Effect of Combined Treatment with Sodium Hypochlorite and α-Tocopherol on Prooxidant and Antioxidant System of the Blood during Experimental Bile Peritonitis

E. A. Petrosyan, V. I. Sergienko, A. A. Sukhinin, I. S. Zakharchenko, and S. S. Oganesyan

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Combined postoperation treatment with sodium hypochlorite and α-tocopherol broke the chain of free radical reactions in lipids and promoted normalization of LPO processes in the plasma and erythrocytes of 250 rats with experimental bile peritonitis.

Key Words: *bile peritonitis; lipid peroxidation; antioxidant system; sodium hypochlorite; α-tocopherol*

Hepatobiliary diseases, including cholelithiasis, acute cholecystitis, and purulent cholecystitis, are most prevalent pathological processes in the abdominal cavity. Bile peritonitis is the most severe complication of these diseases [1,7].

Activation of free radical oxidation resulting in accumulation of toxic LPO products is a pathogenetic factor of bile peritonitis. Therapy of oxidative stress should be directed toward reduction of the concentration of LPO products and inhibition of chain radical reactions.

Sodium hypochlorite (SHC) is extensively used in the therapy of purulent inflammations. This compound modulates detoxification function of cytochrome P-450 in liver hepatocytes and bactericidal activity of myeloperoxidase in neutrophilic granulocytes [5].

Natural antioxidant α-tocopherol is widely used in medical practice. This agent effectively inhibits superoxide anion, peroxide, and OH radicals and breaks the chain of free radical oxidation and LPO in the lipid phase by directly interacting with different radicals ($O_2^{\bullet}$, $HO_2^{\bullet}$, and O_2^{1}) [2,3,8].

Kuban State Medical Academy; Russian Center of Functional Surgical Gastroenterology, Russian Ministry of Health, Krasnodar

Combination therapy of experimental bile peritonitis with α-tocopherol and SHC is pathogenetically substantiated.

Here we studied changes in the prooxidant and antioxidant system during combined treatment of experimental bile peritonitis with SHC and α-tocopherol.

MATERIALS AND METHODS

Experiments were performed on 250 male rats weighing 200-220 g. Experimental bile peritonitis was produced as described elsewhere [6].

The rats were divided into 5 groups. Group 1 intact animals (n=35) served as the control. Group 2 animals (n=35) were examined 24 h after modeling of bile peritonitis. In group 3 animals (n=60) with 24-h bile peritonitis sanation of the abdominal cavity with Furacilin (1:5000) was performed during surgery and single infusion of 0.04% 2.5 ml/kg SHC into the posterior vena cava was made. Group 4 animals (n=60) with 24-h bile peritonitis were subjected to interoperation sanation of the abdominal cavity with Furacilin (1:5000) and intramuscularly received 10% α-tocopherol in olive oil in a single dose of 10 mg per 100 g body weight. Group 5 animals (main group, n=60) with 24-h bile peritonitis were subjected to interoperation sanation of the abdominal cavity with Furacilin

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(1:5000) and received single infusion of 0.04% 2.5 ml/kg SHC into the posterior vena cava and intramuscular injection of 10% α-tocopherol in olive oil in a single dose of 10 mg per 100 g body weight.

The intensity of LPO was estimated by the contents of primary, secondary, and end LPO products in the plasma and erythrocytes [4]. The directionality of oxidation was determined by the oxidation index. It was calculated as the ratio between maximum absorption of nonoxidized lipids and primary LPO products at 215 and 232 nm, respectively.

RESULTS

The number of isolated double bonds (IDB) and concentrations of conjugated dienes (CD), conjugated trienes (CT), conjugated hydroxydienes (CHD), malonic dialdehyde (MDA), and Schiff bases (SB) in the plasma increased by 3, 4, 2.4, 3, 2.3, and 3 times, respectively, 24 h after the incidence of experimental bile peritonitis ($p<0.05$, Table 1).

The intensity of LPO in the plasma progressively decreased starting from the 1st day after surgery (Table 1). These changes were most pronounced in group 5 rats. By the 7th day after surgery, parameters of LPO in these animals did not differ from the control.

The intensity of LPO decreased less significantly in group 3 and 4 animals and surpassed that in intact rats.

Erythrocyte membrane is a typical plasma membrane reflecting structural and functional characteristics of other cell membranes. Therefore, it was important to study the directionality of LPO in erythrocytes under various regimens of postoperation therapy for 24-h bile peritonitis. The concentration of LPO products in erythrocytes of rats with bile peritonitis was higher than in intact animals. We revealed an increase in the number of IDB and concentrations of

TABLE 1. Plasma Concentration of LPO Products during Therapy of Bile Peritonitis ($M \pm m$)

Group		IDB	CD	CT	CHD	MDA	SB
1		1.88±0.10	1.19±0.06	0.67±0.05	0.51±0.07	2.36±0.028	0.33±0.03
2		5.80±0.38*	5.18±0.17*	1.61±0.09*	1.54±0.14*	5.54±0.41*	1.03±0.12*
3	day 1	3.51±0.47*	4.19±0.21*	1.16±0.11*	1.44±0.12*	5.14±0.61*	0.81±0.03*
	day 3	4.10±0.10*	3.36±0.17*	0.93±0.10*	1.20±0.04*	4.80±0.87*	0.61±0.03*
	day 7	3.29±0.31*	3.02±0.23*	0.79±0.10*	0.99±0.05*	4.50±0.41*	0.60±0.02*
4	day 1	4.78±0.15*	4.89±0.14*	1.01±0.12*	1.17±0.04*	5.21±0.49*	0.99±0.03*
	day 3	4.24±0.25*	3.08±0.29*	0.83±0.06*	0.98±0.11*	4.59±0.32*	0.86±0.05*
	day 7	4.48±0.34*	2.35±0.12*	0.81±0.10*	0.95±0.08*	4.34±0.48*	0.59±0.04*
5	day 1	3.91±0.20*	2.47±0.15*	0.94±0.10*	1.06±0.04*	3.41±0.20*	0.61±0.03*
	day 3	3.55±0.11*	2.15±0.30*	0.74±0.07*	0.83±0.11*	3.28±0.31*	0.51±0.05*
	day 7	2.22±0.10	1.53±0.15	0.63±0.03	0.57±0.08	2.34±0.22	0.34±0.04

Note. Here and in Tables 2 and 3: *$p<0.05$ compared to intact animals.

TABLE 2. Concentration of LPO Products in Erythrocytes during Therapy of Bile Peritonitis ($M \pm m$)

Group		IDB	CD	CT	CHD	MDA	SB
1		3.48±0.34	2.95±0.40	1.86±0.13	0.91±0.08	11.84±0.44	2.46±0.22
2		7.86±0.33*	7.11±0.50*	4.30±0.48*	2.19±0.12*	27.0±0.83*	6.95±0.20*
3	day 1	5.41±0.37*	5.16±0.18*	3.26±0.34*	2.17±0.30*	22.66±0.8*	6.87±0.14*
	day 3	5.35±0.50*	4.35±0.12*	2.84±0.30*	1.96±0.12*	22.59±0.7*	5.81±0.24*
	day 7	5.33±0.57*	4.16±0.10*	2.82±0.26*	1.68±0.15*	22.49±1.0*	5.71±0.24*
4	day 1	6.04±0.25*	5.15±0.37*	3.07±0.24*	1.73±0.13*	23.48±0.7*	6.38±0.52*
	day 3	5.89±0.36*	4.03±0.17*	3.32±0.18*	1.42±0.14*	23.09±0.7*	5.79±0.20*
	day 7	4.86±0.13*	3.61±0.18*	2.55±0.26*	1.16±0.10*	21.20±0.7*	5.33±0.34*
5	day 1	5.41±0.37*	5.26±0.14*	2.66±0.24*	1.21±0.13*	16.97±0.3*	6.18±0.26*
	day 3	4.65±0.69*	3.67±0.33*	1.97±0.18*	1.14±0.14*	14.67±0.3*	4.62±0.23*
	day 7	3.86±0.31	2.88±0.26	1.98±0.26	0.97±0.10	12.88±0.2	3.69±0.21

TABLE 3. Comparative Study of Changes in the Concentration of LPO Products in the Plasma and Erythrocytes of Animals with Experimental Bile Peritonitis (*M*±*m*)

Group		Index of plasma oxidation	Relative decrease in the index of plasma oxidation	Index of erythrocyte oxidation	Relative decrease in the index of erythrocyte oxidation
1		0.63±0.09	1.00	0.85±0.06	1.00
2		0.89±0.06*	1.41	0.91±0.07	1.07
3	day 1	1.19±0.11*	1.89	0.95±0.05*	1.12
	day 3	0.82±0.05*	1.30	0.91±0.07	1.07
	day 7	0.92±0.08*	1.46	0.78±0.07	0.92
4	day 1	1.02±0.07*	1.62	0.85±0.04	1.00
	day 3	0.73±0.12	1.16	0.68±0.05	0.80
	day 7	0.52±0.08	0.83	0.64±0.07	0.75
5	day 1	0.63±0.07	1.00	0.97±0.06*	1.14
	day 3	0.61±0.08	0.97	0.79±0.05	0.93
	day 7	0.69±0.06	1.10	0.75±0.06	0.88

CD, CT, CHD, MDA, and SB (by 2.2, 3.4, 2.3, 2.4, 2.3, and 3 times, respectively, Table 2).

Starting from the 1st day after surgery the concentration of LPO products in erythrocytes progressively decreased and by the 7th day this parameter reached the normal only in rats receiving SHC and α-tocopherol (Table 2).

The index of plasma lipid oxidation increased by 1.4 times 24 h after bile peritonitis modeling (Table 3). The index of lipid oxidation significantly increased in animals of group 3 and 4 (1.19 and 1.02, respectively). These changes reflect progression of free radical lipid oxidation. The concentration of oxidized lipids was higher compared to the amount of nonoxidized substances. The index of plasma lipid oxidation in group 3 and 4 rats progressively decreased starting from the 3rd day and did not differ from the control on day 7.

The index of plasma lipid oxidation in group 5 animals decreased to normal on day 1 after surgery and remained unchanged in the follow-up period.

The index of erythrocyte lipid oxidation tended to increase in rats with 24-h bile peritonitis. However, intergroup differences were insignificant. On day 1 after surgery the index of erythrocyte lipid oxidation in animals with 24-h bile peritonitis receiving SHC alone (group 3) or in combination with α-tocopherol (group 5) increased to 0.95 and 0.97, respectively, and surpassed that in control rats. By the 3rd day after surgery the index of erythrocyte lipid oxidation in rats of various groups decreased and did not differ from the control.

During the development and therapy of 24-h bile peritonitis the index of plasma lipid oxidation increased more significantly compared to the index of erythrocyte lipid oxidation. In various periods of observations changes in this index were most significant in group 3 rats and least pronounced in group 5 animals. On day 3 after surgery the index of lipid oxidation in the plasma and erythrocytes from group 5 rats practically did not differ from the control. These data show that free radical oxidation during oxidative stress mainly concerns blood plasma lipids. Damage to erythrocyte membrane lipids is less severe under these conditions.

The index of plasma lipid oxidation increased most significantly in group 3 rats receiving SHC. This compound probably produces an oxidant effect, which results in hydrolysis of double bonds in plasma lipids with the formation of chlorohydrin glycols and lysophospholipids. SHC in the specified doses had no effect on the index of erythrocyte lipid oxidation. Therefore, SHC does not damage formed elements of the blood.

Our results suggest that combined use of SHC and antioxidant α-tocopherol in the postoperation therapy of experimental bile peritonitis breaks the chain of free radical reactions in lipids and promotes normalization of LPO processes in the plasma and erythrocytes. High efficiency of combined treatment with SHC and α-tocopherol is probably associated with their effects on different stages of LPO.

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