Effect of Nitroglycerine on Some Parameters of the Prooxidant-Antioxidant Balance and Functional State of the Liver during Ischemia/Reperfusion

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The effect of nitroglycerine on some parameters of the prooxidant-antioxidant balance and functional state of the liver under conditions of ischemia/reperfusion was studied on rabbits. Hepatic ischemia/reperfusion was accompanied by accumulation of LPO products, depletion of the antioxidant defense system, and increase in blood transaminase activity. Nitroglycerine infusion before the reperfusion period decreased the concentration of LPO products, increased activity of the antioxidant system, and improved liver function.

Key Words: *liver; nitroglycerine; rabbits; lipid peroxidation*

Liver ischemia/reperfusion often accompanies resections and transplantations of this organ and hemorrhagic shock followed by blood transfusion [3,4]. The pathogenetic mechanisms of this syndrome include oxidative stress, inflammatory reaction and migration of leukocytes to the parenchyma, and microcirculatory disturbances. These disorders result in hepatocyte necrosis or apoptosis [2]. Insufficient production of nitric oxide (NO) and predominance of endothelins contribute to microcirculatory disturbances in the liver during reperfusion [1,8]. It should be emphasized that NO in high concentration has cytotoxic effect on hepatocytes. The necessity of maintaining the balance between the damaging and protective effects of NO requires the development of new methods for correction of postischemic liver injury.

Here we studied the effect of nitroglycerine (NG) on the prooxidant-antioxidant balance and function state of the liver during ischemia/reperfusion.

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MATERIALS AND METHODS

Experiments were performed on adult male rabbits weighing 3.5-4.5 kg and maintained in a vivarium under standard conditions. The animals were anesthetized by intravenous infusion of calipsol (1.5 mg/kg/min). Hepatic ischemia was induced by 30-min clamping of *a. hepatic propria*. The reperfusion period was 120 min. A catheter was introduced into *v. hepatica* to obtain hepatic venous blood. Another catheter was inserted into the right atrium to take mixed venous blood samples. Blood samples were withdrawn before, by the end, and 120 min after ischemia to study LPO products and activity of the antioxidant system. Liver tissue was taken by the end of reperfusion to evaluate the prooxidant-antioxidant state. Liver samples obtained before ischemia (n=6) served as the control. The degree of liver injury was estimated by measuring plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities.

The animals were divided into 2 groups. Liver ischemia/reperfusion was induced in group 1 rabbits (n=10). Group 2 animals (n=8) with hepatic ischemia/reperfusion received intravenous infusion of NG (1.5 μg/kg) 5 min before the reperfusion

period [10]. The total concentration of nitrites and nitrates in blood plasma was measured using Griess reagent [6]. Cadmium was used for reduction of nitrates to nitrites.

The concentrations of conjugated dienes (CD), Schiff bases (SB), and α-tocopherol and activity of catalase reflected the prooxidant-antioxidant balance. CD concentration in biological materials was measured by UV spectrophotometry at 233 nm [5]. SB concentration was estimated from fluorescence of the chloroform extract on an F-4010 spectrofluorometer (Hitachi) at excitation and emission wavelengths of 344 and 440 nm, respectively. α-Tocopherol concentration was measured fluorometrically by recording fluorescence of hexane extracts [5]. α-Tocopherol from Sigma served as the control. Catalase activity in biological materials was measured by the spectrophotometric method based on the ability of H_2O_2 to react with molybdenum salts with the formation of a stable colored complex [5].

The results were analyzed by Student's *t* test.

RESULTS

CD concentration in the plasma of hepatic venous blood from group 1 animals was 267.2% higher compared to the basal level ($p<0.05$). SB concentration in the plasma of hepatic venous blood increased by 69% by the end of reperfusion ($p<0.05$). The concentrations of CD and SB in the blood from group 2 rabbits increased less significantly (Table 1). CD concentration in the plasma of hepatic venous blood taken by the end of reperfusion was 57.6% higher compared to the basal level ($p<0.05$). SB concentration in the plasma of hepatic venous blood exceeded the basal level only by the end of ischemia (by 21.4%, $p<0.05$).

Activity of the antioxidant defense system decreased during experimental ischemia/reperfusion (Table 1). By the 30th minute of ischemia, α-tocopherol concentration in the plasma of hepatic venous blood was 93% of the basal level ($p<0.05$). α-Tocopherol concentration in the plasma of mixed venous blood decreased to 75.4% by the 120th minute of reperfusion ($p<0.05$). Erythrocyte catalase activity in group 1 rabbits increased and remained high over all period of ischemia/reperfusion. By the end of reperfusion, enzyme activity in hepatic venous blood exceeded the basal level by 101.1% ($p<0.05$). As distinct from group 1 animals, catalase activity in NG-receiving rabbits increased only in erythrocytes of hepatic venous blood (by 43.7% of the basal level, $p<0.05$). α-Tocopherol concentration in the plasma of various blood samples did not exceed the basal level (Table 1).

The intensity of LPO and activity of the antioxidant system were studied in the liver homogenate. After 120-min reperfusion, CD concentration in group 1 animals increased from 4.97±0.54

TABLE 1. Effect of NG on the Prooxidant-Antioxidant Balance in the Blood during Liver Ischemia/Reperfusion (*M*±*m*)

Parameter		Group 1			Group 2		
		basal level	30th minute of ischemia	120th minute of reperfusion	basal level	30th minute of ischemia	120th minute of reperfusion
Hepatic venous blood							
plasma	CD, ΔE_{233}/ml	0.58±0.05	1.13±0.14*	2.13±0.10*	0.51±0.04	0.59±0.04$^+$	0.81±0.09*$^+$
	SB, U/ml	8.49±0.42	12.75±0.58*	14.35±0.47*	8.89±0.28	10.71±0.49*$^+$	9.38±0.23$^+$
	α-tocopherol, μmol/liter	20.80±0.58	19.34±0.24*	16.54±0.26*	20.45±0.43	19.19±0.35*	19.33±0.38$^+$
erythrocytes							
	catalase, mmol/liter/g hemoglobin	2.77±0.31	3.81±0.33*	5.57±0.06*	2.03±0.17	2.43±0.25$^+$	2.92±0.27*$^+$
Mixed venous blood							
plasma	CD, ΔE_{233}/ml	0.57±0.05	1.56±0.31*	2.58±0.55*	0.63±0.05	1.08±0.18*	1.03±0.20$^+$
	SB, U/ml	9.47±0.12	11.65±0.59*	12.86±0.28*	9.19±0.37	12.06±0.67*	9.95±0.32$^+$
	α-tocopherol, μmol/liter	21.25±0.48	18.56±0.43*	16.02±0.28*	20.12±0.40	18.91±0.29*	19.11±0.30$^+$
erythrocytes							
	catalase, mmol/liter/g hemoglobin	2.50±0.33	3.82±0.37*	6.18±0.02*	2.01±0.15	2.53±0.20$^+$	2.61±0.32$^+$

Note. Here and in Table 2: $p<0.05$: *compared to the basal level; $^+$compared to another group in the same period.

TABLE 2. Effect of NG on Total Concentration of Nitrites and Nitrates and Functional State of the Liver during Ischemia/Reperfusion (*M*±*m*)

Parameter	Group 1			Group 2		
	basal level	30th minute of ischemia	120th minute of reperfusion	basal level	30th minute of ischemia	120th minute of reperfusion
Hepatic venous blood						
ALT, μmol/liter/min	4.81±0.21	5.08±0.16	9.41±0.52*	4.00±0.35	4.32±0.33	4.81±0.27+
AST, μmol/liter/min	3.52±0.19	3.93±0.16	6.85±0.82*	3.84±0.40	4.07±0.29	4.47±0.32+
Nitrates and nitrites	5.89±0.89	6.88±0.89	6.23±0.83	4.15±0.65	7.04±0.61*	4.91±0.65
Mixed venous blood						
ALT, μmol/liter/min	4.48±0.24	4.82±0.21	11.74±1.16*	4.11±0.34	4.25±0.37	4.97±0.47+
AST, μmol/liter/min	3.94±0.37	4.70±0.27	11.25±1.18*	3.78±0.52	4.39±0.30	5.22±0.51+
Nitrates and nitrites	5.19±0.63	5.54±1.00	5.71±0.77	4.83±0.55	7.02±0.77*+	5.94±0.64

to 11.42±1.04 ΔE_{233}/g ($p<0.01$). SB concentration increased from 120.30±12.47 to 404.6±50.2 U/g ($p<0.001$). α-Tocopherol concentration decreased from 19.77±0.68 to 12.17±0.26 nmol/100 mg ($p<0.001$). Catalase activity in the liver homogenate increased from 8.36±0.56 to 11.45±0.35 mmol/g protein/sec ($p<0.01$). By the 120th minute of reperfusion, the test parameters of the prooxidant/antioxidant balance in group 2 rabbits did not differ from the control.

Ischemia/reperfusion in group 1 rabbits was accompanied by an increase in ALT and AST activities in the blood after reperfusion. It should be noted that enzyme activities remained unchanged by the end of ischemia (Table 2). Activities of ALT and AST in the plasma of mixed venous blood increased by 161.3 and 184.8%, respectively, after 120-min reperfusion ($p<0.05$). The increase in activities of these enzymes in blood plasma reflects impairment of hepatocyte membrane integrity during ischemia/reperfusion. Activities of ALT and AST in the plasma of hepatic and mixed venous blood from group 2 animals remained practically unchanged during hepatic ischemia/reperfusion. The total concentration of nitrites and nitrates in the plasma of hepatic and mixed venous blood from NG-treated rabbits increased by the 30th minute of ischemia (by 70.4 and 45.4% of the basal level, respectively, $p<0.05$). However, by the end of reperfusion the total concentration of nitrites and nitrates in the plasma of hepatic and mixed venous blood from these animals exceeded the basal level by only 18.3 ($p>0.05$) and 23%, respectively (Tables 1 and 2). The total concentration of nitrites and nitrates remained unchanged in group 1 animals with hepatic ischemia/reperfusion.

Accumulation of CD and SB in the blood and liver tissue reflects a significant increase in LPO intensity, which is accompanied by a decrease in activity of the antioxidant defense system. These changes contribute to a shift in the prooxidant-antioxidant balance toward activation of free radical processes. The observed changes probably cause liver dysfunction by the end of reperfusion (increase in ALT and AST activities). It should be emphasized that some parameters of LPO and antioxidant system were stabilized in group 2 animals, particularly in mixed venous blood and liver tissue. Our results indicate that NG infusion improves liver function after ischemia. These changes were observed against the background of increased total level of nitrites and nitrates and were probably related to an increase in the concentration of NO and restoration of the balance between vasoconstrictors and vasodilators in the liver during the postischemic period, which improves hepatic microcirculation. It should be noted that NG infusion could improve function of other organs, first of all, of the lungs and heart. Liver reperfusion is often accompanied by damage to these organs [7,9]. The involvement of the L-arginine-NO system (*e.g.*, NG) in the maintenance of the prooxidant-antioxidant balance is mediated by several mechanisms. NO may contribute to antioxidant protection. NO has function of a radical-trapping compound and modifies O_2-binding properties of the blood. It is necessary to take into account that the pathogenesis of hepatic reperfusion injury is mediated by the O_2-dependent mechanism [10].

The data indicate that NG improves the prooxidant-antioxidant balance and liver function in rabbits during ischemia/reperfusion.

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REFERENCES

1. M. N. Khodosovskii and V. V. Zinchuk, *Eksper. Klin. Farmakol*., **66**, No. 3, 39-43 (2003).
2. H. Jaeschke and J. J. Lemasters, *Gastroenterology*, **125**, No. 4, 1246-1257 (2003).
3. G. K. Glantzounis, H. J. Salacinski, W. Yang, *et al*., *Liver Transpl*., **11**, No. 9, 1031-1047 (2005).
4. J. W. Kupiec-Weglinski and R. W. Busuttil, *Transplant. Proc*., **37**, No. 4, 1653-1656 (2005).
5. C. A. Rice-Evans, A. T. Diplock, and M. R. Symons, *Laboratory Techniques in Biochemistry and Molecular Biology: Techniques in Free Radical Research*, Elsevier (1991).
6. K. Schulz, S. Kerber, and M. Kelm, *Nitric Oxide*, **3**, No. 3, 225-234 (1999).
7. P. Taura, J. C. Garsia-Valdecasas, J. Beltran, *et al*., *Anesth. Analg*., **83**, No. 4, 675-680 (1996).
8. D. Uhlmann, S. Uhlmann, and H. U. Spiegel, *J. Cardiovasc. Pharmacol*., **36**, No. 5, Suppl. 1, S212-S214 (2000).
9. A. A. Weinbroum, A. Kidron, E. Hochhauser, *et al*., *Med. Sci. Monit*., **7**, No. 6, 1137-1144 (2001).
10. V. V. Zinchuk and L. V. Dorokhina, *Nitric Oxide*, **6**, No. 1, 29-34 (2002).