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The Effect of Verapamil on Cysteamine-Induced Duodenal Ulcer in the Rat

Choong Ki Lee, M.D., Dae Soon Yim, M.D. and Woo Ho Kim*, M.D.

Departments of Internal Medicine and Pathology, Seoul District Armed Forces General Hospital, Seoul, Korea*

To determine the effect of verapamil on experimental duodenal ulcer, pathologic assessment and secretory study were performed in the rats with ulcerogenic dose of cysteamine. The cysteamine increased gastric acid secretion and produced double duodenal ulcers at the proximal portion of the duodenum. Intramuscular injection of verapamil, 3 hours later, produced a significant decreased in gastric acid secretion which lasted at least 4 hours (cysteamine vs. cysteamine+verapamil; 63.5±18.4 μEq vs. 25.5±9.0 μEq during the 1st hour after verapamil administration, 83.1±24.2 μEq vs. 27.8±12.3 μEq during the 2nd hour, 110.9±14.4 μEq vs. 38.5±25.9 μEq during the 3rd hour, 116.4±12.1 μEq vs. 40.7±29.6 μEq during the 4th hour, p <0.001). However, cysteamine-induced duodenal ulcers were not alleviated by two doses of intramuscular verapamil administration (4 mg/kg×2). It is presumed that suppression of gastric acid secretion may not be sufficient to reduce cysteamine-induced duodenal ulcer formation or that verapamil itself may have aggresive effects against duodenum. To illucidate the exact role of verapamil in cysteamine-induced duodenal ulcer, further studies would be needed.

Key Words: *Verapamil, cysteamine, gastric acid secretion, experimental duodenal ulcer.*

INTRODUCTION

Duodenal ulcer disease in human is more frequent than gastric ulcer and mechanisms of two diseases are thought to be different. In spite of the fact that several gastric ulcer models had been already established, duodenal ulcer model in rats were unsuccessful or inconvinient before the introduction of the cysteamine-induced experimental duodenal ulcer model. Cysteamine produces duodenal ulcers in rats within 24 hours by a single subcutaneous administration (Kirkegaard et al., 1980). The pathogenetic mechanisms leading to ulceration have not yet been fully explained. However, several mechanisms were suggested to be involved, including stimulation of gastric acid, reduced the alkaline mucous secretion from Brunner's gland in the proximal duodenum, and delayed gastric emptying. Serum gastrin concentration is also increased (Lichtenberger et al., 1977a; Lichtengerger et al., 1977b; Kirkegaard et al., 1982b).

Calcium influx seems to play an essential role in the stimulation-secretion coupling in mammalian oxyntic cells. A recent review (Sewing and Hannen, 1983) indicated that calcium channel blocker can inhibit the basal and stimulated gastric acid secretion

Address for Correspondence: *Choong Ki Lee, M.D. Department of Internal Medicine, Seoul District Armed Force General Hospital, 165 Sokeuk-Dong, Chongro-Ku, Seoul 110, Korea. (Tel. (02) 720-2621)*

in vitro. Protective effect of calcium channel blocker against stress-induced gastric erosion and ethanol-or indomethacin-induced gastric lesion were also reported (Ghanayem et al., 1987; Olge et al., 1985). However, Levine et al. (1983) reported the failure of verapamil to alter basal and pentagastrin-stimulated gastric acid secretion. And there is no report whether calcium antagonists have protective effect on duodenal ulcer or not. So we investigated the effect of verapamil on the cysteamine-induced gastric acid hypersecretion and duodenal ulcer in the rat.

MATERIALS AND METHOD

Evaluation of Duodenal Ulcer

Male Sprague-Dawley rats weighting 200-250 g were used throughout the study. The animals were fed freely before the cysteamine injection and then fasted thereafter but with free access to water. Two groups of ten rats each were used. In the first group, 38mg/100g of cysteamine-HCl (Sigma Chemical Co.) was administered subcutaneously. The second group animals received two doses of intramuscular injection of verapamil (4 mg/kg each) at 0th and 5th hour after the cysteamine injection. Twenty-four hours after the cysteamine administration, the rats were killed by cervical dislocation. The stomach and duodenum were opened along the greater curvature and pinned on the cork plate. They were fixed in buffered neutral formalin solution. The mucosal surface was examined by stereomicroscope. All the lesions were measured and mean length of the lesions in each group was calculated. After photographing, histologic examination was done on each lesion. The lesions were classified in one of four stages according to the depth of the involvement; stage 0: normal mucosa, stage 1: mucosal or submucosal ulcers, state 2: transmural ulcer, stage 3: perforating to the peritoneal cavity. And ulcer index (arithmetic mean of stages in each group plus ratio of positive/total animal×2) was calculated as described by Szabo (1979).

Measurement of Gastric Acid Secretion

Before each experiment the rats were kept in raised mesh-bottom cages to prevent coprophage and were fasted for 24 hours but with free access to water. The rats were anesthetized with intraperitoneal injection of urethane (Sigma Chemical Co.) 130 mg/100g body weight, and abdomen was opened by a midline incision. Through a stab wound, placed 8 mm distal to the pylorus, a thin polyethylene catheter was inserted into the antrum of the stomach. The pylorus was ligated and the peritoneum was closed. Ten millimeters of saline were introduced via catheter and then they were aspirated at hourly interval for 7 hours. The aspirates were titrated with 0.02 N NaOH solution using phenolphthalein as a indicator by biuret method. Three groups of 9 rats each were examined. In the first group cysteamine-HCl (38 mg/100g) was administered subcutaneously in a single dose at the start of experiment. The second group received intramuscular injection of verapamil (4mg/kg bogy weight), 3 hours after the cysteamine injection. The third group of rats served as control and were treated with injection of saline. The acidity was expressed as μEq hydrogen ion during one hour interval. Student's test was used for statistical evaluation.

RESULT

Influence of Verapamil on Duodenal Ulcer.

Cysteamine in a dose of 38 mg/100g produced duodenal ulcers in all rats within 24 hours. All of the first group animals had two ulcers situated from gastroduodenal junction and lay logitudinally along both anterior and posterior walls (fig. 1). The lesions at the anterior wall were usually larger than those of posterior wall. The length of the lesion varied from 1 mm to 9 mm. Among ten animals, two had perforating ulcers. The mean length of the lesion was 3.59 mm. On microscopic examination, the lesions were made up of peptic digestive detritus at the upper portion and acute inflammatory cell collections were seen underneath. The proper muscle layer was replaced by edematous granulation tissue with a few inflammatory cells (fig. 2). The perforated lesion showed inflammatory response and foreign body reaction at the serosal surface.

The second group animals, treated with cysteamine and two doses of verapamil, shared basically same pathologic finding as the first group animals (fig. 3). One of the second group animals expired within 5 hours and was excluded in the result. Another one expired within 24 hours due to perforating ulcer. All the animals survived longer than 5 hours had double ulcers. Fourty-four percents of animals showed perforating lesions and they were always at the anterior wall. The mean length of the lesions in the second group was 5.22 mm. The second group showed a greater ulcer index than first group. (4.44 vs. 4.20). Both gross and microscopic examination revealed that the second group animals exhibited

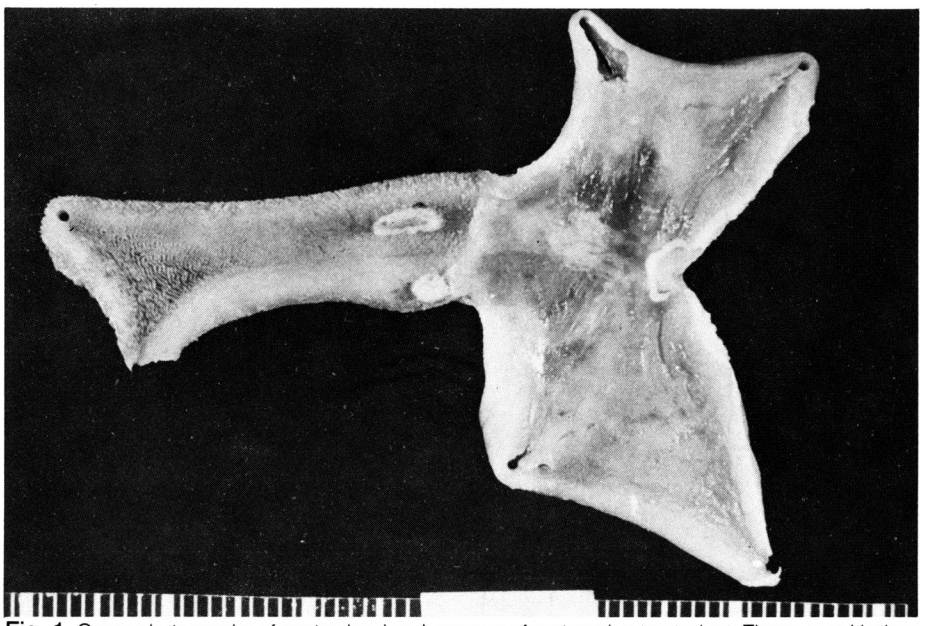

Fig. 1. Gross photography of gastroduodenal mucosa of cysteamine treated rat. There were kissing ulcers at both anterior and posterior wall of duodenum.

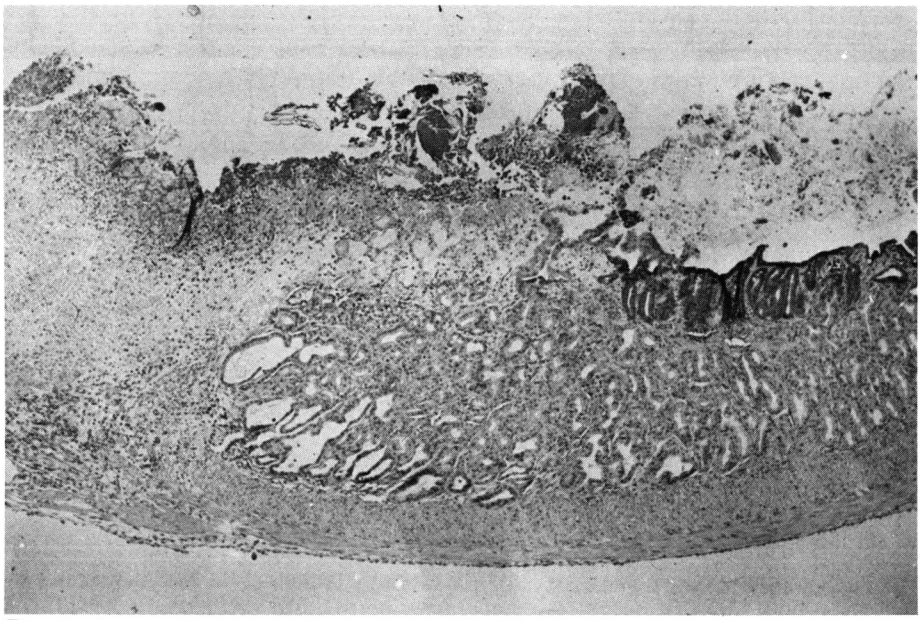

Fig. 2. Microscopic picture of duodenal ulcer. The left half of the duodenal wall is replaced by necrotic detritus admixed with neutrophils. (HE, ×100)

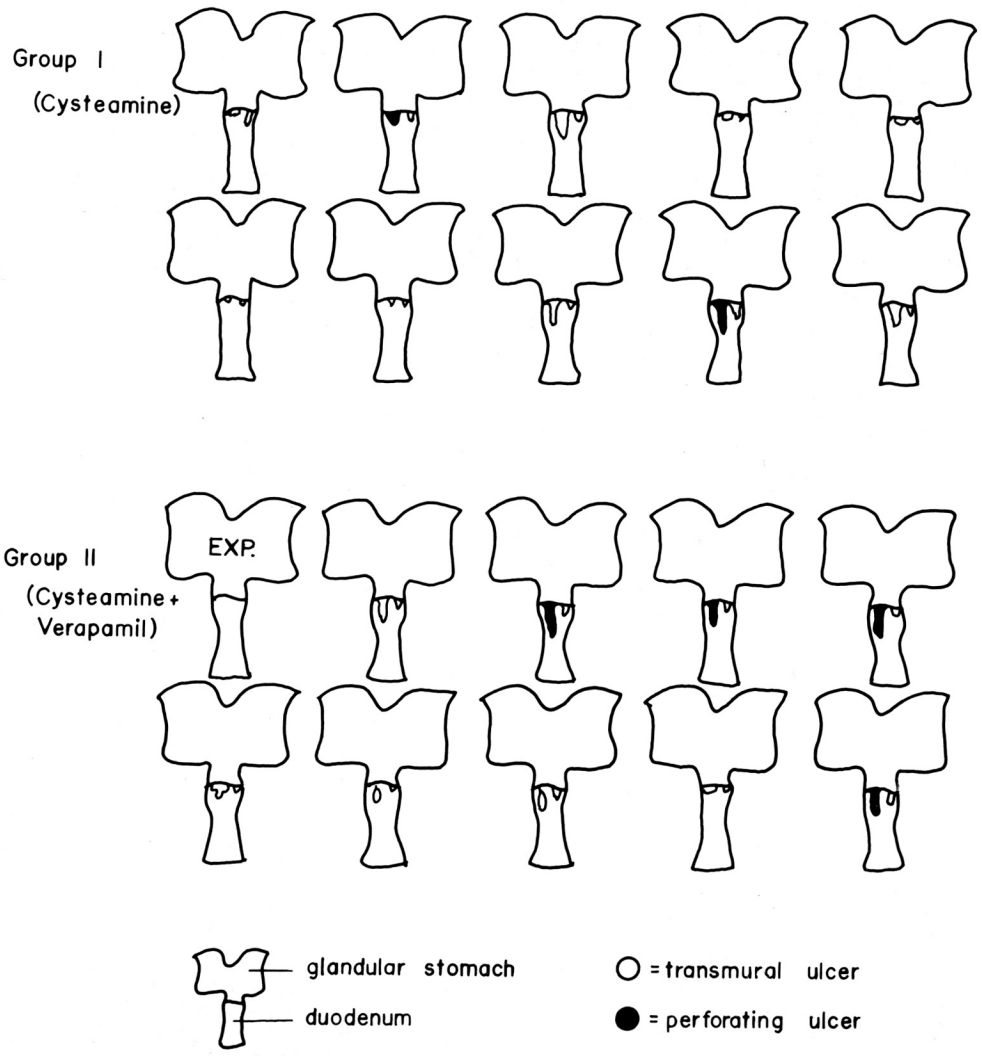

Fig. 3. Diagrams showing extent and location of ulcers in rats receiving cysteamine (38 mg/100g) and cysteamine plug verapamil (4 mg/kg). EXP: expired within 5 hours

greater severity than first group (table 1).

Influence of Verapamil on Gastric Secretion

The administration of cysteamine induced prolonged increase of the gastric acid secretion. The acid secretion persisted at least for 7 hours after injection. The acid outputs at 6th and 7th hour exceed 100 μEq per hour. This cysteamine-stimulated gastric secretion was reduced by intramuscular administration of verapamil, 4 mg/100g ($p < 0.00$); fig. 4). The above effect was evident from the 1st hour after verapamil treatment and lasted at least for 4 hours.

Table 1. Pathological findings of duodenal ulcers in cysteamine and verapamil treated rats

		No. of rats	Duodenal ulcer (%)	Perforation (%)	Ulcer Index	Mean Length of lesions
Cysteamine	38 mg/100g	10	100.0	20.0	4.20	3.95
Cysteamine +verapamil	38 mg/100g 4 mg/kg×2	9	100.0	44.4	4.44	5.22

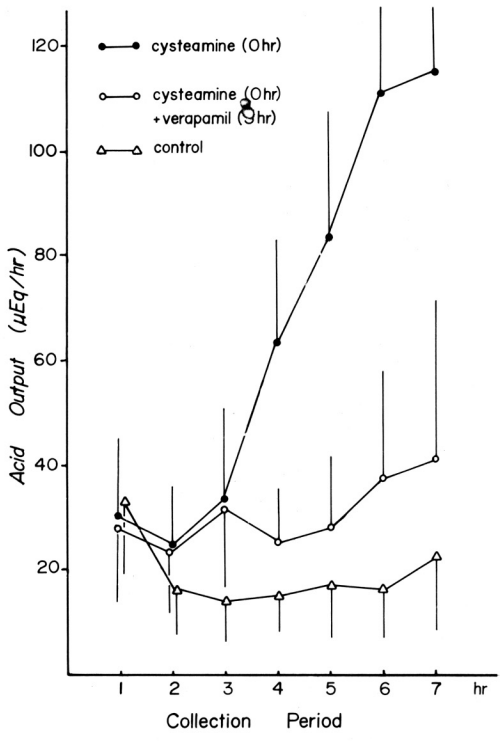

Fig. 4. The time course of gastric acid secretion in acute fistula rats after cysteamine (38 mg/100 g) and cysteamine plus verapamil (4 mg/kg) administration in anesthetized rats (mean±S.D.)

DISCUSSION

Experimental duodenal ulcers in rats induced by subcutaneous Injection of cysteamine were first described by Selye and Szabo (1978). The pathogenic mechanism leading to ulceration have not yet been fully explained, but both protective and aggresive effects on the resistence of the duodenal mucosa seem to be involved. Several studies have shown that the passage of acid gastric content is mandatory for the development of the duodenal ulcer (Robert et al., 1974; Lichtenberger et al., 1977b). Furthermore, it has been reported that cysteamine-induced ulcers can be prevented by anticholinergic agents, antacid and vagotomy (Fujii et al., 1975; Kirkegaard et al., 1980). These facts have led to explain the cysteamine-induced duodenal ulcer as the result of sustained gastric acid secretion.

Calcium channel blocker can inhibit basal and stimulated gastric secretion and protect against certain chemically induced gastric lesions. Olge et al. (1985) reported that gastric hypersecretion as well as ulceration produced by bethanechol were inhibited by intraperitoneally injected verapamil (1,2 or 4 mg/kg). Influx of calcium seems to be an essential step in stimulation-secretion coupling in the mammalian parietal cells (Kirkegaard et al., 1982a). The ability of the calcium channel blocker to reduce gastric acid secretion induced by cysteamine in this study is well agreed with the findings that calcium channel blocker have an inhibitory effect on the histamine, gastrin, pentagastrin and bethanecol-induced stimulation of gastric acid secretion (Sonnenberg et al., 1983). But Levine et al. (1983) reported that verapamil did not alter basal and stimulated gastric acid secretion in human.

Duodenal ulcers were produced in all conscious rats within 24 hours by a single subcuteneous administration of cysteamine. The ulcers developed on both anterior and posterior wall of the duodenum. Injection of verapamil (4 mg/kg×2) failed to reduce the cysteamine-induced duodenal ulcers in the rat, and even aggravated the lesions.

Cysteamine-induced hypersecretion of gastric acid is crucial in ulcerogenesis in rat. But when a gastric acid output equivalent to that produced by the ulcerogenic dose of cysteamine was induced by repeated injections of pentagastin, no duodenal ulcer was induced. These results indicate that although some acid in the duodenum is required for ulcer formation, the hypersecretion of acid induced by cysteamine is not the only factor responsible for the development of duodenal ulcer, and that other pa-

thogenic factors must be involved in ulcerogenesis (Kirkegaard et al., 1980). An important factor in the protection of the duodenal mucosa is the alkaline mucous secretion of Brunner's glands in the proximal duodenum (Hartiala et al., 1950). Epidermal growth factor, a peptide probably identical with urogastron, is known to be a potent inhibitor of gastric acid secretion and stimulator of cell proliferation (Heitz et al., 1978). This epidermal growth factor is present in large amount in Brunner's glands. Cysteamine was proved to interfere with the Brunner's gland secretion (Kirkegaard at el., 1981). Most patients with duodenal ulcer disease have decreased proximal duodenal mucosal bicarbonate production (Isenberg et al., 1987) and significant abnormality in the mucous-bicarbonate barrier in the duodenal cap (Quigley and Turnberg, 1987). Both gastric hypersecretion and decreased alkaline mucosal secretion in proximal duodenum may act synergically in producing the duodenal ulcer.

Cysteamine has been demonstrated to inhibit gastric emptying (Poulsen et al., 1982). Because peristalsis was inhibited, acid gastric secretion cannot be mixed with gastric content. This pool of undiluted gastric secretion may accelerate the formation of duodenal ulcer. Calcium channel blockers also can inhibit the contractility of the gastric muscle (Ochill and Tsai, 1982).

Newman et al. (1983) reported that verapamil inhibited pancreatic secretion in volume and bicarbonate amount in awaked dogs.

It would be possible that verapamil may aggravate the cysteamine-induced duodenal ulcer by inhibiting the pancreatic bicarbonate secretion and by inhibiting gastric emptying or that suppression of gastric acid secretion may not be sufficient to prevent cysteamine-induced duodenal ulcer. The paradoxical effects of verapamil on cysteamine-induced gastric acid secretion and duodenal ulcer formation need further evaluation.

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