ROLE OF LYSOSOMAL ENZYMES IN DRUG-INDUCED ULCEROGENESIS

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The role of lysosomal enzymes (LE), which are generally regarded as being involved in cell damage, has not yet been adequately studied in relation to such a widespread destructive process as ulcer formation in the digestive tract (DT).

Data confirming the role of certain LE in ulcerogenesis induced by stress [9], by a combination of vitamin A with histamine [14], and by several ulcerogenic drugs (UGD), namely acetylsalicylic acid (aspirin) [10], butadione [11], and reserpine [13], have been obtained in the course of experimental studies. An exception is an investigation by Nagorney and co-workers [12], who were unable to confirm the involvement of lysosomes in histamine-induced gastric ulceration in guinea pigs.

In our opinion the study of the role of LE in the pathogenesis of bleeding ulcers of the mucous membranes of DT appearing under the influence of UGD, i.e., in the pathogenesis of drug-induced ulcerogenesis, is a promising trend in the study of the mechanisms of ulcerogenesis on various experimental (pharmacologic) models and in order to explain the effects of UGD, that cause injury to DT, from the standpoint of biochemical pharmacology.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 0.15-0.20 kg. UGD were injected in a single dose (aspirin 200 mg/kg, by the intragastric route, reserpine 5 mg/kg, intramuscularly) or over a period of 1 week (cincophen 500 mg/kg, indomethacin, 2.5 mg/kg, and butadione 150 mg/kg daily by the intragastric route), and for 2 weeks (cincophen 500 mg/kg, prednisolone 10 mg/kg by the intragastric route, prednisolone 10 and 20 mg/kg intramuscularly, daily). After sacrifice the stomach of each animal was opened along the greater curvature and the number of ulcerative hemorrhagic lesions (hemorrhages, erosions, ulcers) in the mucous membrane was counted macroscopically under a magnifying glass, after which the stomach wall was homogenized. A particular feature of the technique used in this investigation of LE activity was that only their free activity was determined. A similar approach also was used in other investigations [13, 14]. Activity of lysosomal cathepsins, however, was studied, as also by Watanabe and co-workers [14], at assigned pH values. Free activity of the following LE was determined in the gastric homogenates: acid phosphatase (AcP) by the method of Bessey et al. in the modification of Levitskii et al. [4], cathepsins at pH 3.5 and 5.5 (C 3.5, C 5.5) by Anson's method in Levitsii's modification [3], and acid nucleases: RNase by the method of Konsvets and Levitskii [2] and DNase by the method of Samoilyuk et al. [7]. Protein was determined by the method of Lowry and co-workers.

EXPERIMENTAL RESULTS

Ulcerative-hemorrhagic lesions of the gastric mucosa were not found in all rats receiving ulcerogenic doses of the drugs. Accordingly the changes in LE activity in the gastric wall were examined separately in animals with ulcers of the mucous membrane and in rats with no macroscopic signs of damage to the stomach.

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TABLE 1. Changes in Relative Free Activity of LE in Gastric Wall of Rats with Ulcers after Course of Intragastric Administration of UGD (in % of control)

Name of UGD	Dose, mg/kg	Exposure, weeks	Relative free activity of LE				
			КФ	К–3,5	К–5,5	RNase	DNase
Cincophen	500	1	160,10	110,33	1261,16*	193,18***	174,71
Cincophen	500	2	23,42***	33,84	798,54*	48,21**	14,52*
Aspirin	100	2	305,4**	376,39***	276,78	296,06**	31,79***
Indomethacin	2,5	1	136,18**	34,79***	1206,71***	79,19	9,54***
Butadione	150	1	113,65	41,48***	1149,66***	73,94	10,67***

Legend. Here and in Tables 2 and 3: *p < 0.05, **p < 0.01, ***p < 0.001.

TABLE 2. Changes in Relative Free LE Activity in Gastric Wall of Rats without Ulcerative Lesions after a Single Dose of UGD (in % of control)

Name of UGD	Dose, mg/kg	Exposure, weeks	Mode of administration	Relative free activity of LE				
				AcP	C 3.5	C 5.5	RNase	FNase
Aspirin	200	4	Intragastric	86,10	121,32	76.84	42,59*	49.85**
Reserpine	5	20	Intramuscular	115,44	128,12	446,18***	82.13	81,18

TABLE 3. Changes in Relative Free LE Activity in Gastric Wall of Rats without Ulcerative Lesions after a Course of Prednisolone (in % of control)

Name of UGD	Dose, mg/kg	Exposure, weeks	Mode of administration	Relative free activity of LE				
				AcP	C 3.5	C 5.5	RNase	DNase
Prednisolone	10	2	Intragastric	58,47***	115,87	76,25	45,81***	23,39
« »	10	2	Intramuscular	109,47	173,24***	93,59	126,78*	144,60
« »	20	2	Intramuscular	86,75	311,63***	135,98	183,52**	370,06

Changes in LE activity in the gastric wall of rats with ulcerative-hemorrhagic lesions of its mucosa after a course of various UGD were discovered in all groups of animals (Table 1), but the character and severity of the changes varied. The following significant changes were found most frequently an increase in relative free activity of C 5.5 and a decrease in DNase activity Changes in activity of the other LE, namely C 3.5, AcP, and RNase, differed in direction, so that no general rule could be established.

To understand the results, changes in LE activity in the gastric wall under the influence of cincophen, used as a model of experimental gastric ulcer, and which can be regarded as a standard UGD, were analyzed (Table 1). Whereas a single dose of cincophen caused a significant increase in relative free activity of C 5.5 and acid RNase, after a 2-week course, when the number of ulcers in the stomach was sharply reduced (the degree of ulceration was reduced by almost 15 times), the increase in C 5.5 activity became less marked, whereas activity of the remaining LE, on the contrary, was reduced: activity of AcP and both acid nucleases, moreover, was significantly reduced. Thus the changes in enzyme activity exhibited a distinct series of stages: initially an increase in activity predominated, but later a tendency was observed for it to decrease, possibly on account of leaking of LE from the injured cells.

Since ulcerative-hemorrhagic lesions of the stomach induced by cincophen are accompanied by both an increase and a decrease in free LE activity, any significant change in it can evidently be regarded as evidence of involvement of LE in ulcerogenesis. The results serve to explain the different trends in changes of enzyme activity found in the other groups of animals also.

In each individual case this trend may also be determined by the choice of UGD and the duration of its administration, and also by the stage at which sacrifice of the animal arrested the process of involvement of the lysosomes in injury to the gastric mucosa.

The character of this involvement also requires an explanation. It has been suggested that lysosomal proteases (cathepsins, capable in particular of destroying gastric mucoprotein) play an important, and indeed in achlorhydria, when peptic activity is absent, the most important role in ulcerogenesis, whether drug-induced or of other etiology, i.e., they can be regarded as effectors of proteolysis, leading to ulcerogenesis [5, 6, 14]. The role of the acid nucleases, however, remains unclear. Another important problem is that of the source of free activity of LE in the stomach wall. It is considered that UGD labilized the

membranes of gastric lysosomes, leading to the release of LE [11, 13]. However the possibility of outflow of LE from extragastric sources, for example, from the salivary granulocytes, which are always present in excess in the gastric contents [8], cannot be ruled out.

In groups of animals in which the ulcerogenic action of the drugs used was not in fact realized, changes also were found in free LE activity in the gastric wall (Tables 2 and 3). After a single dose of the UGD, they resembled those in animals with ulcers: reserpine caused a significant increase in free C 5.5 activity, whereas aspirin caused a decrease in activity of both acid nucleases (Table 2). A 2-week course of prednisolone in large doses caused no macroscopic signs of injury to DT in any animal. Meanwhile, significant but opposite changes in free LE activity were found in the gastric all: C 3.5, AcP, and RNase (see Table 3).

Thus administration of UGD to rats leads to changes in LE activity in the gastric wall, whether ulcerative-hemorrhagic lesions of its mucosa are present or absent. These changes in enzyme activity may perhaps be evidence of a kind of predisposition of the mucosa to ulceration. Other investigators [1, 15] also found changes in LE activity in the gastric wall which preceded ulcer formation in which ulcers were produced by agents other than drugs.

Evidently there exists a premorphologic (biochemical) stage of the damaging action of UGD on the gastric mucosa.

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