EFFECT OF SOME RADIOPROTECTIVE AGENTS ON CONTACT DIGESTION IN IRRADIATED ANIMALS

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UDC 617-001.28-092.9-085.771.8-07:616.34-008.1-072.7

Radioprotectors of the aminomercaptan group, especially mercamine (2-aminoethanethiol), almost completely prevent the inhibition of enzymic activity of the invertase of the small intestine observed in irradiated rats.

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In recent years the attention of gastroenterologists has been drawn to contact digestion, discovered by A. M. Ugolev [14], performed by enzymes fixed to the brush border of the intestinal epithelium and playing a role of considerable importance in the final hydrolysis of food substances.

Contact digestion has been found to be severely disturbed in animals exposed to irradiation [2, 15].

In most investigations the protective action of radioprotectors was estimated from morphological criteria [8, 10, 17, 19, 21]. Only isolated investigations have been made to study the effect of radioprotective substances on changes in the function of the digestive tract in irradiated animals [3, 5, 6, 22].

In the present study an attempt was made to prevent radiation injury to contact digestion by means of radioprotective substances.

EXPERIMENTAL METHOD

Experiments were performed on female albino rats weighing 150-200 g kept on a constant diet. The enzyme activity of the intestinal invertase was used to indicate the state of contact digestion. The material studied consisted of segments of the jejunum taken 20 cm away from the pylorus. The enzyme activity was determined from the increase in reducing substances as a result of hydrolysis of sucrose. Using A. M. Ugolev's method [14], the hydrolysis of sucrose was investigated in the presence of a segment of intestine, giving information on the state of contact digestion; the invertase activity of an intestinal homogenate was determined, to reflect the total activity of the enzyme in that particular region of the intestine; and the invertase activity passing into the solution in which the intestine was incubated was measured. The content of reducing sugars in all samples was determined by Nelson's method as modified by A. M. Ugolev.

The animals were irradiated on an RUM-3 apparatus, with maximal scattering, under the following conditions: 200 kV, 17 mA, filters Cu 0.5 mm and Al 1 mm, distance from anode to center of animals 40 cm, dose rate 55 R/min. The experiments were performed on the 3rd day after irradiation, i.e., when maximal inhibition of contact digestion was observed in the irradiated, unprotected animals.

To prevent radiation damage to the intestine, the most effective radioprotective substances, belonging to different classes of chemical compounds and considered to have different mechanisms of radioprotective action, were used. From the group of aminothiol compounds the following were used: β-mercaptoethylamine (mercamine, 150 mg/kg), S-β-aminoethylisothiouronium (AET, 150 mg kg), and the monosodium salt of β-aminoethylthiophosphoric acid (400 mg/kg) – a compound with a latent mercapto-group, breaking down during hydrolysis in the body with the formation of β-mercaptoethylamine. From the group of the indolylalkylamines, serotonin and its near analog 5-methoxytryptamine (mexamine, 15 mg/kg) were used. The radioprotective preparations were injected intraperitoneally 15 min before irradiation. A combination

Pathophysiological Laboratory, Central Roentgeno-Radiological Research Institute, Leningrad (Presented by Academician V. N. Chernigovskii). Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 65, No. 3, pp. 43-47, March, 1968. Original article submitted June 22, 1966.

TABLE 1. Effect of Some Radioprotective Substances on Intestinal Invertase Activity of Rats Irradiated with X Rays in a Dose of 700 R (M±)

Animals	Invertase activity*		
	of surface of mucous membrane	of intestinal homogenate	of desorbed enzyme
Unirradiated	37,9±2,4	78,8±9,0	6,5±1,6
Irradiated without protection P	3,1±1,0 <0,001	7,4±1,6 <0,001	1,4±0,8 <0,001
Irradiated w/inject. of mercamine P	33,4±4,2 <0,001	66,2±11,4 <0,001	6,5±1,0 <0,01
Irradiated w/injection of AET P	18,4±5,1 <0,01	42,8±8,6 <0,02	5,0±2,4 <0,25
Irradiated with injection of monosodium salt of β-aminoethylthiophosphoric acid P	20,1±6,1 <0,02	39,5±7,4 <0,002	2,2±0,7 >0,5
Irradiated with injection of serotonin P	7,4±1,6 <0,05	13,9±2,8 <0,05	1,4±0,5 >0,5
Irradiated w/injection of mexamine P	11,2±1,8 <0,1	27,6±3,0 <0,001	3,3±0,8 <0,25
Irradiated with injection of monsodium salt of β-aminoethylthiophosphoric acid + mexamine P	26,4±3,3 <0,001	34,3±2,9 <0,001	5,3±1,0 <0,01

*Invertase activity expressed in mg % of reducing sugars.

of the monosodium salt of β-aminoethylthiophosphoric acid in a dose of 350 mg/kg and mexamine in a dose of 10 mg/kg also was used. Each preparation was tested on no fewer than 10 animals.

The radioprotective substances tested all gave a distinct radioprotective effect, but one which varied in degree relative to the intestinal function studied. The highest degree of protection was found when sulfur-containing preparations were used. The most effective compound of this group was β-mercaptoethylamine, after prophylactic injection of which the degree of invertase activity in the irradiated animals in all 3 variants of the experiments was practically indistinguishable from that in the unirradiated animals. The monosodium salt of β-aminoethylthiophosphoric acid and AET gave a somewhat smaller radioprotective effect.

The amine protectors serotonin and mexamine were much weaker in their protective action on contact digestion than the sulfur-containing compounds (Table 1).

After the combined administration of the monosodium salt of β-aminoethylthiophosphoric acid and mexamine no statistically significant differences were obtained compared with the results from the use of monosodium salt of β-aminoethylthiophosphoric acid alone, although hydrolysis of sucrose in the brush border was somewhat higher in the case of combined protection.

Since the radioprotective substances possess high pharmacological activity and are used as a rule in subtoxic doses, experiments were carried out to study the effect of some of the radioprotectors tested on invertase activity of unirradiated animals. For this purpose rats were injected intraperitoneally with mercamine, AET, serotonin, and mexamine in radioprotective doses, after which their contact digestion was investigated on the 3rd day.

Preparations of the aminothiol group were found to have a marked inhibitory action on invertase activity. For instance, the invertase activity of the homogenate from animals receiving mercamine was 74% of the initial value, falling to 47% of its activity in intact animals in the case of preliminary administration of AET. Parallel to this, the increase in reducing substances in solution after incubation of the segment of intestine was also smaller, amounting to 75 and 58% of the initial level respectively.

TABLE 2. Effect of Certain Radioprotectors on Intestinal Invertase Activity of Unirradiated Rats

Substance injected	Invertase activity		
	of surface of mucous membrane	of intestinal homogenate	of desorbed enzyme
Mercamine			
control	37,6±4,6	184,0±18,4	9,4±2,7
experiment	28,1±4,3	135,3±15,6	5,9±1,6
P	<0,5	<0,5	<0,5
AET			
control	37,6±4,6	184,0±18,4	9,4±2,7
experiment	21,8±4,8	86,4±16,7	4,9±1,4
P	=0,05	<0,01	<0,5
Mexamine			
control	37,6±4,6	184,0±18,4	9,4±2,7
experiment	31,3±2,4	184,3±6,8	3,8±0,4
P	<0,5	>0,5	<0,25
Serotonin			
control	58,3±3,5	93,7±9,3	11,9±1,5
experiment	41,6±4,1	84,1±6,3	7,6±1,9
P	<0,05	<0,5	<0,2

In contrast to the sulfur-containing compounds, the radioprotective compounds of the amine group either had no effect on invertase activity (mexamine) or lowered it only very slightly (serotonin). Hydrolysis of sucrose under the influence of invertase of the intestinal homogenates was 100% of the control value on the 3rd day after injection of mexamine, compared with 90% after injection of serotonin. It is an interesting fact that, despite complete preservation of invertase activity in the homogenate, the invertase of the surface of the mucous membrane hydrolyzed 85% ($P < 0.5$) of the sucrose hydrolyzed under the same conditions by the mucous membrane of the intact animals after administration of mexamine, but only 71% ($P < 0.05$) after administration of serotonin (Table 2). It may be postulated that serotonin and, to a lesser degree, mexamine influence the process of translocation of the enzyme from the cell into the brush border without, however, changing the properties of the enzyme itself.

The results thus demonstrate that contact digestion in the small intestine of rats can be protected from disturbance by irradiation by means of radioprotectors. The differences discovered between the degree of radioprotective action of compounds of different chemical groups are in agreement with data in the literature [1, 13, 20].

Comparison of the results given in Tables 1 and 2 shows that the aminothiol compounds, although giving a more marked radioprotective effect, at the same time considerably inhibit invertase activity themselves in unirradiated animals. Conversely, protectors of the amino group themselves have little influence on the invertase activity of the intestine, and prevent changes in its activity during irradiation to a lesser degree.

One possible reason for the phenomena discovered may be differences in the effects of the tested compounds on mitotic activity of the intestinal epithelium. Regarding the aminomercaptans we know that they are inhibitors of cell division [4, 9, 11, 12]. So far as serotonin is concerned, according to data in the literature this preparation has no effect on the mitotic activity of the intestine or on its nuclear metabolism [7, 9]. The decrease in mitotic activity of the intestinal epithelium at the time of irradiation may evidently contribute toward the increase in its radioresistance, and may be one of the causes responsible for maintenance of its functions after irradiation.

Meanwhile, since the radioprotectors are pharmacologically highly active compounds, it is perfectly conceivable that the protection of the enzyme may take place in other ways, for example, by interaction between the protector and functional groups of the enzyme with the formation of temporary complexes protecting it against the action of free radicals produced during irradiation [16, 18], and also by its action on the nervous and humoral regulatory mechanisms, to whose influence enzymic processes are so highly sensitive.*

* The authors are grateful to F. Yu. Rachinskii for providing the preparations.

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