ULCEROSTATIC EFFECT OF TRIS-[2-HYDROXYETHYL] AMMONIUM SALT OF IRON-CONTAINING POLYACRYLIC ACID AND ITS POSSIBLE MECHANISMS

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Initial filling of ulcer defects with granulation and fibrous tissue is essential for restoration of the integrity and function of the mucous membrane [7]. When substances stimulating proliferation and repair are studied, quantitative analysis of the dynamics of connective tissue components is therefore indicated as an objective criterion of the course of repair.

The aim of this investigation was to evaluate the ulcerostatic effect of Tris-[2-hydroxyethyl] ammonium salt of iron-containing polyacrylic acid (APA) in experimental gastric ulcer.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats weighing 180-220 g. Gastric ulcer was produced in groups of animals (each consisting of 10 rats, deprived of food for 24 h) by physicochemical methods. Rats of group 1 were kept in a refrigerator at 2°C in constraining cages for 2 h. In rats of group 2 a gastric ulcer was induced by the serotonin method, in those of group 3 by the histamine method. In all these experiments APA was given perorally in a dose of 40 mg/kg 30 min before the experiment began. The effect of APA was compared with the action of vitamin U, which also was given perorally in a dose of 1 g/kg 30 min before the experiment began [3]. After 24 h the animals were killed with ethyl ether and the stomach was removed, examined, and the number of ulcers on the mucous membrane counted. In a special series of experiments gastric ulcer was produced by the acetate method [8]. Starting with the first day of the experiment the animals were given APA in a dose of 40 mg/kg daily for 10 days, perorally. The effects of APA were compared with the action of hydroxyferriscorbone (HFS), which was injected intraperitoneally in a dose of 30 mg/kg [2], and also of methyluracil (MU), which was given in a dose of 500 mg/kg perorally [1]. Groups of rats were killed on the 3rd, 7th, 10th, 20th, and 30th days after the operation. The stomach was removed and the area of the ulcer measured. Some stomachs of each group were investigated histologically by the traditional methods of fixation and staining, others were used for quantitative analysis of the dynamics of the connective-tissue components [4, 5]. Similar experiments on untreated rats served as the control. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

APA had an ulcerostatic action in all experiments (Table 1). It was more effective than vitamin U. In experiments with acetate-induced ulcer APA accelerated healing of the mucosal defect more than HFS and MU (Table 2).

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TABLE 1. Effect of Preparations on Development of Experimental Gastric Ulcer in Rats (M ± m)

Experimental conditions	Number of ulcers per animal	p
Control 1 – histamine 30 mg/kg	8,8±0,5	
Vitamin U 1000 mg/kg + histamine 30 mg/kg	7,2±0,9	<0,1
APA 40 mg/kg + histamine 30 mg/kg	4,5±0,2	<0,01
Control 2 – serotonin 20 mg/kg	8,0±0,9	
Vitamin U 1000 mg/kg + serotonin 20 mg/kg	5,3±0,8	<0,01
APA 40 mg/kg + serotonin 20 mg/kg	0,5±0,1	<0,001
Control 3 – immobilization	21,2±0,5	
Vitamin U 1000 mg/kg + immobilization	5,8±0,4	<0,01
APA 40 mg/kg + immobilization	2,5±0,2	<0,001

TABLE 2. Dynamics of Healing of Acetate-Induced Gastric Ulcer in Rats Treated with APA and Hydroxyferriscorbone (M ± m)

Preparation	Hydroxyferriscorbone				
	3	7	10	20	30
Control	117,8±9,5	113,8±9,5	36,1±3,5	34,5±4,1	11,0±2,5
APA	90,5±11,0	45,6±11,9	11,8±1,3	4,6±0,4	0
Time of observation, days	98,1±15,7	87,0±24,0	58,7±14,7	13,5±4,4	4,7±3,8

Legend. In all groups of experiments relative to control $p < 0.01$. Values given are area of ulceration, in mm^2.

Biochemical data on the time course of concentrations of collagen (as hydroxyproline) and noncollagen proteins (as tyrosine) in the ulcer tissue are given in Table 3. In the control collagen accumulated rapidly between the 10th and 20th days after the operation, and toward the end of the period of observation its concentration was almost 1.5 times higher than the normal level. The concentration of noncollagen proteins, however, was reduced. As a result of this, at the end of the observations the ratio of the mass of tyrosine to the mass of hydroxyproline was 0.92 compared with 1.91 in the intact gastric wall. In other words, fibrous tissue formed in the region of the ulcer defect, with disturbance of the relative proportions of connective proteins.

In rats treated with HFS accumulation of collagen took place in about the same way as in the control, but at the same time there was a significant change in the dynamics of the noncollagen protein levels, possibly due either to inhibition of their destruction or stimulation of their restoration.

Among animals receiving MU, the dynamics of accumulation and destruction of both collagen and noncollagen proteins did not differ significantly from the corresponding values in rats receiving HFS.

The dynamics of the connective-tissue proteins after administration of APA appeared to be different. Among rats receiving APA the collagen concentration was virtually unchanged during ulcer development. The concentration of noncollagen proteins and the tyrosine/hydroxyproline ratio, also were stable: at the end of the observations the latter was 1.67, i.e., significantly closer to normal. This indicates sufficiently complete restoration of the connective-tissue polymers in the gastric tissue.

The distribution of glycosaminoglycan (GAG) fractions in the intact stomach wall had one distinguishing feature, namely a high concentration of the fraction extractable with 2.1 M sodium chloride solution, i.e., according to data in [6] – the heparin sulfate fraction. Among the rats the total GAG concentration rose on account of the fraction extracted with 0.4 M sodium chloride, i.e., of hyaluronate.

TABLE 3. Concentration of Some Substances in Tissue of Gastric Ulcer (relative units) Treated by APA and Hydroxyferriscorbone in Rats (M ± m)

Time of observation, days	Hydroxyproline (1.44 ± 0.13)			Tyrosine (2.75 ± 0.50)			Sialic acids (0.37 ± 0.4)		
	untreated animals (control)	treated with APA	treated with hydroxyferriscorbone	untreated animals (control)	treated with APA	treated with hydroxyferriscorbone	untreated animals (control)	treated with APA	treated with hydroxyferriscorbone
3	1,39±0,22 —3,5	1,42±0,23 —1,4	1,44±0,17 (sic)	2,12±0,26 —20,9	2,53±0,51 —8,0	2,39±0,3 —13,1	0,83±0,01 +12,4	0,63±0,19 +70,3	0,63±0,1 +70,3
7	1,31±0,29 —9,0	1,26±0,8 —12,5	1,60±0,12 +11,1	2,06±0,3 —25,1	2,70±0,28 —1,8	2,06±0,14 —25,1	0,89±0,01 +140	0,74±0,18 +100	0,63±0,1 +70,3
10	1,37±0,26 —4,9	1,38±0,16 —4,3	1,91±0,26 +32,6	1,92±0,18 —30,2	3,02±0,51 +9,8	2,00±0,2 27,3	0,81±0,1 +119	0,66±0,19 +78,4	0,75±0,0 +103
20	2,15±0,12 +49,3	1,66±0,51 +15,3	2,14±0,15 +48,6	1,79±0,16 —34,9	2,64±0,25 —4,0	2,11±0,12 —23,3	0,48±0,03 +29,7	0,54±0,1 +45,9	0,57±0,0 +54,1
30	2,03±0,30 +38,9	1,51±0,36 +4,9	2,10±0,37 +45,8	1,87±0,21 —32,0	2,53±0,38 —8,0	2,30±0,40 —16,4	0,57±0,17 +54,1	0,58±0,11 +50,8	0,48±0,0 +30,0

Legend. Value of parameter in intact animals shown between parentheses.

TABLE 4. Concentrations of Hexuronic Acids and Distribution of Their Fractions (M ± m)

Group of animals	Time of observation, days	Concentration of hexuronic acids, g/100 g dried, defatted tissue	Fractions extracted by solutions of sodium chloride, g/100 g of dried defatted tissue		
			0,4 M I	1,2 M II	2,1 M III
Intact		0,66±0,12	0,23(35)	0,18(27)	0,25(37)
Untreated (control)	3	0,77±0,13(+16,7)	0,28(36)	0,25(33)*	0,24(31)*
	7	0,81±0,17(+22,7)*	0,29(35)	0,24(30)	0,28(35)
	10	0,78±0,14(+18,1)	0,29(37)	0,20(25)	0,29(38)
	20	0,77±0,13(16,7)	0,31(40)*	0,24(31)	0,22(29)*
	30	0,85±0,15(+25,8)*	0,38(45)*	0,23(27)	0,24(28)*
Treated with APA	3	0,81±0,10(+22,7)*	0,34(42)*	0,20(25)	0,27(33)
	7	0,60±0,04(—9,1)	0,26(44)*	0,16(27)	0,18(29)
	20	0,65±0,1[illegible](—1,5)	0,25(33)	0,15(27)	0,25(40)
	30	0,71±0,20(+7,6)	0,23(32)	0,19(29)	0,28(39)
Treated with hydroxyferriscorbone	3	0,53±0,11(—19,7)	0,26(53)*	0,17(32)*	0,08(15)*
	7	0,55±0,16(—15,7)	0,25(46)*	0,21(37)*	0,09(17)*
	20	0,51±0,10(—22,7)*	0,24(47)*	0,19(38)*	0,08(15)*
	30	0,54±0,22(—18,2)	0,24(45)*	0,19(35)*	0,11(20)*

Legend. Asterisk indicates significance of difference ($p < 0.01$) compared with standard (intact animals). Numbers in parentheses: for hexuronic acid change, in %; for fractions % of total concentration of hexuronic acids; n = 10.

During treatment with HFS the total GAG concentration fell on account of fractions I and II, evidence of the slower development of granulation and fibrous tissue. Consequently, treatment with HFS does not adequately ensure restoration of quantitative ratios between concentrations of connective-tissue glycoconjugates (Table 4). Conversely, treatment with MU, as Table 4 shows, leads to the opposite trend of GAG concentrations, i.e., it stimulates restoration of glycoconjugates. Comparison of the effects of these preparations shows significant differences in the mechanisms of their action.

In response to administration of APA the total GAG concentration initially rises on account of hyaluronate. Later, their dynamics approaches the standard value. This means that under the influence of APA satisfactory restoration of concentrations of collagen, noncollagen proteins, and GAG takes place.

The antiinflammatory (antiexudative) action of all the substances studied (in terms of changes in sialic acid levels) was approximately equal in intensity (Table 3).

It was shown morphometrically that in all experiments the stage of neutrophilic infiltration was replaced toward the 7th-10th day by a stage of active development of granulation and fibrous tissue, with numerous fibroblasts and with the formation of collagen fibers. However, the use of APA accelerated maturation of fibrous tissue and reduced the number of hyaline cells. At the end of the observations, in the case of treatment with APA, definitive tissue formation took place with only a few fibroblasts, ending with epithelization. Among the control rats and rats

treated with MU and HFS, the granulation and fibrous tissue was still rich in cells toward the end of the observations.

Meanwhile monitoring nucleic acid levels in these experiments showed that restoration of the mucous membrane follows a similar course under the influence of APA and MU, suggesting that similar stages occur in the mechanism of action of these substances. Considering information [1] on the action of MU on the genetic apparatus, we also consider that APA may act at this level also.

The results thus indicate that APA is a promising ulcerostatic agent, which can influence the dynamics of important connective tissue components, namely collagen, noncollagen proteins, and glycosaminoglycans, during development of experimental gastric ulcers.

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SERUM ANTIOXIDANT ACTIVITY IN ANIMALS WITH AN EXPERIMENTAL CRUSH SYNDROME

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The crush syndrome (CS) from the pathogenetic point of view has the most complex pathology in traumatology. The most conspicuous feature (after traumatic shock) is given by disturbances of metabolic character, accompanied by the release of toxic substances into the blood. The appearance of these substances is preceded by prolonged tissue ischemia, leading to a disturbance of the oxygen balance in its acute stage and after reperfusion. According to the classification suggested in [3], CS is a form of total ischemia, characterized by cessation of the circulation in the organ and its transition by an open form of existence into a closed form. Under these conditions oxygen deficiency plays the main role in the pathogenesis of the lesion, for active forms of oxygen appear, and lead to the intensification of free-radical oxidation (FRO) [9]. The presence of antioxidants in the tissues and liquid media, inhibiting the development of FRO, can be regarded as the first line of defense the body against the aggressive influence of free

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