USE OF ANTIOXIDANTS TO PREVENT RENAL DAMAGE DURING ACUTE ISCHEMIA AND REPERFUSION

M. V. Bilenko, L. N. Shelenkova,
G. Ya. Dubur, and A. Kh. Velena

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It was shown previously that ischemia and hypoxia of organs and recovery of the blood flow in the ischemic organs (reperfusion) are accompanied by activation of lipid peroxidation (LPO) with the accumulation of its products and lowering of the antioxidative activity (AOA) of lipids [1-4, 9, 11], with corresponding disturbance of cellular homeostasis and of the structure and function of the organ. There have also been reports of the successful use of antioxidants to protect organs against ischemic damage [10, 13-15]. However, the optimal doses, times, and methods of adminstration of antioxidants and also the mechanisms of their antiischemic action have not yet been established, and this has delayed the introduction of this method into clinical practice.

The object of this investigation was to compare the protective effects of the natural antioxidant α-tocopherol and the synthetic antioxidants ionol (dibunol), 6-mercurascan (phenol derivatives), and diludin (a derivative of 1,4-dihydropyridine) during ischemia, lasting 2 h, and subsequent reperfusion of the kidneys. Different doses, times, and modes of adminstration of the compounds were used and the anti-ischemic effect of ionol and its effect on AOA of the renal lipids also were compared.

EXPERIMENTAL METHODS

Experiments were carried out on 413 male August rats weighing 150-180 g. Renal ischemia was created under hexobarbital anesthesia (70 mg/kg) by applying microclips to the vascular bundles of both kidneys isolated from perinephric areolar tissue. The increase in the number of rats (in %) surviving 1 month after the operation was used as integral criterion of renal function and indicator of the positive effect of the treatment. The mean life span of the animals also was determined.

Ionol (2,6-di-tert-butyl-4-methylphenol) was given as a single dose of 30-360 mg/kg body weight, 2-24 h before ischemia, intraperitoneally or by the intragastric route (183 experiments). α-Tocopherol was injected intraperitoneally once a day for 3 days before ischemia in a dose of 50 mg/kg (10 experiments). Diludin (3,5-diethoxycarbonyl-2,6-dimethyl-1,4-dihydropyridine) was given by the intragastric route in a single dose of 60-240 mg/kg, 4 or 24 h before ischemia (68 experiments). Its antioxidant and antiradical effect have been demonstrated previously in experiments on lipids and on model and biological membranes [7, 8]. All lipid-soluble antioxidants were administered in the form of an aqueous suspension in Tween-80, whereas ionol, when given by the intragastric route, was given in the form of a 5% solution in sunflower oil. The water-soluble preparation 6-mercurascan (a mercury derivative of fluorescein) was synthesized in Czechoslovakia and generously provided by Professor P. Malek. The compound was dissolved in cold physiological saline (4°C) and injected intravenously 15-180 min before ischemia or immediately before restoration of the blood flow in the kidneys, in doses of 0.5-1.5 mg/kg (96 experiments). Rats exposed to ischemia for 2 h without administration of antioxidants served as the control (20 experiments).

Sector of Antiischemica Drugs, Research Institute of Biological Testing of Chemical Compounds, Kupavna, Moscow Province. Laboratory of Membrane-Active Compounds and β-Diketones, Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR, Riga. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Kovanov.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 96, No. 9, pp. 8-11, September, 1983. Original article submitted December 17, 1982.

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TABLE 1. Effect of Ionol on Survival of Rats after Renal Ischemia for 2 h (in %)

Time between administration and beginning of ischemia, h	Intraperitoneal injection				Intragastric administration		
	30 mg/kg (n=23)	60 mg/kg (n=28)	120 mg/kg (n=24)	240 mg/kg (n=28)	120 mg/kg (n=15)	240 mg/kg (n=48)	360 mg/kg (n=17)
2	0	0	17	33		17	
4	0	0	50**	17	14	28*	25**
12	0	40**	50**	50**		50**	
24	20	50**	50**	66***	0	0	33

Legend. Here and in Tables 2-3: *P < 0.05, **P < 0.01, ***P < 0.001 compared with control; n) number of experiments.

TABLE 2. Effect of Diludin on Survival Rate of Rats (A, in %) and on Mean Length of Survival of Rats Which Died (B, days, M ± m) after Renal Ischemia Lasting 2 h

Time between administration and beginning of ischemia, h	Dose, mg/kg					
	60 (n=14)		120 (n=36)		240 (n=18)	
	A	B	A	B	A	B
4	50*	14,0±0,1***	73***	14,7±0,8***	50**	2,8±0,2
24	0	9,5±2,7***	68***	5,5±0,5**	63***	4,0±0,3**

TABLE 3. Effect of 6-Mercurascan on Survival Rate of Rats (A, in %) and on Mean Duration of Survival of Rats Which Died (B, in days, M ± m) after Renal Ischemia Lasting 2 h

Time between administration and beginning of ischemia, min	Dose, mg/kg					
	0,5 (n=27)		1,0 (n=36)		1,5 (n=33)	
	A	B	A	B		B
Before reperfusion	11**	5,8±0,9	0	4,4±0,1	0	3,3±0,3
15	33*	2,5±0,7	20**	3,6±0,9	55	1,3±0,3
60	0	3,0±0,6	0	3,0±0,7	22**	1,6±0,2
180	0	3,3±0,8	0	2,3±0,2	25**	5,5±1,8

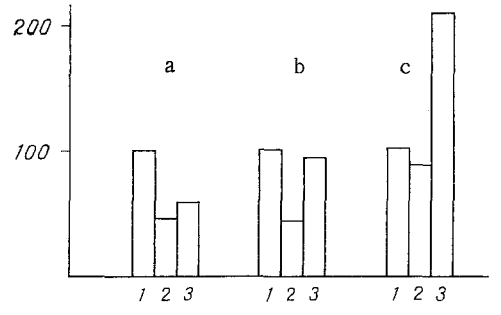

Fig. 1. Effect of ionol on antioxidant activity of lipids in intact (1), ischemic (2), and reperfused (3) kidneys. a) Ischemia for 2 h, b) ischemia + ionol (30 mg/kg), c) ischemia + ionol (120 mg/kg). Ordinate, level of AOA (in % of control).

To compare the anti-ischemic effect of ionol with its effect on AOA of renal tissue lipids, intact kidneys, kidneys after exposure to ischemia for 2 h, and kidneys after reperfusion for 4 h in animals receiving ionol by intraperitoneal injection in doses of 30 and 120 mg/kg, 4 h before removal of the organ or before ischemia, and also from animals not receiving ionol, were removed in 36 acute experiments. Lipids were extracted from the kidneys with a mixture of chloroform and methanol, and AOA was determined on a methyl oleate model. The significance of differences in the survival rates of the rats between the experimental and control series was determined by Pearson's (chi-square) test, that for the mean life span of the rats by Student's test for small samples.

EXPERIMENTAL RESULTS

In control experiments with 2 h of renal ischemia all rats died on average 2 ± 0.2 days after the operation. When α-tocopherol was given the number of surviving rats was 33% ($P < 0.01$) and the duration of their survival was 3.5 ± 0.8 days ($P < 0.05$), evidence of an anti-ischemic effect of the compound, which other workers also have demonstrated on a model of acute hypoxia [16].

Ionol, injected intraperitoneally, led to a significant increase in survival of the rats in doses of 60-240 mg/kg (Table 1); a dose of 120 mg/kg was effective over the widest time interval (4-24 h before ischemia), whereas a dose of 240 mg/kg given 24 h before ischemia gave the greatest increase in the number of surviving animals (66%). When the intragastric route was used a significant anti-ischemic effect was obtained only with higher doses of ionol, namely 240 and 360 mg/kg, given 4-12 h before ischemia, i.e., the compound was less effective than when given intraperitoneally. This fact, together with the better results of later administration of the compound, is evidence that the anti-ischemic effect of ionol is not dependent on its concentration in the organ which, as was shown previously [6], is highest in the first 1-2 h after injection, and is higher when given by the intragastric route than by intraperitoneal injection, but is realized by more complex pathways. The effect of different doses, times, and methods of administration of ionol on the length of survival of the rats was less marked than on the survival rate of the animals and was clearly demonstrated only after intragastric administration in a dose of 360 mg/kg, given 4 or 24 h before ischemia (11.0 ± 2.0 and 9.5 ± 2.6 days; $P < 0.001$).

Administration of diludin 4 h before ischemia gave a high rate of survival of the rats with all doses used; administered 24 h before ischemia it was effective only in doses of 120 and 240 mg/kg; a dose of 120 mg/kg was most effective when administered at both times (Table 2). Comparison of the data in Tables 1 and 2 shows that the number of rats surviving after 2 h of renal ischemia was greater after treatment with diludin than after intragastric administration of the same or higher doses of ionol, and that diludin in a dose of 120 mg/kg was more effective than intraperitoneal injection of the corresponding dose of ionol (survival rate of the rats 73-68% compared with 50%; $P < 0.01$). The anti-ischemic effect of diludin also was manifested as a marked increase in duration of survival of the animals compared with the control; this effect was most marked when the compound was given in a dose of 60-120 mg/kg 4 h before ischemia.

The anti-ischemic action of 6-mercurascan, like that of the lipid-soluble antioxidants, was dose-dependent and time-dependent in character and was most marked when the compound was given 15 min before ischemia (Table 3). Injection of 6-mercurascan immediately before reperfusion of the kidneys was ineffective, and prior administration (1 and 3 h before ischemia) was effective only when a high dose (1.5 mg/kg) was used. The therapeutic effect of the compound also was demonstrated previously in experiments with focal ischemia of the heart [12]. The fact that the compound can be injected intravenously and given a short time before the commencement of ischemia in an organ widens its applicability and facilitates combination with other drugs with a more deferred action.

The study of AOA of the lipids showed that in experiments without ionol and in those in which ionol was given in a dose of 30 mg/kg (ineffective dose), AOA in the kidneys after ischemia for 2 h was much lower than initially, whereas after treatment with ionol in a dose of 120 mg/kg (highly effective dose) it did not differ significantly from initially and rose considerably in the early postischemic period (Fig. 1). The ability of ionol to maintain stable AOA during ischemia evidently plays an essential role in its anti-ischemic effect. As was shown previously, administration of ionol and α-tocopherol also inhibited accumulation of LPO products in membranes of the sarcoplasmic reticulum of the limb muscles and the

endoplasmic reticulum of the liver, and this effect correlated with improvement of the functional and structural state of these membranes [2, 5]. These facts are evidence that the anti-ischemic effect of antioxidants is evidently largely due to their inhibitory action on LPO processes, although other mechanisms cannot be ruled out (inhibition of phospholipases, stabilization of membranes, effect on the rheologic properties of the blood).

The compounds used, when administered prophylactically, thus had a protective action on kidneys exposed to long-term ischemia and reperfusion; the effect depended on the dose, time, and method of administration of the compound and was most marked with ionol and diludin. The anti-ischemic effect of ionol is largely connected with its ability to maintain the AOA level of the lipids and to inhibit accumulation of LPO products during ischemia of the organ.

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