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Influence of Different Oxygen Modes on the Blood Oxygen Transport and Prooxidant-Antioxidant Status during Hepatic Ischemia/Reperfusion

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Summary

Oxygen supply was corrected in rabbits during the hepatic ischemia/reperfusion by means of different breathing mixtures: hypoxic (14.8 % O_2+85.2 % N_2), hyperoxic (78 % O_2+20.2 % N_2+ 1.8 % CO_2), or hypercapnic (5 % CO_2 in air). Hepatic ischemia was induced for 30 min by ligation of hepatic artery, reperfusion period lasted 120 min. Indices of blood oxygen transport ($p50_{act}$, pCO_2, pH, pO_2, etc.) and prooxidant-antioxidant balance (Schiff bases, conjugated dienes, catalase, retinol, α-tocopherol) were measured in the blood and liver. The severity of reperfusion damage was evaluated by the activities of alanine and aspartate aminotransferases (ALT, AST) in the blood. Hepatic ischemia/reperfusion resulted in higher $p50_{act}$ in hepatic venous and mixed venous blood in all experimental groups. The changes of $p50_{act}$ were most marked in the hypercapnic group and were the weakest in the hypoxic group. The rise in $p50_{act}$ was accompanied by higher levels of lipid peroxidation products, ALT and AST in blood and liver homogenates, and by a simultaneous fall of α-tocopherol and retinol concentrations, except in the hypoxic group. Catalase activity at the end of reperfusion increased under normoxia, decreased under hyperoxia or hypercapnia and did not change under hypoxia. The moderate hypoxia during reperfusion was accompanied by a better balance between the mechanisms of reactive oxygen species production and inactivation that may be observed by optimal changes in $p50_{act}$ and reduced the hepatic damage in this pathological condition.

Key words
Hemoglobin-oxygen affinity • Hepatic ischemia • Reperfusion • Hypoxia • Hyperoxia • Hypercapnia • Rabbits

Introduction

Hepatic injuries induced by transient ischemia and following reperfusion have a high incidence during liver resection or grafting (Lentsch *et al.* 2000). The enhanced radical generation with imbalance between reactive oxygen species (ROS) production and factors of antioxidant defense is considered to be the main cause of reperfusion damage. An alteration of mechanisms of oxygen transport in a tissue during reperfusion promotes intensifying the damages caused by ROS (Kaneda *et al.* 2001, Zinchuk and Dorokhina 2002). However, the results of this damage correction by change in oxygen regimen are inconsistent. The preliminary hyperoxia was shown to attenuate the liver and heart ischemia/ reperfusion injury (Chen *et al.* 1998, Tahepold *et al.* 2001), whereas hyperoxia in the reperfusion phase can aggravate the damage in the liver, kidneys and the heart

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(Matsumoto *et al.* 1997, Zwemer *et al.* 2000, Kaneda *et al.* 2001). Red blood cells are an important contributor to the blood antioxidant capacity measured as the specific intracellular enzyme activities (superoxide dismutase, catalase) and the state of glutathione system. Blood oxygen-binding properties take part in the antioxidant system by the establishment of tissue oxygen transport conditions and tissue pO_2 values (Zinchuk and Borisiuk 1998, Zinchuk and Dorokhina 2002). The role of blood oxygen transport including hemoglobin-oxygen affinity (HOA) changes during the ischemia/reperfusion has not been sufficiently studied with exception of occasional investigations (Grocott *et al.* 1998, Pagel *et al.* 1998), which did not allow to elucidate the involvement of blood oxygen transport in a pathogenesis of reperfusion injury. The present investigation aimed to study the influence of different body oxygen delivery conditions on the blood oxygen transport and prooxidant-antioxidant balance during ischemia/reperfusion.

Methods

The adult male Chinchilla rabbits (body weight 3.5-4.5 kg) were kept for two weeks in a constant-climate environment with respect to the temperature (22±2 °C), humidity (50±10 %) and daylight cycle (light, 7:00-19:00 h). Animals were fed a laboratory diet with water and food *ad libitum* until use and fasted overnight with free access to water before the operation. Surgical procedures were performed between 8.00-12:00 h to avoid chronobiological variations. All the experimental procedures described in this paper are in accordance with the *Guiding Principles for the Care and Use of Animals* recommended by the Ethical Committee of Grodno Medical University.

All surgical steps were performed under combined intravenous anesthesia (diazepam 1.5 mg/kg, hexenalum 30.0 mg/kg, ketamine 1.5 mg/kg/min). Hepatic ischemia was induced by ligation of the hepatic artery for 30 min and reperfusion lasted 120 min. Two catheters were introduced: into the hepatic vein for sampling of hepatic venous blood and into the right atrium for mixed venous blood sampling. Blood sampling for estimation of blood oxygen transport and prooxidant-antioxidant balance indices was performed before ischemia and at 0 and 120 min after its ceasing. The hepatic tissue was sampled at the end of reperfusion for evaluation of the prooxidant-antioxidant balance. The degree of liver damage was evaluated by assessing activities of alanine and aspartate aminotransferases (ALT and AST, respectively) in the blood according to Reitman and Frenkel (1957).

The change of oxygen mode during the reperfusion was carried out by inhalation of different gaseous mixtures from 5 min before the end of ischemia to 1 h of reperfusion. Rabbits were subdivided into four groups: first (n=10) – normoxic (breathing by ambient air during the hepatic ischemia and reperfusion), second (n=6) – hypoxic (breathing by 14.8 % O_2 + 85.2 % N_2), third (n=7) – hyperoxic (inhalation of mixture with 78 % O_2, 20.2 % N_2 and 1.8 % CO_2), fourth (n=4) – hypercapnic (breathing by 5 % CO_2 in air). Hepatic tissues taken before ischemia (n=6) and immediately after (n=5) served as the controls.

The blood oxygen transport indices – pO_2, pCO_2, blood pH, actual base excess (ABE), bicarbonate (HCO_3^-) and total carbon dioxide (TCO_2) concentrations, standard buffer base deficiency (SBE), and standard bicarbonate concentration (SBC) – were evaluated with gas micro-analyzer ABL-330 "Radiometer". HOA was determined as p50 (blood pO_2 at its 50 % oxygen saturation) by modified 'mixing' method (Scheid and Meyer 1978). $p50_{stand}$ was measured under standard conditions (pH 7.4, pCO_2 40 mm Hg, and temperature 37 °C), and $p50_{act}$ was calculated for actual values of these parameters.

The following indices of prooxidant-antioxidant state were measured: conjugated dienes (CD), Schiff bases (SB), retinol, α-tocopherol and catalase activity. CD content in the biologic materials was determined with UV spectrophotometry at wavelength of 233 nm, characteristic for conjugated diene structure of lipid hydroperoxides (Rice-Evans *et al.* 1991). SB levels were measured as the intensity of chloroform extract fluorescence at excitation and emission wavelengths of 344 and 440 nm, respectively (Fletcher *et al.* 1973), with spectrofluorimeter F-4010 (Hitachi). The α-tocopherol content was evaluated by fluorescence intensity of hexane extract at excitation and fluorescence wavelengths of 292 and 325 nm (Aruoma and Coppett 1997) with the same spectrofluorimeter. Retinol levels were also measured by the fluorescence method (Rice-Evans *et al.* 1991) with spectrofluorimeter F-4010 (Hitachi). The α-tocopherol and retinol ("Sigma") were used as the standards. Catalase activity in biological materials was estimated spectrophotometrically by Aruoma and Coppett (1997). The protein content was measured by Lowry *et al.* (1951).

All values are given as means ± S.E.M. Statistical evaluation of the data was performed with one-way analysis of variance (ANOVA) followed by

Student's *t*-test, where $p<0.05$ was considered as significant.

Results

Hepatic ischemia/reperfusion increased the ALT and AST activities in both samples of blood at the end of reperfusion in normoxic, hyperoxic and hypercapnic groups of rabbits (Fig. 1). In the hypoxic group at 120 min of reperfusion such rise of ALT activity was observed only in hepatic venous blood and was by 43.9 % lower than in normoxic animals. Mixed venous ALT activity and AST activity in both kinds of venous blood displayed only the trend to increase under hypoxia at 120 min of reperfusion (Fig. 1).

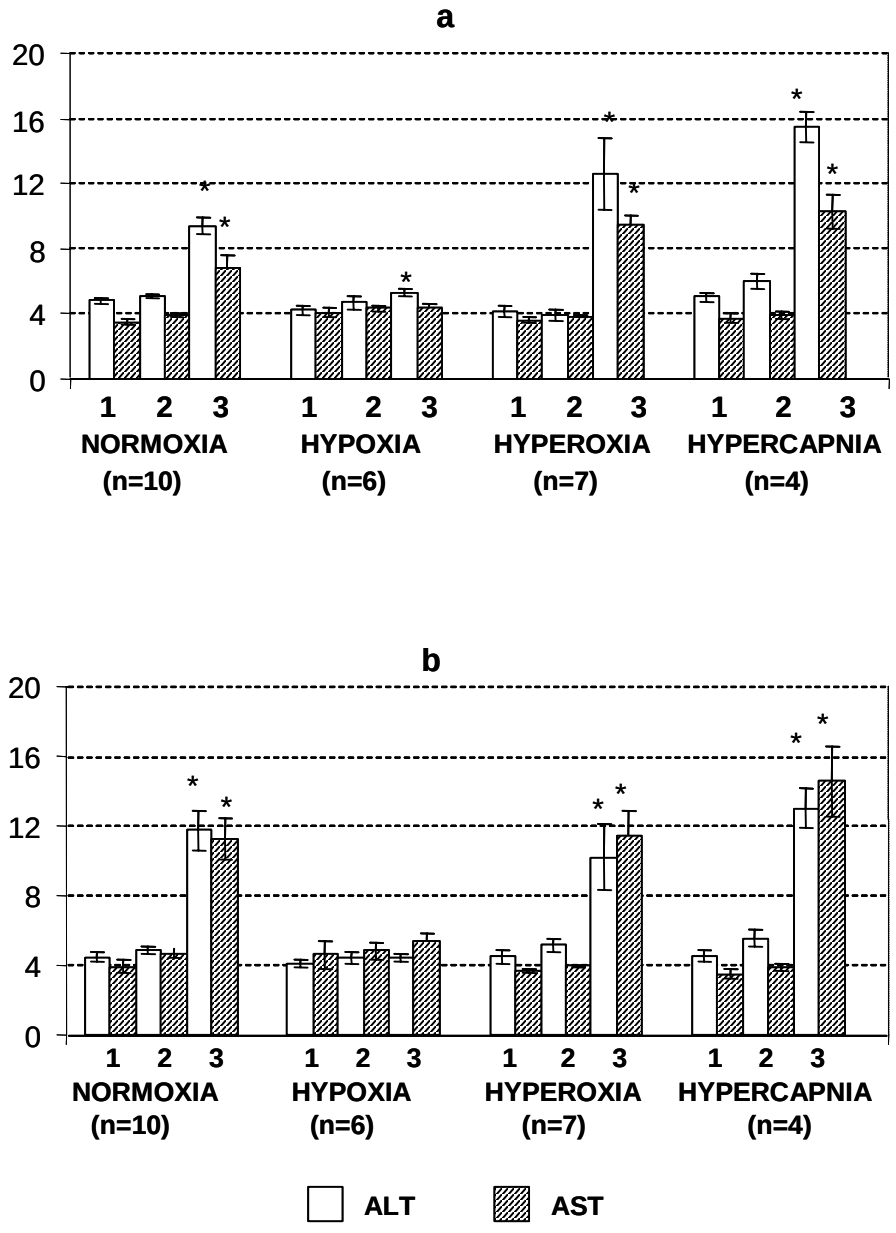

Fig. 1. *Activities of plasma alanine and aspartate aminotransferases (ALT, AST) in rabbit hepatic venous (a) and mixed venous (b) blood during the hepatic ischemia /reperfusion: (μmol/min): 1 – basal level, 2 – after ischemia, 3 – after reperfusion, * $p<0.05$ vs. basal level in all groups.*

Table 1. Influence of different oxygen modes on the blood oxygen transport indices of hepatic venous blood during hepatic ischemia/reperfusion in rabbits.

Parameter	Normoxia			Hypoxia			Hyperoxia			Hypercapnia		
	Basal level	30 min of ischemia	120 min of reperfusion	Basal level	30 min of ischemia	120 min of reperfusion	Basal level	30 min of ischemia	120 min of reperfusion	Basal level	30 min of ischemia	120 min of reperfusion
Number of animals	10	9	7	6	6	6	7	7	7	4	4	4
$p50_{act}$ *mm Hg*	32.12 ± 0.86	35.76 ± 1.4*	40.06 ± 1.83*	31.44 ± 1.35	33.52 ± 1.06	37.82 ± 1.69*	32.66 ± 0.61	37.05 ± 1.42*	40.73 ± 0.79*	32.5 ± 1.21	42.76 ± 1.48*	44.84 ± 1.23*
$p50_{stand}$ *mm Hg*	26.85 ± 0.57	28.35 ± 0.8	28.0 ± 0.66	30.12 ± 0.83	28.48 ± 1.39	28.9 ± 2.06	31.53 ± 0.67	29.04 ± 0.95	30.18 ± 0.96	31.49 ± 0.8	30.75 ± 1.4	31.01 ± 1.29
pO_2 *mm Hg*	30.4 ± 3.04	22.42 ± 2.25	25.09 ± 3.67	36.33 ± 1.93	23.98 ± 1.76*	28.03 ± 2.52*	37.13 ± 3.47	41.4 ± 1.87	25.79 ± 1.74*	37.65 ± 3.06	30.2 ± 2.88	32.95 ± 3.97
pH	7.308 ± 0.024	7.262 ± 0.017	7.156 ± 0.035*	7.411 ± 0.022	7.294 ± 0.035*	7.201 ± 0.017*	7.404 ± 0.016	7.227 ± 0.015*	7.203 ± 0.018*	7.352 ± 0.039	7.151 ± 0.023*	7.115 ± 0.05*
pCO_2 *mm Hg*	53.73 ± 2.67	58.73 ± 3.41	62.07 ± 1.76*	51.77 ± 3.84	62.2 ± 5.65	53.62 ± 3.92	55.7 ± 2.62	57.37 ± 2.31	53.33 ± 1.64	52.4 ± 2.46	66.03 ± 3.49*	56.05 ± 6.37
HCO_3^- *mmol/l*	26.23 ± 1.05	25.5 ± 0.84	21.37 ± 0.86*	32.27 ± 1.48	28.54 ± 1.56	20.55 ± 2.17*	36.25 ± 1.94	23.16 ± 1.13*	20.3 ± 0.87*	29.13 ± 3.89	22.13 ± 1.05	17.03 ± 1.09*
TCO_2 *mmol/l*	27.88 ± 1.08	27.32 ± 0.92	23.21 ± 0.84*	33.85 ± 1.56	30.44 ± 1.64	22.18 ± 2.28*	37.98 ± 1.99	24.93 ± 1.17*	21.93 ± 0.89*	30.75 ± 3.94	24.1 ± 1.11	18.73 ± 1.12*
ABE *mmol/l*	−0.27 ± 1.2	−1.72 ± 0.72	−6.96 ± 1.27*	6.67 ± 1.24	1.88 ± 1.6*	−6.88 ± 2.24*	9.9 ± 1.82	−4.36 ± 1.13*	−7.19 ± 1.08*	3.05 ± 4.0	−6.58 ± 1.38	−11.38 ± 1.46*
SBE *mmol/l*	0.24 ± 1.17	−1.1 ± 0.74	−6.26 ± 1.18*	7.1 ± 1.32	2.08 ± 1.67*	−6.62 ± 2.19*	10.6 ± 1.9	−3.74 ± 1.16*	−6.74 ± 1.02*	3.43 ± 3.98	−5.93 ± 1.2	−11.1 ± 1.52*
SBC *mmol/l*	23.48 ± 1.01	22.11 ± 0.57	17.99 ± 1.0*	29.98 ± 1.16	24.86 ± 1.56*	18.03 ± 1.64*	33.22 ± 1.69	20.37 ± 0.95*	17.76 ± 0.83*	26.98 ± 3.73	18.33 ± 1.12	14.75 ± 0.99*

*Data are means ± S.E.M. * Significantly different from basal level for each group ($p<0.05$)*

Table 2. Influence of different oxygen modes on the blood oxygen transport indices of mixed venous blood during hepatic ischemia/reperfusion in rabbits.

Parameter	Normoxia			Hypoxia			Hyperoxia			Hypercapnia		
	Basal level	30 min of ischemia	120 min of reper-fusion	Basal level	30 min of ischemia	120 min of reper-fusion	Basal level	30 min of ischemia	120 min of reper-fusion	Basal level	30 min of ischemia	120 min of reper-fusion
Number of animals	10	9	7	6	6	6	7	7	7	4	4	4
$p50_{act}$ mm Hg	32.55 ± 1.05	36.39 ± 1.06*	40.48 ± 1.89*	30.88 ± 1.88	34.1 ± 1.26	38.14 ± 1.98*	32.11 ± 0.53	36.55 ± 0.83*	39.74 ± 0.76*	32.84 ± 0.81	42.44 ± 1.12*	45.18 ± 1.82*
$p50_{stand}$ mm Hg	26.78 ± 0.7	28.76 ± 0.74	27.96 ± 0.76	29.28 ± 1.07	28.92 ± 1.39	29.26 ± 1.62	29.59 ± 0.44	28.33 ± 0.98	29.85 ± 0.89	30.71 ± 0.49	29.3 ± 0.59	31.2 ± 0.99
pO_2 mm Hg	35.26 ± 2.0	38.77 ± 2.18	28.89 ± 2.58	35.33 ±1.47	27.08 ± 1.65*	31.27 ± 3.43	32.01 ± 2.24	38.33 ± 2.32	30.04 ± 1.96	38.3 ± 3.13	33.65 ± 2.66	36.1 ± 3.11
pH	7.293 ± 0.015	7.260 ± 0.018	7.146 ± 0.036*	7.401 ± 0.054	7.294 ± 0.036*	7.208 ± 0.023*	7.375 ± 0.011	7.212 ± 0.015*	7.214 ± 0.015*	7.340 ± 0.027	7.119 ± 0.015*	7.111 ± 0.038*
pCO_2 mm Hg	55.76 ± 2.11	56.94 ± 2.92	60.2 ± 2.12	52.63 ± 3.48	59.53 ± 3.93	53.42 ± 2.18	49.4 ± 3.25	61.34 ± 2.43*	53.09 ± 1.96	48.33± 1.05	70.0 ± 4.17*	58.0 ± 4.35
HCO_3^- mmol/l	26.62 ± 0.89	24.66 ± 0.6	20.64 ± 0.87*	32.2 ± 1.88	28.13 ± 1.56	20.52 ± 1.85*	28.6 ± 2.32	23.93 ± 1.25	20.73 ± 0.68*	25.8 ± 1.93	21.55 ± 0.67	17.38 ± 0.36*
TCO_2 mmol/l	28.36 ± 0.91	26.62 ± 0.67	22.5 ± 0.83*	33.78 ± 1.93	29.98 ± 1.56	22.13 ± 1.91*	30.0 ± 2.31	25.66 ± 1.29	22.34 ± 0.7*	27.3 ± 1.96	23.7 ± 0.78	19.15 ± 0.28*
ABE mmol/l	–0.22 ± 0.92	–2.59 ± 0.63	–7.73 ± 1.28*	6.42 ± 1.7	1.42 ± 1.81*	–6.9 ± 1.96*	3.0 ± 2.04	–4.04 ± 1.27*	–6.56 ± 0.81*	0.1 ± 2.16	–7.63 ± 0.59*	–11.03 ± 0.89*
SBE mmol/l	0.39 ± 0.91	–1.89 ± 0.63	–7.1 ± 1.21*	6.85 ± 1.77	1.73 ± 1.79*	–6.5 ± 1.99*	3.34 ± 2.16	–3.29 ± 1.28*	–6.16 ± 0.75*	0.35 ± 2.14	–6.93 ± 0.53*	–10.83 ± 0.9*
SBC mmol/l	23.66 ± 0.8	21.67 ± 0.54	17.43 ± 1.0*	29.78 ± 1.58	26.33 ± 2.17	18.2 ± 1.53*	26.56 ± 1.92	20.56 ± 1.01*	18.39 ± 0.66*	24.18 ± 1.82	17.5 ± 0.42*	15.1 ± 0.71*

*Data are means ± S.E.M * Significantly different from basal level for each group ($p<0.05$)*

Tables 1 and 2 present the blood oxygen transport indices for hepatic and mixed venous blood. Both kinds of rabbit blood had decreased values of pH, ABE, HCO_3^-, TCO_2, SBE, and SBC throughout the hepatic ischemia-reperfusion in all experimental groups, especially in hypercapnic group. In normoxic conditions, hepatic ischemia led to a fall of pO_2 in hepatic outflow by 26.3 % from basal level. Simultaneously, a rightward shift in the oxyhemoglobin dissociation curve was observed (rise of $p50_{act}$ by 11.3 % ($p<0.05$) in hepatic and by 11.8 % ($p<0.05$) in mixed venous blood). After restoring the hepatic arterial inflow, the oxyhemoglobin dissociation curve shift to the right continued, and after 120 min of reperfusion the hepatic and mixed venous values of $p50_{act}$ increased by 24.7 % and 24.4 %, respectively. The moderate hypoxia at the end of ischemia (second group) resulted in a lowering of pO_2 in hepatic and mixed venous blood by 34 % ($p<0.05$) and 23.3 % ($p<0.05$), respectively. In hypoxic group at 30 min of ischemia the $p50_{act}$ did not significantly change for both samples of venous blood, but increased after 120 min of reperfusion. The breathing of a hyperoxic mixture at the end of ischemia led to higher mixed venous pCO_2 (by 24.2 %, $p<0.05$). Hepatic venous pO_2 decreased at the end of reperfusion by 30.6 % ($p<0.05$).

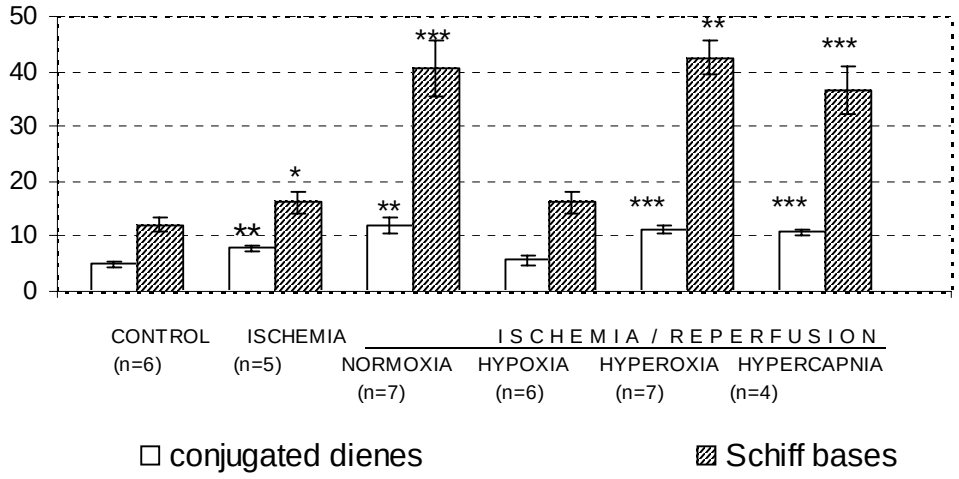

Fig. 2. *Conjugated diene (ΔE233/g) and Schiff bases (U/100 mg) levels during the hepatic ischemia/ reperfusion. * p<0.05 vs. controls, ** p<0.01, *** p<0.001.*

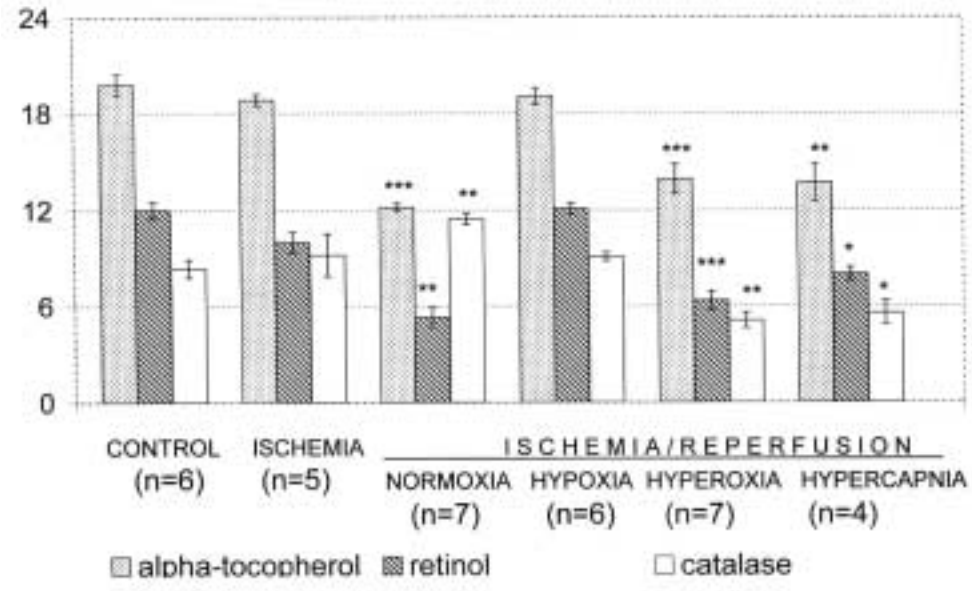

Fig. 3. *Indices of hepatic antioxidant system during ischemia/reperfusion: α-tocopherol (nmol/100 mg), retinol (nmol/100 mg), catalase activity - (mmol/s*g protein).*
**p<0.05 vs controls,*
*** p<0.01,*
**** p<0.001.*

Table 3. Influence of different oxygen modes on the prooxidant-antioxidant balance parameters of hepatic venous blood during hepatic ischemia/reperfusion in rabbits.

Parameter	Normoxia			Hypoxia			Hyperoxia			Hypercapnia		
	Basal level	30 min of ischemia	120 min of reperfusion	Basal level	30 min of ischemia	120 min of reperfusion	Basal level	30 min of ischemia	120 min of reperfusion	Basal level	30 min of ischemia	120 min of reperfusion
Number of animals	10	9	7	6	6	6	7	7	7	4	4	4
CD_{PL} ΔE_{233}/ ml	0.58 ± 0.05	1.13 ± 0.14*	2.13 ± 0.1*	0.65 ± 0.09	1.69 ± 0.13*	0.79 ± 0.09	0.6 ± 0.05	1.12 ± 0.06*	1.61 ± 0.09*	0.61 ± 0.05	1.01 ± 0.07*	1.76 ± 0.05*
CD_{RBC} ΔE_{233}/ml	5.02 ± 1.12	12.88 ± 2.1*	20.3 ± 1.28*	5.21 ± 0.5	8.66 ± 0.89*	5.9 ± 0.57	5.3 ± 0.18	7.9 ± 0.33*	10.46 ± 0.64*	5.31 ± 0.2	7.02 ± 0.3*	10.86 ± 1.29*
SB_{PL} U/ml	8.49 ± 0.42	12.75± 0.58*	14.35± 0.47*	9.46 ± 0.5	11.75 ± 0.33*	9.56 ± 0.51	8.51 ± 0.41	11.63 ± 0.65*	13.49 ± 0.81*	8.36 ± 0.42	10.97 ± 0.37*	12.54 ± 1.42*
SB_{RBC} U/ml	40.66 ± 2.63	58.52 ± 5.38*	83.91 ± 6.7*	38.66 ± 1.89	50.9 ± 1.66*	38.46 ± 1.92	40.64 ± 3.33	57.22 ± 1.77*	68.97 ± 4.54*	40.44 ± 1.46	52.67 ± 3.83*	70.17 ± 6.15*
$Retinol_{PL}$ μmol/l	2.29 ± 0.03	1.97 ± 0.05*	1.47 ± 0.08*	2.22 ± 0.05	2.22 ± 0.05	2.15 ± 0.05	2.17 ± 0.04	1.92 ± 0.07*	1.56 ± 0.1*	2.39 ± 0.07	2.28 ± 0.22	1.93 ± 0.16*
$Retinol_{RBC}$ μmol/l	8.81 ± 0.24	8.2 ± 0.23	6.81 ± 0.12*	8.87 ± 0.92	7.95 ± 1.24	8.72 ± 0.9	9.26 ± 0.89	7.55 ± 0.8	5.44 ± 0.54*	7.97 ± 0.61	6.48 ± 0.47	6.11 ± 0.76
α-$tocopherol_{PL}$ μmol/l	20.8 ± 0.58	19.34 ± 0.24*	16.54± 0.26*	21.08 ± 0.48	18.44 ± 0.54*	20.66 ± 0.54	20.21 ± 0.15	18.57 ± 0.3*	16.69 ± 0.59*	20.39 ± 0.29	19.27 ± 0.35*	18.14 ± 0.35*
α-$tocopherol_{RBC}$ μmol/l	115.82 ± 2.17	111.19 ± 2.54	90.65 ± 1.34*	112.25 ± 5.28	98.26 ± 5.01	110.95 ± 4.96	117.3 ± 0.94	106.6 ± 2.02*	96.96 ± 3.37*	118.34 ± 2.02	113.75 ± 2.17	104.33 ± 2.98*
$Catalase_{RBC}$ μmol/s*gHb	2.77 ± 0.31	3.81 ± 0.33*	5.57 ± 0.06*	2.64 ± 0.02	2.89 ± 0.1*	2.65 ± 0.03	3.07 ± 0.27	3.97 ± 0.14*	1.85 ± 0.3*	2.29 ± 0.15	3.11 ± 0.11*	4.81 ± 0.12*

*Data are means ± S.E.M., PL – plasma, RBC – red blood cells, * Significantly different from basal level for each group ($p<0.05$)*

Table 4. Influence of different oxygen modes on the prooxidant-antioxidant balance parameters of mixed venous blood during hepatic ischemia/reperfusion in rabbits.

Parameter	Normoxia			Hypoxia			Hyperoxia			Hypercapnia		
	Basal level	30 min of ischemia	120 min of reperfusion	Basal level	30 min of ischemia	120 min of reperfusion	Basal level	30 min of ischemia	120 min of reperfusion	Basal level	30 min of ischemia	120 min of reperfusion
Number of animals	10	9	7	6	6	6	7	7	7	4	4	4
CD_{PL} ΔE_{233}/ ml	0.57 ± 0.05	1.56 ± 0.31*	2.58 ± 0.55*	0.66 ± 0.07	1.48 ± 0.18*	0.85 ± 0.12	0.62 ± 0.04	1.13 ± 0.05*	1.53 ± 0.06*	0.66 ± 0.07	1.07 ± 0.09*	1.49 ± 0.06*
CD_{RBC} ΔE_{233}/ml	6.56 ± 1.27	11.76 ± 2.09*	21.77 ± 0.44*	5.67 ± 0.42	9.32 ± 1.01*	6.11 ± 0.58	5.35 ± 0.24	7.95 ± 0.5*	10.01 ± 0.44*	5.49 ± 0.32	7.32 ± 0.5*	9.06 ± 0.3*
SB_{PL} U/ml	9.47 ± 0.12	11.65 ± 0.59*	12.86 ± 0.28*	9.55 ± 0.55	17.01 ± 3.98	9.64 ± 0.51	8.66 ± 0.16	10.94 ± 0.21*	13.39 ± 0.72*	8.34 ± 0.18	11.03 ± 0.42*	11.6 ± 0.99*
SB_{RBC} U/ml	40.48 ± 1.93	60.31± 4.84*	77.55 ± 5.67*	35.69 ± 1.97	51.85 ± 0.88*	36.31 ± 1.81	40.11 ± 0.28	57.06 ± 2.04*	68.55 ± 4.72*	40.67 ± 1.5	52.01 ± 2.98*	60.6 ± 6.73*
$Retinol_{PL}$ μmol/l	2.29 ± 0.03	1.98 ± 0.06*	1.43 ± 0.06*	2.22 ± 0.04	2.22 ± 0.04	2.23 ± 0.05	2.23 ± 0.07	1.95 ± 0.06*	1.52 ± 0.06*	2.3 ± 0.02	2.08 ± 0.21	1.81 ± 0.07*
$Retinol_{RBC}$ μmol/l	8.8 ± 0.18	8.01 ± 0.37	6.82 ± 0.05*	8.67 ± 0.9	9.05 ± 0.77	8.82 ± 0.88	8.73 ± 0.83	7.34 ± 0.8	5.37 ± 0.47*	8.69 ± 0.49	6.76 ± 0.3*	6.45 ± 0.69*
α-$tocopherol_{PL}$ μmol/l	21.25 ± 0.48	18.56 ± 0.43*	16.02 ± 0.28*	20.88 ± 0.57	18.56 ± 0.92	20.32 ± 0.51	19.77 ± 0.25	18.43 ± 0.34*	16.36 ± 0.55*	20.28 ± 0.24	19.31 ± 0.22*	17.97 ± 0.3*
α-$tocopherol_{RBC}$ μmol/l	118.9 ± 2.68	110.25 ± 3.28	95.11 ± 0.91*	116.69 ± 4.58	96.17 ± 4.5*	113.67 ± 4.37	117.91 ± 0.71	107.85 ± 2.62*	98.16 ± 4.08*	117.69 ± 2.02	112.86 ± 2.74	103.65 ± 2.37*
$Catalase_{RBC}$ μmol/s*gHb	2.5 ± 0.33	3.82 ± 0.37*	6.18 ± 0.02*	2.64 ± 0.02	2.69 ± 0.02	2.64 ± 0.02	3.01 ± 0.19	4.02 ± 0.18*	1.7 ± 0.15*	2.32 ± 0.06	3.09 ± 0.1*	4.56 ± 0.19*

*Data are means ± S.E.M., PL – plasma, RBC– red blood cells, * Significantly different from basal level for each group ($p<0.05$)*

Hyperoxia resulted in $p50_{stand}$ decrease by 9.8 % ($p<0.05$) in hepatic venous blood at 30 min of ischemia, but $p50_{act}$ significantly increased during that time in both samples of venous blood: by 11.2 % ($p<0.05$) in hepatic and by 13.8 % ($p<0.001$) in mixed venous blood. At 120 min of reperfusion, the values of $p50_{act}$ in the two samples of venous blood were higher than basal levels by 19.5 % ($p<0.001$) and 21 % ($p<0.001$), respectively. The inhalation of hypercapnic mixture before the start of reperfusion led to rise of $p50_{act}$ in hepatic and mixed venous blood at 30 min of ischemia by 25.9 % ($p<0.01$) and 25 % ($p<0.01$), respectively. At 120 min, the values of $p50_{act}$ in these samples of blood were higher than basal level by 32 % ($p<0.01$) and 33 % ($p<0.01$), respectively.

Hepatic ischemia resulted in a considerable rise of CD and SB in the plasma and red blood cells for both samples of blood in all animal groups (Tables 3 and 4). Thus, the plasma and red blood cells in the hepatic outflow CD levels increased after ischemia by 94.8 % ($p<0.01$) and 156.6 % ($p<0.01$), respectively, in the normoxic group, by 161 % ($p<0.001$) and 66.3 % ($p<0.01$) in hypoxic group, by 88 % ($p<0.05$) and 82.9 % ($p<0.05$) in hyperoxic group, and by 66.9 % ($p<0.05$) and 33.3 % ($p<0.05$) in hypercapnic group. After 30 min of hepatic ischemia, hepatic tissue CD levels increased from 4.97±0.54 to 7.8±0.7 ΔE_{233}/g ($p<0.01$). Simultaneously, SB content increased in liver homogenates (Fig. 2). The level of antioxidant indices in hepatic tissue during this time was not significantly different from that in the control group, whereas in plasma of hepatic and mixed venous blood, α-tocopherol content decreased by 7.0 % ($p<0.05$) and 12.7 % ($p<0.01$), respectively, in normoxia; by 12.5 % ($p<0.01$) and 11.1% ($p<0.05$) in hypoxia; by 8.1 % ($p<0.05$) and 6.8 % ($p<0.05$) in hyperoxia; by 5.5 % ($p<0.05$) and 4.8 % ($p<0.05$) in hypercapnia. The level of lipid peroxidation (LPO) products in blood and hepatic tissues at 120 min of reperfusion continued to rise in first, third and fourth group of rabbits (Tables 3 and 4, Fig. 2). Hepatic venous CD levels at the end or reperfusion were higher than basal level: under normoxia by 267.2 % ($p<0.001$) in plasma and by 304.4 % ($p<0.001$) in red blood cells, under hyperoxia by 169.9 % ($p<0.001$) and 97.4 % ($p<0.001$), and under hypercapnia by 190.9 % ($p<0.001$) and 104.5 % ($p<0.01$), respectively. Hepatic tissue displayed similar changes in CD and SB contents at the end of reperfusion (Fig. 2). In the hypoxic group, the CD content in blood and liver homogenates after 120 min of reperfusion did not differ from basal values. Plasma α-tocopherol level fell in hepatic and mixed venous blood at the end of reperfusion: under normoxia by 20.5 % ($p<0.001$) and 24.6 % ($p<0.001$), under hyperoxia by 17.4 % ($p<0.001$) and 17.3 % ($p<0.001$), under hypercapnia by 11 % ($p<0.01$) and 11.4 % ($p<0.01$), respectively. The α-tocopherol levels in liver homogenates after the end of reperfusion decreased in normoxic, hyperoxic and hypercapnic groups of rabbits compared to the controls from 19.77± 0.68 to 12.2±0.22 ($p<0.001$), 13.93±0.91 ($p<0.001$), and 13.69±1.19 ($p<0.01$) nmol/100 mg, respectively. In the hypoxic group, α-tocopherol content in homogenate at the end of reperfusion had only a decreasing trend (Fig. 3). Hepatic tissue catalase activity at 120 min of reperfusion rose in the first group vs. controls from 8.36±0.56 to 11.45±0.35 ($p<0.01$), in the hypoxic group to 9.1±0.26 ($p>0.05$), but in the hyperoxic and hypercapnic groups it fell to 5.11±0.49 ($p<0.05$) and to 5.57±0.75 mmol/s*g protein ($p<0.05$), respectively (Fig. 3).

Discussion

Our experiments have shown that the present model of ischemia/reperfusion was associated with deterioration of hepatic function and prooxidant-antioxidant balance in all series. However, this worsening had different degrees, the least being observed in a hypoxic environment. Hepatic reoxygenation was followed by higher values of $p50_{act}$ in hepatic and mixed venous blood of all groups, this effect appeared to be induced by metabolic acidosis, judging by acid-base balance indices. The HOA changes were most prominent in the hypercapnic group and the least in hypoxic groups. The increase of $p50_{act}$ was accompanied by higher blood and hepatic levels of LPO products and lower α-tocopherol and retinol concentrations in all experimental groups, except hypoxic one. The higher catalase activity in red blood cells and homogenates taken from normoxic rabbits during the reperfusion may obviously be the result of endogenous H_2O_2 accumulation (Zhou *et al.* 2001). The fall of hepatic tissue catalase activity in hyperoxic and hypercapnic groups at the end of reperfusion indicates a marked distortion of prooxidant-antioxidant balance.

Perhaps, the hepatic tissue after ischemia cannot normally utilize oxygen and the restored blood flow with usual or increased O_2 content promotes the enhanced ROS generation, with the following damage of cellular

and subcellular structures by LPO. This suggestion is supported by the fact that a shift of prooxidant-antioxidant balance to radical generation in normoxic, hyperoxic, and hypercapnic groups was accompanied by higher blood ALT and AST activities at the end of reperfusion. Taking into account the enhancement of oxygen supply to tissues with the oxyhemoglobin dissociation curve shift to the right (Hsia 1998, Zinchuk and Borisiuk 1998), the observed increase $p50_{act}$ during the reperfusion may promote the enlargement of the O_2 fraction used for the free radical generation.

The moderate hypoxia aided the normalization of prooxidant-antioxidant balance in our experiments. The value of $p50_{act}$ increased less, and AST activities did not differ from the basal level (ALT activity was higher in hepatic blood only). The decreased blood pO_2 during the reperfusion appears to limit the "electron leakage" and ROS generation in hepatic mitochondria. In addition, O_2 is necessary for activation of the hepatic hypoxanthine/xanthine oxidase system which is an important ROS source during the reperfusion (Matsumoto *et al.* 1997, Togashi *et al.* 2000). The moderate hypoxia seems to result in the lower O_2 utilization in the free radical reaction, as evidenced by a more normal prooxidant-antioxidant state in experimental animals.

Hypercapnia during the reperfusion of isolated rabbit lung is known to reduce the damage, perhaps through the inhibition of endogenous xanthine oxidase (Shibata *et al.* 1998). However, breathing a mixture with 5 % CO_2 did not normalize the prooxidant-antioxidant balance, acidosis became more severe, because its respiratory component was added to the metabolic one. Higher value of $p50_{act}$ under hypercapnia appeared to promote the free radical processes to a larger extent than the antioxidant effect of CO_2 inhibited them.

The decrease of HOA with a synthetic modifier (2-[4-[[(3,5-dimethylanilino) carbonyl]methyl]phenoxyl]-2-methylpropionic acid) ameliorated the damage after incomplete cerebral ischemia, but was ineffective after almost total ischemia (Grocott *et al.* 1998). In our experiment, the considerable HOA decrease in non-hypoxic groups was accompanied by deterioration of prooxidant-antioxidant imbalance and exaggeration of hepatic injuries during the reperfusion. In the hypoxic group, the oxyhemoglobin dissociation curve shift to the right and severity of hepatic injury during the reperfusion were the least. Perhaps, the inadequate oxyhemoglobin dissociation curve shift to the right and excessive tissue O_2 delivery can be the stage of hepatic reperfusion damage pathogenesis in conditions of mismatch between tissue oxygen delivery and utilization (Zinchuk 1999a, Zinchuk and Dorokhina 2002). The oxygen-dependent nature of free radical generation suggests that blood oxygen transport influences the LPO activity in biological systems. The shift of oxyhemoglobin dissociation curve correlated with the free radical oxidation indices, which allows considering HOA as one of the factors participating in maintenance of body prooxidant-antioxidant balance (Zinchuk 1999b). The prooxidant capacity may be decreased by hemoglobin modification (pyridoxalated hemoglobin-polyoxyethylene conjugate) (Privalle *et al.* 2000). In our experiments, the moderate oxyhemoglobin dissociation curve shift to the right can promote the maintenance of optimal prooxidant-antioxidant state through the reduction of imbalance between electron donors and acceptors, under conditions of such imbalance the respiratory chain cannot properly reduce O_2 resulting in the free radical process activation (increase in LPO products and depletion of the antioxidant system).

Thus, the data obtained indicate that the HOA decrease during the hepatic reperfusion is accompanied by marked activation of LPO processes and lower content of several antioxidants. The higher oxygen delivery during the reperfusion induces a prooxidant-antioxidant imbalance and exacerbates the liver damage. The moderate hypoxia during the reperfusion is associated with optimal HOA changes and promotes the minimization of hepatic injuries in this pathological condition.

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