removed, trimmed free of adipose tissue, and then opened by cutting along the greater curvature. The gastric juice was collected. The specimens were then washed under tap water. The severity of the ulcer was graded from 1+ to 4+ by composite macroscopic and microscopic findings. Grade 1+ indicated a few hemorrhagic spots which consisted of focal superficial ulceration, 2+ was used for several discrete mucosal sloughings which involved the mucosa and submucosa, 3+ for wide area of mucosal sloughings which extend into muscular layer and 4+ for wide ulceration with perforation. Free acid and total acid from the juice were titrated in the usual manner using Töpfer's and phenolphthalein as the indicators and N/20 NaOH for titration. Pepsin activity was determined by the method of Anson (1938).

RESULTS

Ulcerative gastric lesions in rats were produced by the procedure of Shay et al (1945). As in the previous report (Chang et al., 1965) the ulceration develops mostly in the rumen, less often in the antrum, and least frequently in the body of the stomach. The lesions are most marked in the rumen.

The administration of the olive oil into the duodenum was very effective in preventing the occurrence of gastric lesions produced by pyloric ligation in the fasting rats (Table 1). The incidence of gastric ulceration was 10% in the olive oil group while the incidence was 100% in untreated controls. Furthermore, the lesion of the stomach in one animal which had an ulcer was quite slight. Although the occurrence of ulceration was more frequent than in that of the olive oil group, mineral oil was also effective for

prevention of ulceration. Introduction of acid into duodenum was slightly effective but alkali was not at all effective in preventing the gastric lesion.

The occurrence of gastric ulcer produced by pyloric ligation and starvation was greatly diminished by the administration of the specific duodenal extracts, secretin and cholecystinin-pancreozymin (Table 2). The efficacy by Vitrum secretin and Boots pancreozymin were the highest and by Boots secretin the lowest.

In the third series of experiments pretreatment with atropine or hexamethonium was effective in preventing the gastric lesions produced by ligation after starvation. Acetazolamide and procaine were also effective but to a lesser degree in preventing the ulceration (Table 3).

The acidity of gastric juice was significantly lower in the animals treated with specific duodenal extracts than control animals (Fig. 1).

The acidity of the stomach contents was not decreased in the animals receiving intraduodenal mineral oil, by which the occurrence of gastric lesions was well prevented. Pepsin activity in gastric juice was decreased in the animals treated with olive oil or the specific duodenal extracts except Boots secretin (Fig. 2). Hexamethonium, atropine or mineral oil also showed lower pepsin activity. However acetazolamide brought a marked increase of the activity. In the animals which had pyloric ligation for 20 hours the pepsin activity of stomach contents was not much different between the treated and untreated control groups, although the gastric acidity in the animals treated with specific duodenal extracts, atropine or hexamethonium was low.

Table 1. Effect of intraduodenal olive oil, acid or alkaline solution on experimentally-induced gastric ulcer

Group	Starved S(hr.)	Ligated (hr.)	Treated	No. of rats used	No. of rats died	No. of rats had ulcer Degree of ulcer*					Ulcer Incidence (%)
						1+	2+	3+	4+	Total	
1	48	20	Control	11	1	2	2	5	1	10	100.0
2	48	20	Olive oil	11	1	1				1	10.0
3	48	20	Mineral oil	15	2	6				6	46.1
4	48	20	0.1 N HCl	10	3	2	2	1		5	71.5
5	48	20	0.1 N NaOH	10	0	4	3	3		10	100.0

* 1+: Slight, 2+: Moderate, 3+: Severe, and 4+: The most severe degree of ulcer.

Table 2. Effect of secretin or cholecystokinin-pancreozymin preparation on experimentally-induced gastric ulcer

Group	Starved (hr.)	Ligated (hr.)	Treated	No. of rats used	No. of rats died	No. of rats had ulcer Degree of ulcer*				Ulcer Incidence (%)	
						1+	2+	3+	4+		
1	48	20	Control	12	2	2	1	3	3	9	90.0
2	48	20	Secretin(Vitrum)	10	0		1			1	10.0
3	48	20	Secretin(Boots)	10	2	2	2	1		5	62.5
4	48	20	Cecekin(Vitrum)	10	0		1	2		3	30.0
5	48	20	Pancreozymin (Boots)	11	0	1				1	8.3

* 1+: Slight, 2+: Moderate, 3+: Severe, and 4+: The most severe degree of ulcer.

Table 3. Effect of atropine, hexamethonium, acetazolamide or procaine on experimentally-induced gastric ulcer

Group	Starved (hr)	Ligated (hr)	Treated	No. of rats used	No. of rats died	No. of rats had ulcer Degree of ulcer*				Ulcer Incidence (%)	
						1+	2+	3+	4+		
1	48	20	Control	12	0	3	1	4	2	10	83.3
2	48	20	Atropine	11	1	1		2		3	30.0
3	38	20	Hexamethonium	10	0	2				2	20.0
4	48	20	Acetazolamide	10	2	6				6	75.0
5	48	20	Procaine (intraduodenal)	10	5	2	1	1		4	80.0

* 1+:Slight, 2+: Moderate, 3+: Severe, and 4+:The most severe degree of ulcer.

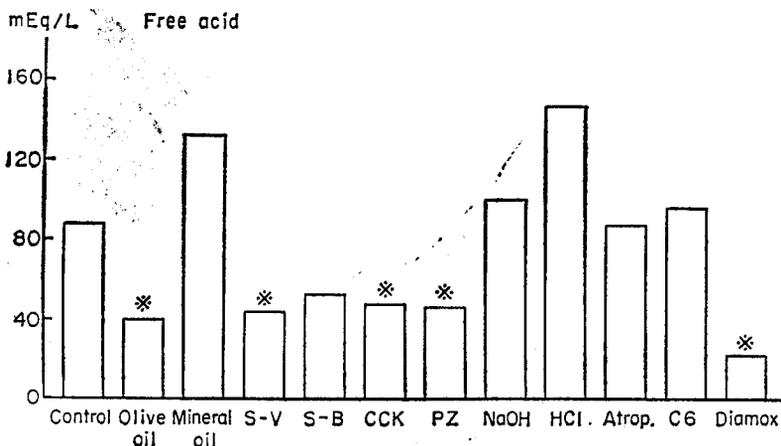


Fig. 1. Free gastric acid content in each experimental group. S-V: Secretin (Vitrum), S-B: Secretin (Boots), CCK: Cecekin (Vitrum), PZ: Pancreozymin (Boots), Diamox: Acetazolamide, and Asterisk: Difference from control group, $P < 0.05$.

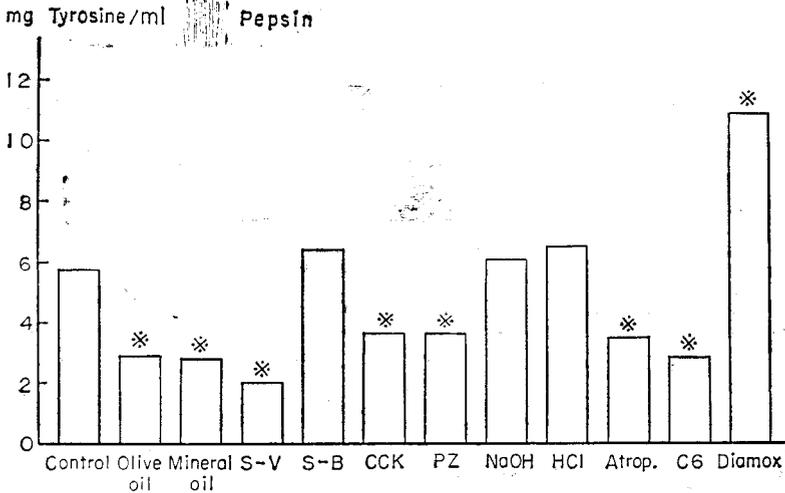


Fig. 2. Pepsin content in each experimental group. Abbreviations same as Fig.1.

DISCUSSION

The discovery of enterogastrone derived from the fact that ingested fat inhibits both motor and secretory activity of the stomach. The proof that this effect is carried out by a hormonal mechanism was provided by experiments with the auto-transplanted pouch, on gastric motility by Farrell and Ivy (1926) and on secretion by Feng et al. (1929). Kosaka and Lim (1930) found that an extract of duodenum made after exposure to olive oil inhibited secretion and suggested the name enterogastrone for the active principle. However, the principle has never been purified, nor has it been characterized chemically.

Inhibition of gastric secretion by acid in the duodenum is well documented(Wormsley and Grossman, 1964; Pincus et al., 1944). The evidence also shows that this inhibition is at least in part hormonal in nature (Andersson, 1960;Code and Watkinson, 1955). Recently Johnson and Grossman (1968) found that a physiological dose of secretin was

able to account for all the inhibition of gastric secretion seen with acid in the duodenum and concluded that secretin is probably the only enterogastrone released by acid in the duodenum. The effect of alkali in the duodenum on the other hand had been little studied. Brown et al. (1966) found that when alkaline solutions were introduced into the duodenum the activity of denervated and of transplanted pouches was increased. By this and other evidence they postulated two mechanisms of action for the alkaline buffer effect; inhibition of the release of an inhibitory substance from duodenal mucosa or release of a humoral stimulating agent.

Recent studies showed that the specific extracts of duodenal mucosa containing secretin or cholecystokinin-pancreozymin were found to inhibit gastric acid secretion (Greenlee, et al., 1957; Jordan and Peterson, 1962; Wormsley and Grossman, 1964) and its motility (Johnson and Magee, 1965; Johnson, et al., 1966).

In this study the administration of olive oil into the duodenum or of the specific

duodenal extracts into a vein was very effective in prevention of gastric lesions produced by pyloric ligation after starvation. Furthermore, the acidity and pepsin activity of gastric contents were significantly decreased by secretin or olive oil administration. Therefore doubtless the anti-ulcer effect was brought on by the inhibitory action on the acid-pepsin secretion of the stomach. The anti-ulcer effect of Boots secretin was far less that of Vitrum. This may be due to the differing potency of brands since that of the former is 8 to 9 times less than of the Vitrum secretin, according to Stening et al. (1968). The pepsin activity was rather increased by the Boots preparation, which may be in part due to the other ingredients in the available preparation, since the combinations of histamine or gastrin pentapeptide with pure secretin produced higher pepsin outputs than did histamine or pentapeptide alone, according to the study of Nakajima et al. (1969). Recently Johnson and Magee (1965) suggested that the preparation of pancreozymin available to them was contaminated with enterogastrone, or that enterogastrone and pancreozymin are identical hormones. On the other hand Johnson and Grossman (1968) claimed that secretin is probably the only enterogastrone. From our results it is felt that both secretin and pancreozymin may participate the physiological role of enterogastrone which is released from the duodenal mucosa by contact with olive oil. Why intraduodenal mineral oil was somewhat effective in preventing the ulceration, is not known. It may depend upon either a different mechanism from olive oil, such as the greasiness itself or an activation of anthelone (Sandweiss, 1943), or on the fact that mineral oil also elaborates secretin/pancreozymin from the

duodenal mucosa. However the latter is unlikely since it is denied by other experimenters (Feng, et al., 1929; Ivy, 1934).

The incidence of gastric lesions produced by pyloric ligation after starvation was considerably reduced by the instillation of acid and the lesions were not affected by alkali in our experiment. The results are understandable and are in part elicited by humoral mechanism according to the studies made by Code and Watkinson (1955) and others (Andersson, 1960; Brown, et al., 1966). In relation to duodenal acidification, Gold and Poth (1968) claimed that the experimental expedient of directing the acid gastric chyme into the distal duodenum to increase the acidity of the duodenal mucosa, augmented the synthesis of secretin and enterogastrone and fortified inhibition of gastric secretion in the treatment of duodenal ulcer. Recently Uvnäs (1971) claimed that bulbogastrone, a chalone, released from the duodenal bulb by an acid medium has also an important role in the inhibitory mechanism of gastric secretion.

The administration of atropine or hexamethonium was found to reduce significantly the ulceration induced by pyloric ligation after starvation but did not change the acidity of stomach contents. The pepsin content in both groups was considerably decreased. The anti-ulcer effect of these drugs is known to be due to their inherent capacity to inhibit gastric secretion as well as motility. Acetazolamide and procaine were less effective in preventing the ulceration and the former showed a significant decrease of acid output and a marked increase of pepsin content, which reminds us of the observations by Heitmann et al. (1967) in the canine Heidenhain pouch.

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