0007-4888/93/0001-0038$12.50

Aggressive Role of Lysosomal Enzymes in Gastric Ulceration

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UDC 616.33-002.44-07:616.153.1:577.152.321

Translated from *Byulleten' Experimental'noi Biologii i Meditsiny*, Vol. 115, No. 1, pp. 37-38, January, 1993
Original article submitted June 25, 1992

Key words: *rat lysosomes, gastric ulceration*

The involvement of lysosomal enzymes of the gastric mucosa in the mechanisms of ulceration has been indicated recently in several reports [3, 13, 14]. As established, ulceration in the gastroduodenal zone in human beings and experimentally induced ulceration in animals is associated with elevated serotonin and histamine production. When administered in excessive doses, these biogenic amines can induce an ulcerogenic effect [3-6, 11]. In addition, steroid hormones in certain doses also provoke an ulcerative process [5, 6]. Gastric juice secreted in response to histamine in ulceration manifests much higher aggressiveness in comparison with that secreted by the intact stomach [1]. According to the accumulated data [3, 10, 12-14], gastric ulceration induced by stress, serotonin, or salts of bile acids leads to impairment of the lysosomal membrane stability, resulting in a release of lysosomal enzymes capable of inducing primary destruction of the mucous membrane tissue of the stomach.

Under physiological conditions, biogenic amines, hormones, and other biologically active substances maintain tissue homeostasis in the organism. However, under conditions of hyperproduction and focal increase in concentration, these compounds switch from physiological regulators to their exact opposites and begin to act as mediators of the early period of local aseptic inflammation [8, 9]. It is probable that in such a situation impairment of the lysosomal membrane permeability takes place followed by a further release of tissue proteases initiating the ulcerative process.

In the present study we made an attempt to experimentally investigate the ulcerogenic role of lysosomal proteases administered after premedication with excessive doses of biogenic amines and glucocorticosteroids.

MATERIAL AND METHODS

The experiments were performed on nonpedigree albino rats with a body weight of 220-240 g. The animals were divided into several groups, 15 rats in each. During three days before administration of lysosomal material they were placed on daily intraperitoneal injections of histamine (100 mg/kg), prednisolone (30 mg/kg), ACTH (10 U), serotonin (10 mg/kg), a mixture of serotonin and histamine, or serotonin and histamine administered at the same time but separately. The total volume of the preparations was adjusted to 1 ml with physiological solution. The rats in the control group received 1 ml of physiological solution.

The lysosomal material was obtained from the mucous membrane of resected stomach of rats with duodenal ulcer. We used the method of fractional sedimentation of lysosomes in a sucrose solution in EDTA [7] in consecutive centrifugation. The lysosomal fraction was administered between the serosal and mucosal layers of the stomach in a volume of 0.1 ml. The effect of both intact lysosomes and free lysosomal enzymes was tested. The lysosomal material was obtained after freezing-and-thawing of lysosomes repeated 5 times to destroy the lysosomal membrane. The release of lysosomal enzymes was monitored according to the appearance of proteolytic activity determined by the hemoglobin method.

One day after administration of the lysosomal material the rats were killed with ether, their stomachs were resected along the greater curvature and washed in water, and the condition of the gastric mucosa was assessed in each case.

RESULTS

Administration of lysosomes as well as free lysosomal enzymes between the serosal and mucosal layers of intact rat stomachs did not provoke injuries in the mucous membrane.

Injection of histamine during three days before the experiment did not result in ulceration either, while serotonin premedication induced the formation of ulcerative lesions of up to 40 mm^2. Histamine and serotonin, when administered as a mixture, slightly reduced mucosal sensitivity to lysosomal enzymes

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compared to that induced by serotonin. In this case we observed general hyperemia of the mucous membrane and the formation of lesions of 13 mm^2. The effect of premedication with histamine and serotonin administered separately proved to be the same as that obtained when the substances were injected together: we registered a reduced mucosal reactivity to the serosal component, hyperemia of the mucous membrane and formation of separate lesions of 13-15 mm^2.

ACTH and prednisolone in the doses used did not induce any noticeable changes in the state of the gastric mucosa, which remained pink, with normal folds, without edema or petechiae. Administration of physiological buffer did not affect the state of the gastric mucosa either.

Administration of lysosomes between the serosal and mucosal layers of the stomach did not cause any significant changes in the state of the gastric mucosa in any experimental group of rats.

When free lysosomal enzymes were used as the lysosomal material, the state of the gastric mucosa in rats that underwent premedication with histamine, serotonin, histamine plus serotonin injected together or separately, ACTH, or physiological buffer did not significantly differ from that described after premedication only or after premedication followed by administration of lysosomes.

Administration of free lysosomal enzymes after prednisolone premedication led to the formation of ulcerative lesions with an average area of 8 mm^2 at the sites of enzyme injection in all the animals tested. On the remaining area the gastric mucosa retained the normal appearance, and no edema or petechiae were observed.

Thus, among all the types of premedication tested, only prednisolone premedication favored manifestation of the ulcerogenic effect of exogenous lysosomal enzymes.

The protective properties of the organism in respect to lysosomal enzymes are well developed. This may be associated, first, with their active involvement in cell metabolism and, second, with the ensuing requirement of the organism to protect healthy cells from their destructive influence. A high resistance of the gastric mucosa against lysosomal enzymes probably explains the absence of lesions in intact rat stomachs after administration of lysosomal material. Independently of the type of lysosomal material - native lysosomes or free lysosomal enzymes - the mucosal tissue with unimpaired homeostasis proved highly resistant to its aggressive effect.

When homeostasis is impaired, the resistance of the organism to the factors of aggression changes, the character of the changes probably being dependent on the nature of the factors which caused the impairment of homeostasis.

Contrary to our expectations, in the experiments performed histamine did not promote manifestation of the ulcerogenic effect of exogenous lysosomal enzymes. It may be a high resistance of the gastric mucosa to histamine in rats that led to the situation where thrice-repeated administration of histamine proved insufficient for disturbing homeostasis of the mucosal tissue. It is probable that histamine accomplishes its role in ulceration through some other unknown mechanisms.

On the other hand, administration of serotonin even in a dose three times lower than that used for modeling experimental stomach ulcer [2, 3] led to such a generalized affection of the gastric mucosa that it was impossible to differentiate the effect of lysosomal enzymes in this process. The observed inhibitory influence of histamine on the serotonin effect raises a new problem concerning the interrelation between the serotonin and histamine mechanisms of ulcerogenesis as well as their role in the formation of the ulcerative defect.

Manifestation of the ulcerogenic effect of lysosomal proteins against the background of prednisolone testifies that prednisolone premedication in doses insufficient for the induction of ulcerative lesions significantly lowers the resistance of the gastric mucosa to agressive factors. It should be pointed out that lesions are of a local nature that may reflect the exictence of a specific correlation between the reaction of the organism to prednisolone and the action of lysosomal enzymes.

Since ACTH achieves its effect through the adrenal cortex, we expected to generate an effect analogous to that of prednisolone by stimulating production of corticosteroids. The absence of an ulcerogenic effect of lysosomal enzymes against the background of ACTH pretreatment in our experiments indicates that, although the dose of the agent was rather high, production of corticosteroids was not high and stable enough compared to the release of ACTH from the pituitary, so that the homeostasis of the mucosal tissue remained undisturbed.

Two conclusions may be drawn from the data obtained: 1) exogenous lysosomal enzymes administered between the serosal and mucosal layers are capable of generating an ulcerative process; 2) exogenous lysosomal enzymes provoke ulceration only when the organism's reactivity is reduced; in other cases their action is inhibited by some unknown protective systems.

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0007-4888/93/0001-0040$12.50

The Decrease of Acute Toxicity of Ethanol by Means of Zn-Metallothionein Preparation

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UDC 615.917:547.262].036.11.085

Translated from *Byulleten' Experimental'noi Biologii i Meditsiny*, Vol. 115, No. 1, pp. 39-40, January, 1993
Original article submitted July 23, 1992

Key Words: *Zn-metallothionein; ethanol: acute toxicity*

Metallothioneins (MT) are low-molecular cytoplasmic proteins which are able to bind selectively anions of heavy metals and contain up to 30% cysteine. MT synthesis induction results from the effect of toxic factors [3, 8, 11]. These substances have been proved to increase the organism's resistance to metals and to in the metabolic regulation of Zn and Cu levels [8]. The known increase of endogenous MT content in the liver of rodents influenced by low-molecular alcohols [3, 11] is considered to be a nonspecific response which leads to a decrease of the toxic effect of these compounds. There are few data about the effect of exogenous MT on the toxicity of both inorganic and organic substances. Thus, it was demonstrated that the preliminary injection of Zn-MT reduces the harmful influence of Cd-MT in rats [14].

In the present study it was demonstrated that exogenous Zn-MT is capable of reducing the acute toxicity of organic compounds, for example, that of ethanol.

MATERIAL AND METHODS

Mice ($(CBAxC_{57}Bl)F_1$ (25-30 g weight) and nonpedigree albino rats (300-400 g weight) were used in the experiments. All solutions were injected intraperitoneally. To induce MT synthesis 20 rats were injected with $ZnCl_2$ solution (10 mg of Zn ions per kg) and killed after 24 hours; the liver was extracted and washed with physiological solution. The tissue mass was mixed with 2.5 volumes of 10 mM Tris-HCl buffer at pH 7.4 ("standard buffer"), made homogeneous and centrifuged twice for 30 min at 15 000 g. The supernatant was heated for two minutes at 80°C and centrifuged under the same conditions. Cytoplasmic extract was concentrated by the ultrafiltration method up to 40 ml of volume and loaded onto a Sephadex G-75 column (3.2x95 cm) (Pharmacia, Sweden). The column was equilibrated and eluted with standard buffer. Protein concentration [10] and MT content using the cadmium-Hb method (from the quantity of labeled Cd bound) [5] were determined in the fractions. ^{109}Cd with a specific radioactivity of 11.3 GBq per mg (Izotop) was used. Absorption spectra of Zn-MT were registered in standard buffer. Electrophoresis under denaturation conditions was performed as previously described [9] on Pharmacia-LKB-2011 apparatus (LKB, Sweden) on polyacrylamide gel (1 mm thickness of layer) in a concentration gradient from 4 to 20%. Protein molecular mass standards

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