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Superoxide anion production by the mitochondrial respiratory chain of hepatocytes of rats with experimental toxic hepatitis

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Abstract The progression of toxic hepatitis is accompanied by the activation of oxidative processes in the liver associated with an enhancement of the mitochondrial respiratory chain activity and superoxide anion production ($O_2^{\cdot-}$). The purpose of this study was to examine our previously formulated assumption concerning the predominant contribution of the complex I to $O_2^{\cdot-}$ production increase by the mitochondrial respiratory chain of hepatocytes in toxic hepatitis (Shiryaeva et al. Tsitologiia, 49, 125–132 2007). Toxic hepatitis was induced by a combined application of CCl_4 and ethanol. Respiratory chain function analysis was executed with submitochondrial particles (SP) in the presence of specific inhibitors. It was shown that the rate of $O_2^{\cdot-}$ production by SP of animals with toxic hepatitis, when NADH was delivered, was 2.5-fold higher as compared with the control. The rates of $O_2^{\cdot-}$ production by SP of rats with toxic hepatitis in the presence of NADH or NADH + rotenone were similar. The $O_2^{\cdot-}$ production rate by control SP in the presence of NADH + rotenone corresponded to the $O_2^{\cdot-}$ production rate by toxic hepatitis SP when only NADH was delivered. When NADH + myxothiazol were delivered to the incubation system, $O_2^{\cdot-}$ production by toxic hepatitis SP was 72% higher than for the control. Conversely, in the presence of antimycin A, the production of $O_2^{\cdot-}$ by toxic hepatitis SP was lower compared to the control. Collectively, the presented data indicate that the $O_2^{\cdot-}$ production rate was enhanced by the complex I of the hepatocyte mitochondrial respiratory chain in experimental toxic hepatitis. Complex III contribution to the production of $O_2^{\cdot-}$ was insignificant. We assume that the increase in $O_2^{\cdot-}$ production by the respiratory chain may be considered not only as the mechanism of pathology progression, but also as a compensatory mechanism preserving the electron transport function of the mitochondrial respiratory chain when complex I functioning is blocked in part.

Keywords Toxic hepatitis · Mitochondrion · Respiratory chain · Submitochondrial particles · Superoxide anion

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Introduction

Hepatitis progression is accompanied by the intensification of both processes of degeneration and regeneration of liver tissue. The interrelation of these processes has an influence on the clinical outcome. Regeneration success depends on the efficacy of hepatocyte energetic metabolism which by-turn is determined completely from respiratory chain

functioning. The typical feature of toxic hepatitis is an activation of the oxidative processes in the liver which is associated with an increase of reactive oxygen species (ROS) production (Bailey 2003).

It is known that the respiratory chain enzymes NADH-CoQ-oxidoreductase (complex I) and CoQ-cytochrome c-oxidoreductase (complex III) can implement oxygen one-electron reduction in $O_2^{\cdot -}$ (Liu 1997; Han et al. 2001; Skulachev 2001). The intensity of this process is insignificant in native liver, and in addition, mitochondrial superoxide dismutase eliminates generated $O_2^{\cdot -}$ (Agol 1996; Andreev et al. 2005). The mechanism of $O_2^{\cdot -}$ production enhancement with hepatitis is insufficiently studied. Previously, we had found that toxic hepatitis progress is associated with an increase in oxygen consumption by rat hepatocyte mitochondria, which may be dependent on complex I functioning (Le Couteur et al. 1999; Shiryaeva et al. 2007, 2008).

The respiratory rate in state 3 according Chance in the presence of malate + glutamate was significantly higher in toxic hepatitis than in control. In addition, rotenone suppression of isolated hepatocytes respiration was significantly lower in toxic hepatitis as compared to the control (Shiryaeva et al. 2007). These data evidenced the presence of the obstacle in complex I that limited electron transport. However, the degree of mitochondrial oxidation and phosphorylation coupling was high both in hepatocytes and mitochondria isolated from toxic hepatitis animals. Additionally, cytochrome oxidase *c* activity appeared to be significantly higher in toxic hepatitis.

We surmised that cause of the increase in oxygen consumption by hepatocytes' mitochondria in toxic hepatitis might be electron leakage in oxygen from the complex I that resulted in the $O_2^{\cdot -}$ production increase. Alterations which were found in the functioning of the other respiratory chain complexes play a compensatory role and purpose to preserve the energy producing capacity of the mitochondrion (Shiryaeva et al. 2007). The goal of this work was to examine our speculation relating to the predominant contribution of the complex I to $O_2^{\cdot -}$ production enhancement by the mitochondrial hepatocyte respiratory chain in toxic hepatitis. In addition, to determine the integral picture of the mitochondrial hepatocyte respiratory chain functioning in toxic hepatitis, the antioxidant system of mitochondria and their energy production efficacy were studied.

Materials and methods

Materials

Rotenone and 2,4-dinitrophenol were from ICN (Aurora, Ohio). Tris-(hydroxymethyl) aminomethane was from Baker (Phillipsburg, N.J.). Sodium dihydrogen phosphate, disodium hydrogen phosphate, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, hydrochloric acid and sulfuric acid were purchased from Merck (Darmstadt, Germany). Other chemicals were purchased from Sigma (St. Louis, Mo.) and Serva (Heidelberg, Germany).

Experimental animals and toxic hepatitis induction

Thirty male Wistar rats (180–200 g) were kept on standard rat chow and tap water ad libitum under 12-h light-dark cycles. All animals were divided into two groups. They were intact, control rats (group 1) and experimental hepatitis rats (group 2). Control animals were kept without any treatment. The experimental hepatitis was induced as described (Strubelt et al. 1978). Briefly, the combination of drinking water with 5% ethanol and intragastric delivery of a 50% solution of CCl_4 in vaseline oil (0.2 mg/kg body mass) twice weekly over 4 weeks via gastric cannula was used. All animals received human care in compliance with the International Guiding Principles for Animal Research. One week after the last treatment, animals were sacrificed under thiopental anaesthetic for analysis.

Isolation of mitochondria

Mitochondria of the excised liver were prepared as previously described (Johnson and Lardy 1969) with slight modifications (Yang et al. 2004). Briefly, the liver samples were immediately processed to obtain the functional mitochondria. After the fat removing the tissue samples were rinsed and homogenized in 30 ml of isolation buffer (250 mM sucrose without Ca^{2+}, 3 mM Tris-HCl, 0.5 mM EGTA, pH 7.3). The nuclei and the cell debris were removed by centrifugation at 700 g for 5 min. Then supernatants were centrifuged at 10,000 g for 10 min and the second supernatants and fat were eliminated. Pellets were resuspended in 25 ml isolation buffer without EGTA and centrifuged at 10,000 g for 10 min. This last procedure was repeated twice. The mitochondrial pellets were resuspended in 1 ml of isolation buffer without EGTA. All of the above procedures were performed at 4°C. The mitochondrial protein was measured as described (Bradford 1976). The final mitochondrial suspensions were placed into ice and immediately used for submitochondrial particles isolation.

Isolation of submitochondrial particles

The submitochondrial particles (SP) were isolated from the hepatocyte mitochondria by differential centrifugation (Herrero and Barja 2000). The isolated mitochondria were suspended in 1 ml of 10 mM Tris-HCl buffer, pH 7.4 contained 220 мМ mannitol, 70 мМ sucrose, 1 мМ Na_2EDTA (buffer 1) in an ice bath. Then the mitochondrial suspension was sonicated with 50-W Vibra Cell apparatus (2×30 s and 1-min interval between sonications). Further

15 ml of buffer 1 was delivered into sonicated mitochondria and the suspension was centrifuged at 10,000 g for 10 min. The supernatant was collected and centrifuged at 100,000 g for 40 min by the ultracentrifuge Beckman Optima XL 900. The pellet was suspended in 15 ml buffer 1 and centrifuged once more at 100,000 g during 40 min. The final pellet that contained SP was suspended in 0.3 ml buffer 1.

Superoxide anion production rate measurement

Superoxide anion production rate measurement was accomplished in accordance with Misra and Fridovich (1972). This method is based on adrenalin oxidation to adrenochrome in the presence of $O_2^{\cdot -}$ that leads to optical density alteration at 480 nm. The measurements were done in a spectrophotometer cuvette containing 1 ml buffer 1 supplemented with SP (0.1–0.2 mg protein), 2 μM catalase, substrates (NADH and succinate), mitochondrial respiratory chain inhibitors and 2 mM adrenaline. 50 U/ml SOD were delivered into some samples to eliminate $O_2^{\cdot -}$. 50 mM adrenaline solution (pH 2.0) was prepared ex tempore and kept in an ice bath. An optical density was measured every 15 s after adrenalin solution delivery over 3 min. For $O_2^{\cdot -}$ production calculation, the optical density rate alterations of samples with SOD were subtracted from the optical density rate alterations of samples without SOD. The coefficient of adrenochrome molar extinction was taken to be equal to $4.0 \cdot 10^3\ M^{-1} \cdot cm^{-1}$ (Tarpey and Fridovich 2001). Substrate and inhibitor concentrations used were as follows: NADH – 10 mM, succinate – 5 mM, rotenone – 5 μM, antimycin A – 10 μM, myxothiazol – 10 μM.

Determination of mitochondrial superoxide dismutase activity

The activity of the mitochondrial superoxide dismutase was determined as described (Flohe and Ötting 1984). 1.45 ml of the solution A (0.001 N NaOH) contained 0.76 mg xanthine was delivered to 100 ml 50 мМ KH_2PO_4-Na_2HPO_4 buffer, pH 7.8 containing 24.8 mg cytochrome *c* and 0.1 mM Na_2EDTA. 25 μl supernatant (25 μg protein), collected after SP sedimentation, was delivered into 1 ml solution A. The enzymatic reaction was initiated by delivering 25 μl solution B freshly prepared and containing 0.02 U xanthine oxidase in 0.1 mM Na_2EDTA. The optical densities of samples at 550 nm were measured after 5-min incubation at 25°C.

Determination of mitochondrial glutathione peroxidase activity

The activity of mitochondrial glutathione peroxidase was analyzed as described (Flohe and Günzler 1984). The reaction medium was prepared in a spectrophotometer. It included 0.1 M KH_2PO_4-Na_2HPO_4 buffer, pH 7.0, containing 1 mM Na_2EDTA, 10 μl supernatant (10 μg protein) collected after SP sedimentation, 100 μl (0.24 U) freshly prepared glutathione peroxidase and 100 μl 10 mM GSH prepared in the same buffer. The mixture was preincubated for 10 min at 25°C and then 100 μl 1.5 mM NADPH in 0.1% $NaHCO_3$ was added. To initiate the enzymatic reaction, 100 μl 12 mM t-butyl hydroperoxide was added. The optical density of samples was measured at 340 nm after 5-min incubation at 37°C.

Determination of mitochondrial oxidized and reduced glutathione

The glutathione was analyzed according to Tietze's procedure (Tietze 1969) in Hazelton's modification (Hazelton and Lang 1980). This procedure is based on the fact that the rate of the reduction of 5,5′-dithiobis-(2-benzoic acid) is proportional to the concentration of GSH or GSSG introduced into the sample. When analyzed, 500 μl mitochondrial lysate was added into the medium containing 0.5 U glutathione reductase, 0.5 μM 5,5′-dithiobis-(2-benzoic acid) and 0.1 M KH_2PO_4-Na_2HPO_4 buffer, pH 7.5 containing 0.005 M Na_2EDTA. The total volume of the incubation medium was 1 ml. Samples were preincubated for 2 min at 22ºC to permit the reaction between thiol components and 5,5′-dithiobis-(2-benzoic acid). To initiate the enzymatic reaction, 20 μl of 0.2 mM NADPH was delivered into the incubation mixture. The rate of 5,5′-dithiobis-(2-benzoic acid) reduction was measured at 412 nm. The calculations were executed on the basis of standard plots for known concentrations of GSH and GSSG. The total glutathione content was calculated as a sum of GSH and GSSG.

Determination of adenylic nucleotides

The determination of adenylic nucleotides was accomplished by means of ion-exchange chromatography on the liquid chromatograph Knauer (Germany) equipped with columns packed with Separon SGX NH_2 (150×3.3 mm, 5 μm) и Diasorb amine (120×1 mm, 5 μm) at 260 nm. The linear gradient of 0-0.35 M KH_2PO_4-Na_2HPO_4 buffer, pH 6.8 was used to separate ATP, ADP and AMP. Injected sample volume was 20 μl and the speed of eluent solution delivery was 0.3 ml per min. Nucleotides peaks were identified starting from positions of appropriate standards.

Statistical analysis

All calculations were made using the Statistica 6.0 program. The data obtained was presented as the mean +/- S.E. The

statistical significance of the results was determined using Student's *t*-test. A $P<0.05$ was required for the results to be considered statistically significant.

Results

Superoxide anion production by the submitochondrial particles of hepatocytes

The results of $O_2^{\cdot-}$ production measurement are presented in Fig. 1. In the presence of NADH (the substrate of the complex I of the mitochondrial respiratory chain), the rate of $O_2^{\cdot-}$ production by SP of toxic hepatitis animals appeared 2.5-fold higher compared to the control SP. If succinate (the substrate of the complex II of the mitochondrial respiratory chain) was added, the rate of $O_2^{\cdot-}$ production by toxic hepatitis and control SP did not differ from proper values of $O_2^{\cdot-}$ production by control SP in the presence of NADH. However, when succinate was delivered, $O_2^{\cdot-}$ production rate by toxic hepatitis SP was similar in magnitude to the control and was significantly lower than in the presence of NADH.

The delivery of the combination of rotenone (inhibitor of the complex I) and NADH into the incubation system resulted in a 4.7-fold increase in $O_2^{\cdot-}$ production by the control SP and had no influence upon $O_2^{\cdot-}$ production by toxic hepatitis SP. The combination of myxothiazol (the inhibitor of CoQ oxidation-reduction) and NADH increased $O_2^{\cdot-}$ production by up to 7.3 and 4.9 times that of the control and toxic hepatitis SP respectively. Combined delivery of antimycin A (inhibitor of CoQ reduction) and NADH resulted in an 8-fold $O_2^{\cdot-}$ production increase by control SP as compared with $O_2^{\cdot-}$ production in the presence of NADH only, and failed to change significantly $O_2^{\cdot-}$ production by toxic hepatitis SP. The combination of myxothiazol and antimycin A in the presence of NADH caused a 6-fold and 5.4-fold $O_2^{\cdot-}$ production increase by control and toxic hepatitis SP, respectively, compared with $O_2^{\cdot-}$ production against a NADH background. Under the influence of myxothiazol and antimycin A combination, the $O_2^{\cdot-}$ production rate by toxic hepatitis SP appeared 2-fold higher as compared to control SP.

Antioxidant system of mitochondria

Antioxidant system of mitochondria is presented mainly by its mitochondrial superoxide dismutase and glutathione cycle components (Andreev et al. 2005). Superoxide dismutase and glutathione peroxidase activities were decreased by 34% and 26%, respectively, in toxic hepatitis mitochondria as compared to the control (Table 1). Mitochondrial oxidized and reduced glutathione concentrations had no significant differences in both groups of animals.

Adeninyl nucleotides concentrations

AMP and ADP concentrations were similar in toxic hepatitis and control livers while ATP concentration was decreased by 45% in the liver of toxic hepatitis animals (Fig. 2).

Discussion

Progress in toxic hepatitis is accompanied by an enhancement in the mitochondrial respiration, providing an increase in the production of ATP needed for liver regeneration. Respiration enhancement can result in an increase in ROS generation and the oxidative damage of molecular components of the mitochondrial respiratory chain (Harvey et al. 2000; Bailey

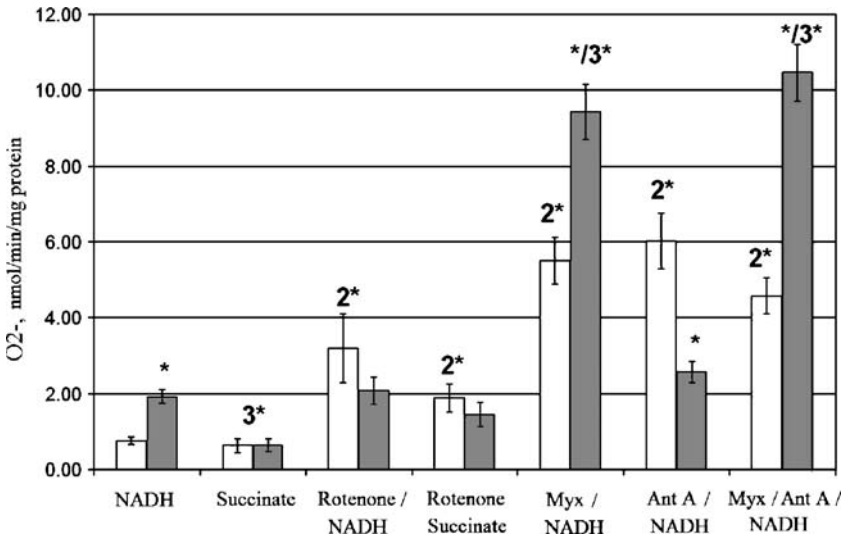

Fig. 1 $O_2^{\cdot-}$ production by the submitochondrial particles of rat hepatocytes in control (*white bars*) and toxic hepatitis (*grey bars*). X ± S.E.; $N=15$; * $P<0.05$ vs. control; [2]* $P<0.05$ vs. control with NADH; [3]* $P<0.05$ vs. toxic hepatitis with NADH. Myx – myxothiazol; Ant A – antimycin A

Table 1 Antioxidant state of hepatocyte mitochondria of rats in the control and toxic hepatitis

Indices	Control	Toxic hepatitis
Superoxide dismutase, U/mg protein	5.8±0.4	3.8±0.1 **
Glutathione peroxidase, nmol/min/mg protein	466±14	345±32 **
Glutathione, nmol/mg protein	2.6±0.3	2.1±0.1
Glutathione reduced, nmol/mg protein	2.0±0.3	1.6±0.1
Glutathione oxidized, nmol/mg protein	0.60±0.06	0.50±0.06

X ± S.E.; N=15; ** P<0.01 vs. control

2003; Andreev et al. 2005). However, injury of the mitochondrial respiratory chain complexes may decrease its capacity to produce ATP. The mitochondrial respiratory chain is a multi-component structure consisting of five complexes that are localized in the inner mitochondrial membrane: NADH-CoQ-oxidoreductase (complex I), succinate-CoQ-oxidoreductase (complex II), CoQ-cytochrome *c*-oxidoreductase (complex III), cytochrome *c* oxidase (complex IV) and ATP-synthase (Fig. 3).

Previously, we have shown that oxygen consumption by hepatocytes and mitochondria isolated from the liver in toxic hepatitis induced by CCl_4 and ethanol combination in the rats, was significantly higher as compared to the control (Shiryaeva et al. 2007, 2008). Oxygen consumption rates by toxic hepatitis mitochondria in states 3 (V_3) and 4 (V_4) according to Chance appeared to be significantly higher in toxic hepatitis than in the control. In addition, distinctions between respiration rates in toxic hepatitis and the control in state 3 was significantly higher with respiratory chain complex I substrates (Shiryaeva et al. 2007). Moreover, the level of rotenone suppression of hepatocyte respiration in toxic hepatitis was significantly lower compared to control hepatocytes respiration suppression. Meanwhile, the degree of the mitochondrial oxidation and phosphorylation coupling was high in toxic hepatitis. Cytochrome *c* oxidase activity was higher by 80% in toxic hepatitis (Shiryaeva et al. 2007). Is all oxygen consumed by the mitochondria used for ATP synthesis? If not, what other purpose is the hepatitis mitochondria using the oxygen consumed for?

Mitochondria concentrate the main portion of oxidative metabolic pathways and contain multiple redox-transporters and sites potentially capable of one-electron reduction of

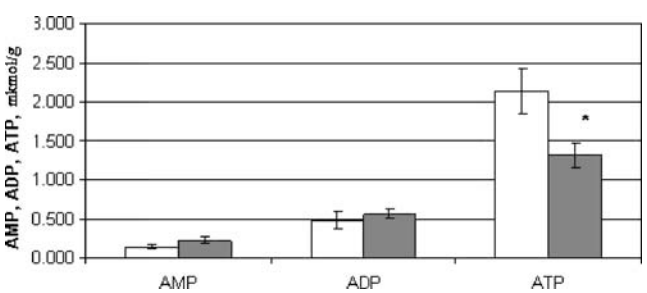

Fig. 2 Adenylic nucleotides content in the liver of control (white bars) and toxic hepatitis (grey bars) rats. X ± S.E.; N=15; * P<0.01

oxygen to $O_2^{\cdot-}$ which is a precursor of the other ROS (Cadenas et al. 1977; Lenaz 2001; Andreev et al. 2005). Mitochondrial respiratory chain complexes I and III are considered to be the main producers of $O_2^{\cdot-}$ (Boveris and Chance 1973; Cadenas et al. 1977; Turrens and Boveris 1980; Lenaz 2001; Andreev et al. 2005). In complex I $O_2^{\cdot-}$ production is thought to be connected with the work of the iron-sulfur cluster and semiquinone (Herrero and Barja 2000; Andreev et al. 2005). As a site of $O_2^{\cdot-}$ production in complex III, the unstable semiquinone radical $Q^{\cdot-}$ in the Q_o center is assumed (Rich and Bonner 1978). However, existence of this radical has not been demonstrated until now (Turrens 2003).

$O_2^{\cdot-}$ production rate by the normal liver hepatocytes is insignificant. For example, mitochondria produce 0.6-1 nmol H_2O_2/min per mg protein, amounting to about 2% of the total oxygen consumed (Liu 1997). $O_2^{\cdot-}$ produced can be involved in cell metabolism regulation or eliminated by mitochondrial SOD (Liu 1997; Turrens 1997). $O_2^{\cdot-}$ production by hepatocytes increases in liver pathologies (Yang et al. 2004; Andreev et al. 2005) but the mechanism of $O_2^{\cdot-}$ production enhancement is poorly studied.

Previously we have suggested that the increase in $O_2^{\cdot-}$ production by the toxic hepatitis mitochondria is connected with electron transport impairment within the mitochondrial respiratory chain complex I (Shiryaeva et al. 2007, 2008). This could explain the enhanced oxygen consumption by hepatocyte mitochondria in toxic hepatitis. Presented data indicate that there is an obstacle in electron transport via complex I in experimental hepatitis. For instance, the $O_2^{\cdot-}$ production rate by toxic hepatitis SP in the presence of NADH was 2.5-fold higher compared to control. Delivery of rotenone, which suppressed the electron moving via complex I from iron-sulfuric clusters to oxidized ubiquinone (Herrero and Barja 2000; Andreev et al. 2005), failed to change the $O_2^{\cdot-}$ production rate to toxic hepatitis SP in the presence of NADH. The level of $O_2^{\cdot-}$ production by control SP when combined with NADH + rotenone was similar to $O_2^{\cdot-}$ production rate by toxic hepatitis SP in the presence of NADH only (Fig. 1). The breach in electron transport along complex I transporters could stimulate electron leakage to oxygen and $O_2^{\cdot-}$ production (Fig. 3).

To examine the contribution of the other respiratory chain components to $O_2^{\cdot-}$ production in toxic hepatitis, we

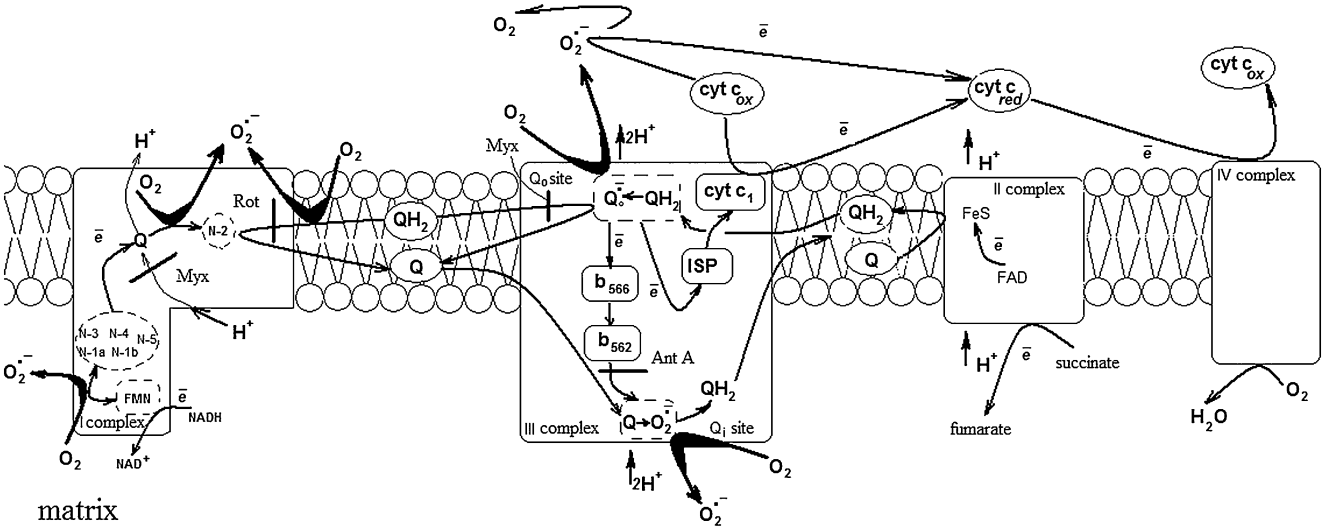

Fig. 3 $O_2^{\cdot-}$ production plausible sites in the mitochondrial respiratory chain and sites of the respiratory chain inhibitors action. Myx – myxothiazol; Ant A – antimycin A; Rot – rotenone

measured the $O_2^{\cdot-}$ production rate in the presence of respiratory chain inhibitors myxothiazol and antimycin A, which have action sites different from rotenone (Fig. 3). Myxothiazol has two sites of influence in the respiratory chain of mitochondria: it inhibits CoQ oxidation in the Q_o center of complex III and partial CoQ reduction in complex I (Ward 2003; Ortega-Sáenz et al. 2003). Antimycin A suppresses an electron moving from cytochrome b556 to oxidized ubiquinone in complex III, preventing ubiquinone reduction (Andreev et al. 2005). Normally, myxothiazol delivery to SP results in a manifold increase in $O_2^{\cdot-}$ production by complex I, while antimycin A addition to SP also markedly stimulated $O_2^{\cdot-}$ production by complex III (Andreev et al. 2005). Myxothiazol and antimycin A in combination induces a small decrease in $O_2^{\cdot-}$ production by complex III (Andreev et al. 2005). The similar effects of myxothiazol and antimycin A on $O_2^{\cdot-}$ production rate were revealed with control SP in our study (Fig. 1). However, the $O_2^{\cdot-}$ production rate by toxic hepatitis SP in the presence of myxothiazol +NADH was higher by 72% as compared to control SP. This indicates that electron transport intensity along the mitochondrial respiratory chain is by far higher in toxic hepatitis compared with the control. Therefore, myxothiazol data are also indicative of the complex I to be the main $O_2^{\cdot-}$ production site in the mitochondrial respiratory chain of hepatocytes in toxic hepatitis.

Controversially, the rate of $O_2^{\cdot-}$ production by toxic hepatitis SP in the presence of antimycin A was lower than in the control, but similar to the rate of $O_2^{\cdot-}$ production by toxic hepatitis SP when rotenone and NADH were delivered. Hence, it follows that either $O_2^{\cdot-}$ production by complex III in toxic hepatitis is very small, or practically all $O_2^{\cdot-}$ produced by complex III is utilized by means of the compensatory mechanism with cytochrome *c* and cytochrome oxidase involvement. Cytochrome *c* can oxidize $O_2^{\cdot-}$ produced from oxygen and transport it to cytochrome oxidase that reduces the oxygen to H_2O (Korshunov et al. 1999; Andreev et al. 2005). The previously found significant increase in cytochrome oxidase activity in toxic hepatitis conforms to such a possibility (Shiryaeva et al. 2007).

It could be suggested that the combined effect of myxothiazol and antimycin A on toxic hepatitis SP $O_2^{\cdot-}$ production depends on electron transport inhibition by myxothiazol in complex I. The putative mechanism of $O_2^{\cdot-}$ utilization in toxic hepatitis mitochondria, involving cytochrome *c* and cytochrome oxidase, might generate the additional electrochemical potential that can provide additional possibilities for ATP synthesis. Therefore, the mitochondrial respiratory chain $O_2^{\cdot-}$ production increase in complex I in toxic hepatitis could be considered to be a compensation mechanism of the electron transport impairment via this complex.

Our data regarding the $O_2^{\cdot-}$ production rate by the mitochondrial respiratory chain complex II in the presence of succinate (complex II substrate) failed to reveal some distinctions between toxic hepatitis and control. Therefore, in toxic hepatitis complex II contribution to $O_2^{\cdot-}$ production is negligible. Other studies also provide evidence of the small importance of complex II as a generator of $O_2^{\cdot-}$ compared to the other ROS sources in hepatitis (McLennan and Degli-Esposti 2000). Some authors express doubt regarding any contribution of complex II to ROS production by the mitochondrial respiratory chain of hepatocytes in the normal liver (Andreev et al. 2005).

Mammalian mitochondria have an effective defense against ROS (Li et al. 1995). Elimination of primary ROS ($O_2^{\cdot-}$ and H_2O_2) is accomplished by antioxidant enzymes Mn-SOD and glutathione peroxidase. Mn-SOD allocated in

the mitochondrial matrix, accelerates $O_2^{\cdot-}$ dismutation to H_2O_2 and protects iron-sulfuric clusters from $O_2^{\cdot-}$ attack (Gardner et al. 1995). Glutathione peroxidase utilizes H_2O_2 by means of reduced glutathione oxidation (Li et al. 1995). The activity of an antioxidant system providing ROS elimination is high in native mitochondria, but can be insufficient in pathologies associated with ROS production increase. In addition, the oxidative metabolic products themselves can inhibit components of the antioxidant system (Bota and Davies 2002). The oxidative modifications of the respiratory chain proteins can be also generated that can enhance oxidative stress and result in additional damage in the mitochondrial respiratory chain and result in an ATP synthesis decrease. We revealed that ATP concentration declined by 45% in toxic hepatitis rat liver. In toxic hepatitis, SOD and glutathione peroxidase activities decreased by 34% and 26%, respectively. However, oxidized and reduced glutathione contents in this pathology had no significant distinctions from control values (see Table). Other liver pathologies also associated with antioxidant enzymes decrease activity. For example, glutathione peroxidase decline was found in chronic alcoholic injury of the liver and in cirrhosis of the liver (Bailey et al. 2001; Balkan et al. 2001). Therefore, the mitochondrial antioxidant system of hepatocytes fails to provide an appropriate $O_2^{\cdot-}$ elimination that can intensify progression of the pathology.

Collectively, the presented data allows one to conclude that $O_2^{\cdot-}$ production by the complex I of the mitochondrial respiratory chain of hepatocytes is enhanced in toxic hepatitis. Hepatitis mitochondria reduce to $O_2^{\cdot-}$ a significant portion of the oxygen consumed. We surmise that the $O_2^{\cdot-}$ production increase could be considered as not the only pathologic process component, but also the compensatory mechanism providing the preservation of the electron-transport function of the mitochondrial respiratory chain when complex I functioning is blocked in part. Data received in relation to the respiratory chain complex III contribution to $O_2^{\cdot-}$ production in toxic hepatitis failed to provide a possible explanation for this contribution.

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All experiments described in the manuscript were performed in accordance with the current laws of The Russian Federation.

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