ANIMAL MODEL OF HUMAN DISEASE

Duodenal Ulcer Disease

Animal Model: Cysteamine-Induced Acute and Chronic Duodenal Ulcer in the Rat

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Gastroduodenal or peptic ulcer disease is a widespread yet poorly understood disorder. In the United States the lifetime prevalence of duodenal ulcer disease is 8 to 10% while that of stomach ulceration is 1%.[1] In contrast to gastric ulcers which are easily induced in experimental animals by stress, hormones, and drugs, there has been no reliable and simple model of duodenal ulcer in rodents.

Animal Model

We reported that injection of propionitrile[2] or cysteamine[3] causes rapidly progressing duodenal ulceration in normal rats (without fasting or other preparations). This unexpected finding was confirmed and extended by others.[4-6] More recently, we recognized that the duodenal ulcerogenic potency of these chemicals seems to be associated with two carbon (-C-C-) groups containing reactive radicals, (eg, -SH,-CN,-NH_2, -CH_3, -Cl). Unsaturation (-C=C-) emphasizes adrenocorticolytic action, eg, acrylonitrile.[7] Cysteamine, in particular, causes rapid duodenal ulceration and is one of the best agents for producing acute and chronic duodenal ulcer disease.

Biologic Features

Chemically induced duodenal ulcers can be produced in either male or female rats of various strains. We use Sprague-Dawley–derived Charles River CD female rats (Wilmington, Mass.) with an initial body weight of 200 g with *ad libitum* access to Purina Lab Chow (Ralston Purina Co., St. Louis, Miss.) and tap water. One to three injections of cysteamine-HCl in 10% aqueous solution (Aldrich Chem. Co., Milwaukee, Wis.) are given in

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a single day either orally by rubber stomach tube or subcutaneously at 3 to 4-hour intervals. To obtain *acute ulcers* animals are killed on the third day.

The dose-response studies with cysteamine have shown that the chemical is active after either oral (Figure 1) or subcutaneous (Figure 2) administration; a linear relationship exists between the dose and mortality (Figures 1 and 2). Ulcerogenesis, however, appears to be unimodal (especially following one full dose of cysteamine). If two or three small doses of the drug are administered, high ulcerogenic response and low mortality can be obtained. Our standard techniques call for the oral route: 28 mg/100 g × 3, 40 mg/100 g × 2, or 75 mg/100 g × 1; and, with the subcutaneous injection, 20 mg/100 g × 2.

Another method for producing acute and subacute duodenal ulcer has become available with the introduction of slow releasing ALZET capsules (ALZA Corp., Palo Alto, Calif.) Two osmotic minipumps of 150-μl capacity filled with 70% aqueous cysteamine-HCl (approximately 1.5 μl/hr release for 100 hours) were implanted subcutaneously in 100-g female rats which were killed on the seventh day. The development of capsules with larger reservoirs and releasing capacities will probably permit the implantation of only one capsule and/or the use of 200-g rats.

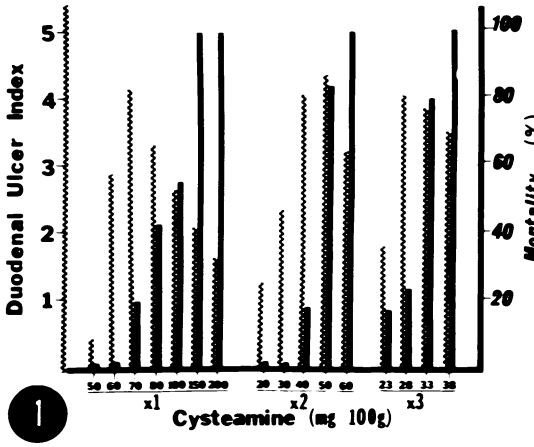
Chronic ulcers were induced by following an acute ulcerogenic regimen on the first day, eg, cysteamine-HCl, 28 mg/100 g orally × 3 or 40 mg/100 g orally × 2) and then giving the rats access to drinking water containing 0.2, 0.05, or 0.01% cysteamine-HCl. The low amount of cysteamine in drinking water is sufficient to maintain gastric hyperacidity; thus the cicatrization and chronicity of the duodenal ulcer can be followed for at least 21 to 60 days.[8]

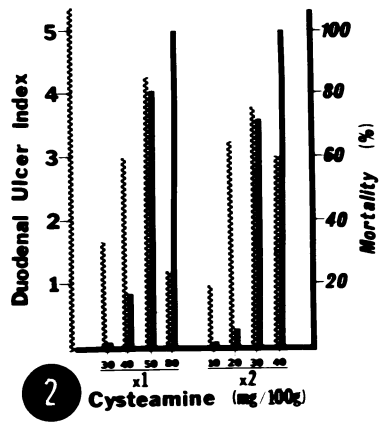
The ulcer develops 2 to 4 mm from the pylorus on the anterior (antimesenteric) wall of the duodenum and frequently perforates or penetrates the liver. A small ulcer is usually present on the posterior wall ("kissing ulcer") of the duodenum, and it invariably penetrates the pancreas. The lesion appears to start in the absorptive cells of villus folds of the proximal duodenum and progresses downward, resulting in an avillous area.[9] The eventual ulcer crater contains necrotic debris and is sharply demarcated and infiltrated by inflammatory cells by the third day. If perforation occurs, localized peritonitis ensues, or, as after penetration, necrosis and hemorrhage are evident in the corresponding parts of the liver and/or pancreas (Figures 3 and 4). Granulation tissue and (in 2 to 3 weeks) dense fibrous connective tissue are seen around the ulcer, and early mucosal epithelial regeneration on the edge of the ulcer can be detected (Figures 5 and 6). Active ulceration is evidenced by the presence of necrotic material and acute inflammatory response on the lumenal layers of the crater.

The duodenal ulceration is associated with increased gastric acid output, delayed gastric emptying,[10] and elevated serum gastrin levels which are further enhanced by peptone or food intake [11] and can be modulated by antacids, anticholinergic agents,[4] and cimetidine or vagotomy.[12]

Comparison With Human Disease

Duodenal ulcers in rats given cysteamine and in humans have similar pathomorphologic history and are on the anterior and/or posterior wall ("kissing ulcers"), frequently penetrating the pancreas. Functionally, the





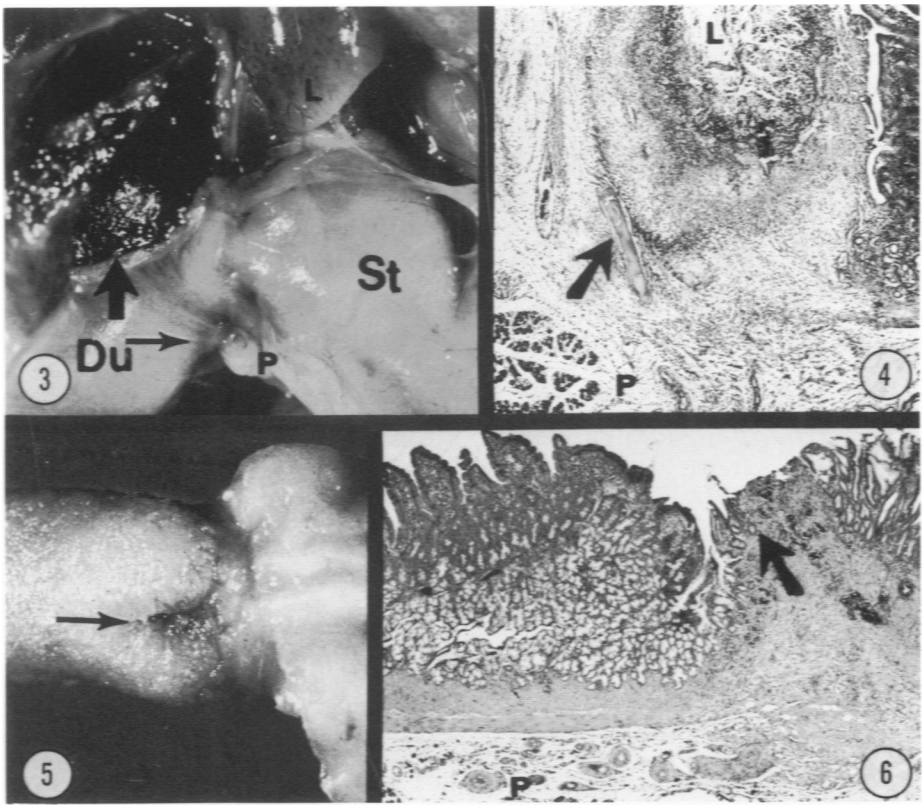


Figure 1—Dose-response for duodenal ulcerogenic action of cysteamine given orally in the rat. The ulcers were scored for intensity using a scale of 0 to 3, where 0 = no ulcer, 1 = superficial mucosal erosion, 2 = deep ulcer or transmural necrosis, 3 = perforated or penetrated (into the pancreas or liver) ulcer, and for incidence, ie, positive/total. The ulcer index is the sum of the arithmetic mean of the intensity in a group and the ratio of positive/total multiplied by 2, eg, 2.1 + (9/10 × 2). **Figure 2**—Dose-response for duodenal ulcerogenic action of cysteamine injected subcutaneously in the rat. **Figure 3**—*In situ* appearance of acute (2-day-old) duodenal ulcer in a rat treated with cysteamine. Note the large, fresh blood clot (*vertical arrow*) at the site of perforation, ie, penetration into the liver. Another ulcer (diametrically opposite to the first one) tends to penetrate the pancreas (*horizontal arrow*). *St* = stomach, *Du* = duodenum, *P* = pancreas, *L* = liver. **Figure 4**—Light microscopy of acute duodenal ulcer penetrating the pancreas. One large blood vessel (*arrow*) is in the middle of necrotic crater. *L* = lumen of duodenum, *P* = pancreas. (× 40) **Figure 5**—Gross (mucosal surface) presentation of a chronic (3-week-old) duodenal ulcer (*arrow*), which is sharply demarcated from the surrounding mucosa. **Figure 6**—Histologic section of chronic ulcer localized in the proximal duodenum at the pyloric junction. The dense fibrous connective tissue is partially covered with regenerating epithelium (*arrow*). *P* = pancreas. (× 40)

human duodenal ulceration is accompanied by increased gastric acid output and fasted serum gastrin levels, although these correlations are frequently challenged. Some experiments suggest that these associations exist in the course of cysteamine-induced duodenal ulcer as well.[4-6,10] The similarity of the food-sensitive hypergastrinemia in patients and rats with duodenal ulcer is striking.[11] The cysteamine-induced duodenal ulcer is frequently accompanied by adrenocortical necrosis. Although the ulcerogenic property of glucocorticoids in humans is also documented, histologic examination is almost impossible. The association of duodenal and adrenal lesions, the strict localization of duodenal ulcers on the anterior and posterior wall, the implications of central and/or peripheral nervous system and neuroendocrine interactions in duodenal ulcerations are probably the key and urgent questions in the elucidation of the mechanism of this disease.

Potential Usefulness of the Model

The chemically induced acute and chronic duodenal ulcer models in rodents are the first easily reproducible, economic models of peptic ulcer in which the ulcerogenic agent can be maintained for indefinite periods. At least three immediate applications of this model are noted: a) a model to study the pathogenesis of duodenal ulceration, b) a test to find antiulcerogenic regimens, and c) since some of these chemicals might have a role in the etiology of human duodenal ulcer as well,[13] the structure-activity conclusions may help to identify ulcerogenic chemicals in our food and environment.

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