PROTECTIVE EFFECT OF IONOL IN ISCHEMIC INJURY TO THE SMALL INTESTINE

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A phenolic antioxidant 4-methyl-2,6-di-tert-butylphenol (ionol) has been proved to be an effective tissue (membrane) protector in various pathological states, and the sphere of its use is continually expanding [1-7]. The most promising method of its administration in medical practice is the peroral route. Meanwhile, the effect of ionol on the intestinal mucosa has not been studied.

This paper gives the results of a study of the action of ionol on the transport and barrier properties of the epithelium of the small intestine in rats under normal conditions and after temporary ischemia. Ionol was found to increase absorption in the normal intestine, to prevent a sudden increase in permeability and cell loss induced by ischemia, and also to increase the survival rate of the animals in the postischemic period.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 200-250 g. The barrier properties of the mucosa were assessed by determining the transepithelial resistance (R), the relative ionic permeability (P_{Na}/P_{Cl}), and the resistance of the epithelial lining to induced cell loss (ICL). The value of R was determined by passing dc pulses with a density of under 50 $\mu A/cm^2$, the values of P_{Na}/Cl was calculated from the results of measurement of the transepithelial diffusion potential (E) by the equation for a steady field. The resistance of the epithelial lining to ICL was considered to be inversely proportional to the number of single enterocytes (N) isolated from tissue exposed under standard conditions to a flow of fluid directed tangentially to the surface of the intestinal tube. The transport properties were assessed from the short-circuiting current (SCC), which is proportional to the velocity of active Na transport — the chief type of transport activity of the intestinal epithelium. All measurements were made on the same part of the small intestine, which was separated every time 15 cm away from the ileocecal angle. The time from sacrifice of the animals to the beginning of the measurements was constant at 20 min. Methods of measuring R, P_{Na}/P_{Cl}, SCC, and ICL were described previously [6, 8]. Total ischemia was produced by temporary ligation of the vessels of the small intestine [6]. After the specified time the ligatures were removed and the abdominal wall closed by a continuous suture. In all the experiments ionol was used in a dose of 240 mg/kg body weight. Control rats received the solvent — 0.01 ml of Tween-80 in 0.5 ml distilled water — by intraperitoneal injection or 0.5 ml of vegetable oil for intragastric administration. The results were subjected to statistical analysis by the use of the Wilcoxon—Mann—Whitney nonparametric sign tests, Fisher's accurate method, and Student's test.

EXPERIMENTAL RESULTS

Values of the selected parameters in the control and experiment 1 h after administration of ionol by gastric tube are given in Table 1. An appreciable drop in R, i.e., an increase in permeability of the epithelium, and a sharp rise in SCC, due to stimulation of active Na transport, indicate an increase in absorption. The value of P_{Na}/Cl corresponding to the mean control value of E = 5 mV, was 2.0. After administration of ionol the relative ionic permeability increased to 2.7, but this difference from the control was not statistically significant. Ionol also had virtually no effect on ICL.

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TABLE 1. Effect of Ionol on Transport and Barrier Properties of Epithelium of Intact Rat Small Intestine (M ± m)

Parameter	Control	Experiment — administration of ionol
R, $\Omega \cdot cm^2$	61,2±3,8	49,3±3,0*
E, mV	5,0±0,7	7,2±1,3
SCC, $\mu A/cm^2$	22,5±2,1	42,4±4,7*
ICL, percent	100±11,3	131±8,5

Legend. Number of experiments was 12. *P < 0.05 compared with control.

TABLE 2. Effect of Preliminary Intragastric Administration of Ionol on Changes in Transport and Barrier Properties of Small Intestine after Total Ischemia for 50 min (M ± m)

Parameter	Control	Ischemia	Ionol + ischemia
R, $\Omega \cdot cm^2$	61,5±6,3 (20)	33,3±4,0 (18)**	50,8±6,3 (18)***
E, mV	7,5±0,5 (16)	—2,3±0,1 (11)**	—0,9±0,2 (12)****
SCC, $\mu A/cm^2$	22,0±2,0 (20)	4,2±1,4 (18)*	12,8±2,1 (17)***
ICL, percent	100±6,9 (18)	628±52,1 (15)*	390,8±26,8 (18)**

Legend. Number of experiments shown in parentheses. *P < 0.001, **P < 0.01 compared with control; ***P < 0.01, ****P < 0.001 compared with ischemia.

Ischemia of the small intestine for 50 min induced a sharp decrease in R, P_{Na}/P_{Cl}, and SCC and in increase in ICL (Table 2). The value of E under these circumstances changed its sign, i.e., the epithelium became anion-selective. Intragastric administration of ionol 1 h before intestinal ischemia had a marked protective action.

Disturbance of the transport and barrier properties of the small intestine during its ichemia was probably the main cause of the complications in the postischemic period. The degree of these disturbances was proportional to the duration of ischemic injury [6, 8]. Substances helping to preserve the integrity of the epithelial lining and preventing an increase in its permeability can abolish some complications of the postischemic period and, in particular, they can increase the survival rate of the animals. To verify this hypothesis, the effect of ionol on the survival rate of the rats after temporary total ischemia of the small intestine was studied. After ischemia for 50 min this was impossible, for under those circumstances about 90% of the animals survived. However, when the duration of ischemia was increased to 75 min the mortality rose to 90%. The animals died during the first two days after the operation. Ionol was shown to cause a real increase in the survival rate and the maximal effect was observed after intragastric administration of the compound 1 h before ischemia (Table 3).

Previously ionol was found to have the ability to protect the gastric mucosa against the formation of stress ulcers [3], to protect membranes of the sarcoplasmic reticulum and lysosomes of the limb muscles and membranes of the endoplasmic reticulum of the liver against ischemic and reoxygenation injuries, and also to increase the survival rate of animals after ischemic damage to the kidney and liver [1-3]. In the present investigation the corresponding property of ionol was discovered in relation to ischemic damage to the small intestine. However, intraperitoneal injection which, according to data in the literature [2], is more effective than intragastric administration, proved to be much less effective, on the other hand, in the present experiments.

The results are thus evidence that even comparatively high doses of ionol, given by gastric tube, have no harmful action on the epithelial lining of the small intestine. Moreover, this antioxidant can increase absorption and prevent disturbance of the transport and barrier

TABLE 3. Effect of Preliminary Administration of Ionol on Survival of Animals after Total Ischemia of the Small Intestine for 75 Min

Mode of administration	Number of animals dying during first day after ischemia, percent		
	ischemia	ionol + ischemia	P
Intraperitoneally 2 h before ischemia	90 (18 of 20)	75 (12 of 16)	
By gastric tube			
2 h before ischemia	90 (9 of 10)	70 (7 of 10)	
1 h before ischemia	86 (6 of 7)	22 (2 of 9)	<0,05

properties of the mucosa induced by ischemia. This last effect of ionol is evidently largely responsible for the increase in the survival rate of the animals in the postischemic period.

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LITERATURE CITED

1. Yu. V. Arkhipenko, M. V. Bilenko, S. K. Dobrina, et al., Byull. Éksp. Biol. Med., No. 6, 683 (1977).
2. M. V. Bilenko, in: Bioantioxidants in the Regulation of Metabolism under Normal and Pathological Conditions [in Russian], Moscow (1982), p. 195.
3. M. V. Bilenko, V. E. Kagan, D. M. Velikhanova, et al., in: Prospects for Bioorganic Chemistry in the Creation of New Therapeutic Preparations [in Russian], Riga (1982), p. 200.
4. T. A. Devyatkina, Dokl. Akad. Nauk SSSR, 242, 449 (1978).
5. F. Z. Meerson, Adaptation, Stress, and Prophylaxis [in Russian], Moscow (1981).
6. A. G. Melikyants and V. G. Kucheryanu, Izd. Akad. Nauk SSSR, No. 4, 527 (1981).
7. N. M. Émanuél', Dokl. Akad. Nauk SSSR, 209, 1213 (1973).
8. A. G. Melikyants (A. G. Melikjants), V. P. Coulic and G. V. Kucheryanu (V. G. Koutcherianu), Gastroent, Clin. Biol., 4, 265 (1980).