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Opposite effects of endotoxin on mitochondrial and endoplasmic reticulum functions

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Abstract

In this study, we determined functional integrity and reactive oxygen species generation in mitochondria and endoplasmic reticulum in liver of rats subjected to endotoxic shock to clarify whether intracellular reactive oxygen species (ROS) destabilize cellular integrity causing necrosis in rats challenged with lipopolysaccharide (LPS). LPS caused drastically increased plasma levels of alanine aminotransferase, suggesting damage to plasma membranes of liver cells. Liver necrosis was confirmed by histological examination. LPS induced a significant increase in ROS production in rat liver mitochondria (RLM), but did not impair mitochondrial function. In contrast to mitochondria, enzymatic activity and ROS production of cytochrome P450 were lower in microsomal fraction obtained from LPS-treated animals, suggesting the dysfunction of endoplasmic reticulum. Protein patterns obtained from RLM by two-dimensional electrophoresis showed significant upregulation of mitochondrial superoxide dismutase by LPS. We hypothesize that upregulation of this enzyme protects mitochondria against mitochondrial ROS, but does not protect other cellular compartments such as endoplasmic reticulum and plasma membrane causing necrosis.

Keywords: Endotoxic shock; Mitochondria; Endoplasmic reticulum; Reactive oxygen species; Cytochrome P450

The systemic inflammation developed under septic conditions finally culminates in multiple organ failure, which is the direct cause of death. Oxidative stress is a major factor contributing to the high mortality rates associated with several diseases including endotoxic shock. This condition can be controlled to a certain degree by antioxidant therapies [1,2]. Increased production of reactive oxygen species (ROS) is very often associated with mitochondrial dysfunction [3]. Alterations of oxygen metabolism during sepsis and other critical illness have been well documented and are characterized by impaired tissue oxygen extraction and other "pathologic" consequences of altered oxygen consumption [4–6]. Septic/endotoxic shock has previously been shown to affect mitochondrial function and may cause multiple organ failure [7]. Though mitochondrial function during sepsis and endotoxic shock has been studied extensively, results are contradictory. While some investigators have failed to find any abnormality in mitochondrial function in septic/endotoxic shock [8–10], others have found that mitochondria isolated from septic/endotoxic tissues have reduced state 3 activity and ATP synthesis activity [11–13]. Moreover, some investigators have found that mitochondrial function is improved under septic conditions

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[14–16], at least during the early phase of shock [16]. In our recent study we have shown that mitochondrial response to lipopolysaccharide (LPS)-induced shock is not equal in different tissues: liver mitochondria respond with an improvement and heart mitochondria with an impairment of their respiratory function [17]. Another important subcellular organelle, endoplasmic reticulum, is also influenced by endotoxic shock. It has been shown that hepatic cytochrome P450-mediated drug metabolism is heavily affected by sepsis in rats [18]. The hepatic cytochrome P450 isoform CYP1A2 is downregulated and inhibition of cytochrome P450 enzyme system has been associated with an exacerbated inflammatory response in sepsis [19]. Taking into account that the cytochrome P450 system is another important source of ROS in liver cells, we assumed that intracellular damage of plasma membrane and endoplasmic reticulum could arise from ROS generated by cytochrome P450. Therefore, in this study we have compared function and ROS generation of mitochondria and endoplasmic reticulum in order to define the intracellular source of ROS responsible for oxidative damage of the liver.

Methods

Animal model of endotoxic shock. Adult male Sprague–Dawley rats weighing 280 ± 21 g were divided into two groups to receive saline intraperitoneally (control group; $n = 8$), or 20 mg/kg LPS (*Escherichia coli* 026:B6; $n = 8$). The rats were sacrificed by decapitation 16 h after injections, heparinized blood was withdrawn for biochemical analysis, and livers were quickly extracted and placed in an ice-cold preparation buffer. The study was approved by the local Committee on Animal Experiments of Vienna, Austria, and was performed in accordance with the Guide for the Care and Use of Laboratory Animals (Publication NIH 86-23; revised 1985).

Blood analysis and histological examination. Plasma levels of alanine aminotransferase (ALT) were measured as described previously [20]. Liver tissue was fixed in 10% formaldehyde solution for 1–2 weeks. The paraffin blocks and later sections were prepared. The sections were stained with hematoxylin and eosin.

Preparation of liver homogenates and isolated mitochondria. The rats were killed by decapitation, livers were quickly extracted and moved to ice-cold preparation buffer (containing 0.25 M sucrose, 10 mM Tris–HCl, 0.5 mM EDTA (pH 7.2), and 0.5 g/l essentially fatty-acid free bovine serum albumin). A piece of left liver lobe was weighted, minced with scissors and homogenized in a motor-driven Potter–Elvehjem homogenizer in the preparation buffer (1/9 = w/v). Rat liver mitochondria (RLM) were prepared as described previously [21].

Isolation of microsomes. A part of each rat liver was rinsed with buffer (150 mM KCl, 50 mM Tris, pH 7.4), minced with scissors, and homogenized on ice in a motor-driven Potter–Elvehjem homogenizer with three volumes of buffer. The crude homogenate was centrifuged at 8750*g* for 15 min at 4 °C. Microsomes were sedimented from the 8750*g* supernatant by centrifugation at 165,000*g* for 38 min at 4 °C [22]. After two washing steps, microsomes were dispersed in the buffer to provide a protein concentration of approximately 30 mg/ml.

Mitochondrial function. Respiratory parameters were determined in liver homogenates and suspensions of isolated mitochondria with a Clark-type oxygen electrode (Oroboros Ltd, Austria). Liver homogenates (5 mg wet tissue/ml) and RLM (0.5 mg protein/ml) were incubated in a buffer consisting of 105 mM KCl, 20 mM Tris–HCl, 1 mM diethylenetriaminepentaacetic acid, 5 mM KH_2PO_4, and 1 mg/ml fatty acid-free bovine serum albumin (pH 7.4, 25 °C). Respiration was stimulated either by the addition of 5 mM glutamate plus 5 mM malate (homogenates; RLM) or 10 mM succinate (RLM) in the presence of rotenone (1 μg/ml). The latter was used to prevent electron transport from and to complex I. The transition to state 3 respiration was induced by the addition of 200 μM ADP.

Cytochrome P450 function. The activity of the microsomal cytochrome P450 system was assessed from the *O*-dealkylation of 7-ethoxycoumarin to the fluorescent molecule 7-hydroxycoumarin using fluorescence spectroscopy [23]. Microsomal protein (1.25 mg) was resuspended in 2 ml of 0.1 M Tris-buffer (pH 7.6) supplemented with 100 μM of 7-ethoxycoumarin. The suspension was placed in a fluorescence cell and a time scan was initiated to obtain the baseline slope (excitation 360 nm, emission 460 nm). After 150 s NADPH (50 μM) was added to start the metabolic reaction increasing fluorescence due to the formation of 7-hydroxycoumarin. At 500 s 1 μM 7-hydroxycoumarin was added for calibration purposes. The results were expressed in nanomoles 7-ethoxycoumarin dealkylated per minute and per milligram microsomal protein.

Measurement of cytochrome P450 and cytochrome b_5 contents. Microsomal protein (2 mg) was suspended in 1 ml air-saturated buffer (100 mM P_i, pH 7.0). A reference spectrum of the suspension from 350 to 650 nm was recorded in a cell with 1 cm light path using an SLM Aminco dual-wavelength photometer. After addition of dithionite (10 mM) and bubbling the solution with CO gas for 20 s the cytochrome P450 differential spectrum was obtained. The amount of cytochrome P450 was calculated from the absorption difference between 450 and 490 nm using an extinction coefficient of 91 cm^{-1} mM^{-1} [24]. The content of cytochrome b_5 was determined upon addition of NADH (0.2 mM) and calculated from the absorption difference 424 nm minus 409 nm using an extinction coefficient of 185 cm^{-1} mM^{-1} [24].

ROS generation. The rates of ROS generation in mitochondrial and microsomal suspensions were detected in the presence of 250 μM of 1-hydroxy-3-carboxy-pyrrolidine (CPH). Upon reaction with ROS, CPH is transformed into a stable $CP^{\cdot}$ radical (3-carboxy-proxyl) which can be detected by electron paramagnetic resonance (EPR) spectroscopy as described previously [25]. EPR spectra were recorded at 22 °C with a Bruker EMX spectrometer (BioSpin GmbH, Germany) in a flat cell. The general settings were: microwave frequency 9.777 GHz, modulation frequency 100 kHz, modulation amplitude 2 G, microwave power 1 mW, gain 5×10^5, time constant 40 ms, sweep time 41.9 s. Mitochondria (0.55 mg/ml) were incubated for 20 min at 22 ± 1.5 °C in the incubation buffer identical to the buffer used for determination of respiratory parameters. The microsomal reaction mixture (5.5 mM glucose-6-phosphate, 0.67 U/ml glucose-6-phosphate dehydrogenase, 1.5 mg protein/ml microsomes, 5 mM diethylenetriaminepentaacetic acid, and 250 μM CPH, final volume 500 μl) was saturated with air and incubated for 20 min at 25 °C. The reaction was started by the addition of 0.38 mM $NADP^+$.

2-DE DIGE and protein identification. Samples were diluted with 30 mM Tris–HCl, 9 M urea, 4% CHAPS (w/v), pH 8.5, and labelled with CyDyes® according to the instructions of the manufacturer (GE Health Care, Uppsala, Sweden; 8 nmol dye/mg protein). Two samples (labelled with Cy3 and Cy5, respectively) and one internal standard (a pool of all samples, labelled with Cy2) were applied on each gel. 2-DE was performed as has been previously described [26] as a combination of isoelectric focusing in the pH-range of 4–10 in the first and of SDS–PAGE in 10–15% *T* gradients in the second dimension. Following electrophoresis, gels were scanned on a Typhoon 9400 imager and evaluated with DeCyder Software V5.02 (all GE Health Care), including filtering out of differentially expressed protein spots and statistical evaluation. Potentially interesting spots were identified by nanoHPLC–MS/MS protein sequencing as described previously [26].

Statistical methods. The data are presented as means ± SEM. The calculations were made with the software Origin 6.1 (Microcal Inc.) and MS Excel (Microsoft Corp.). Kolmogorov–Smirnov test was used to test that a variable is normally distributed. Significance was determined by *t* test for normally distributed values and by Mann–Whitney test for other values. The significance level was set at 0.05.

Results

Sixteen hours after LPS injection typical signs of organ failure were confirmed by histological examination. Multiple focal necrosis and cell infiltration were observed in livers obtained from LPS-treated rats (Fig. 1A). The levels of ALT, a common marker of necrosis were drastically increased in blood (Fig. 1B).

Fig. 2A shows a typical curve of oxygen uptake by mitochondria. Fig. 2B shows that respiratory control values measured in mitochondrial suspensions were always lower than those in fresh liver homogenates. Respiratory control value was always higher in the corresponding LPS sample compared to control in both mitochondrial suspension and in homogenate.

A significant increase in ROS generation rate in mitochondria from LPS-treated rats was observed in the presence of both mitochondrial complex I and complex II substrates (Fig. 3A). This increase was more pronounced with the complex I substrates (glutamate/malate) than with the complex II substrate (succinate). In contrast to mitochondria, we found that the rate of ROS formation was significantly lower in microsomes from LPS-treated rats compared to controls (Fig. 3B).

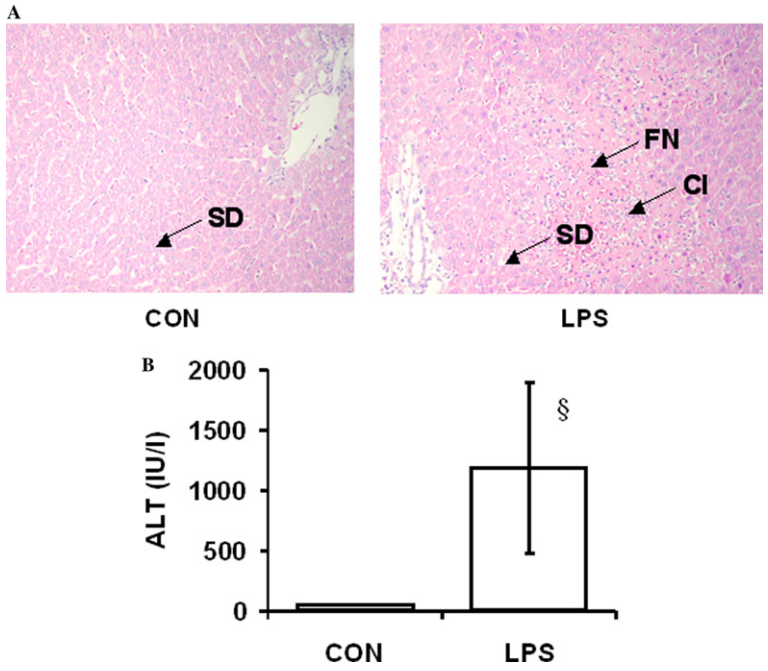

Fig. 1. Histological and biochemical markers of necrosis in control rats (CON) and rats treated with lipopolysaccharide (LPS). (A) A typical result of histological examination of liver by means of light microscopy with hematoxylin and eosin staining. *Abbreviations:* SD, distension of Disse's spaces; FN, focal necrosis; CI, cellular infiltration (polymorphonuclear neutrophils). (B) Levels of alanine aminotransferase (ALT) in plasma of control and LPS challenged rats. $^{\S}p < 0.05$.

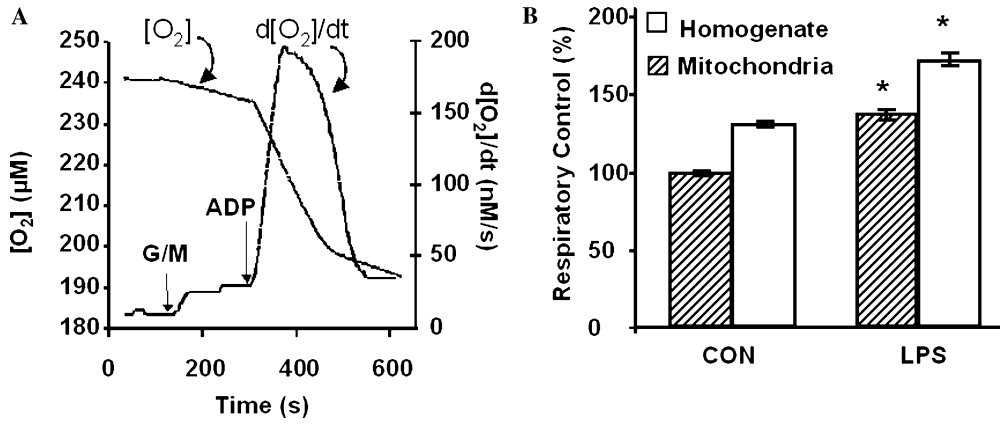

Fig. 2. Respiratory activity of mitochondria determined in liver homogenates and liver mitochondria obtained from control rats (CON) and rats treated with lipopolysaccharide (LPS). The experiments were performed in the presence of glutamate/malate (5 mM/5 mM). (A) Typical curves of oxygen concentration ($[O_2]$) and oxygen consumption ($[d[O_2]/dt$) observed in liver homogenate or mitochondrial suspension. (B) Effect of LPS challenge on the respiratory control values of liver mitochondria determined in homogenates and isolated mitochondria. $^{*}p < 0.01$.

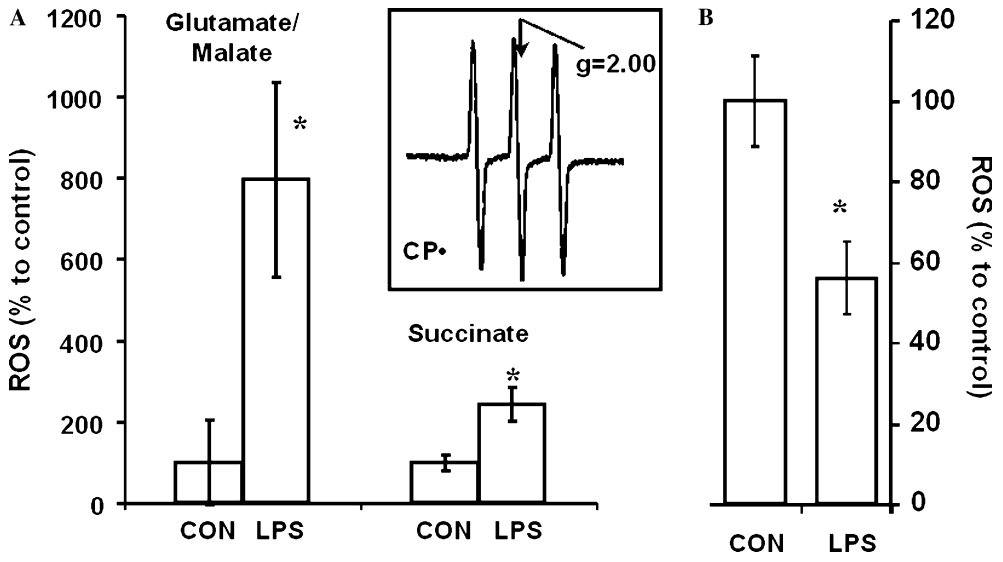

Fig. 3. Effect of LPS challenge on the generation of reactive oxygen species (ROS) in rat liver mitochondria (A) and in rat liver microsomal fraction (B). Mitochondrial ROS were determined in the presence of either glutamate/malate (5 mM/5 mM) or succinate (10 mM). Microsomal ROS were determined in the presence of glucose-6-phosphate dehydrogenase and 0.38 mM $NADP^+$. (Inset) Electron spin resonance signal of the 3-carboxy-proxyl ($CP^•$). $^*p < 0.01$.

There was no significant difference between mitochondria from LPS-treated and control animals in state 4 respiration (Table 1). In contrast, the respiration in state 3 was significantly faster in mitochondria from LPS-treated animals compared to controls (Table 1) with both glutamate/malate and succinate. In contrast to elevated mitochondrial function we observed a decrease in P450 and b_5 contents (Table 1). Monooxygenase activity of P450 (CYP1A2) was decreased in LPS-treated animals compared to controls (Table 1).

In mitochondrial proteomes analysed by 2D-electrophoresis we have found few spots that were significantly changed after LPS treatment compared to control. The spot encircled in Fig. 4A was one of them; it was previously identified as mitochondrial superoxide dismutase (Mn-SOD) [26]. A quantitative estimation revealed that this enzyme was definitely upregulated on the protein level suggesting an increase in SOD activity (Fig. 4B).

Table 1
Effect of LPS challenge on functional parameters of rat liver mitochondria and endoplasmic reticulum

	Control	LPS	p^*
State 4 respiration (nmol O_2/mg protein/min)			
Glutamate/malate	6.3 ± 0.5	4.9 ± 0.6	0.1133
Succinate	13.5 ± 0.6	13.6 ± 0.9	0.8926
State 3 respiration (nmol O_2/mg protein/min)			
Glutamate/malate	46.4 ± 2.4	61.8 ± 3.9	0.0018
Succinate	62.8 ± 2.9	85.2 ± 5.0	0.0004
Cytochrome content (nmol/mg total protein)			
Cytochrome P450	1.237 ± 0.074	0.791 ± 0.046	0.0001
Cytochrome b_5	0.350 ± 0.012	0.281 ± 0.015	0.0035
P450 activity (pmol 7-hydroxycoumarin/min/mg total protein)	13.5 ± 2.0	5.9 ± 1.8	0.0117

* Significance level.

Discussion

The present study demonstrated an opposite behavior of two intracellular ROS sources in a model of endotoxic liver injury: an increased generation of ROS in mitochondria and a decreased ROS generation in endoplasmic reticulum. Since antioxidants ameliorate the tissue damage [2,27,28] we can expect that enhanced ROS production in LPS-treated animals can contribute to the damage of liver cells and focal necrosis formation as it was confirmed by increased levels of ALT in blood (Fig. 1A) and histological examination (Fig. 1B), respectively. The examination of mitochondrial and microsomal function revealed an improvement of mitochondrial function in LPS-treated compared to control animals. The latter is in agreement with our previous publication [17]. In contrast the activity of cytochrome P450 was reduced (Table 1), which is in line with previous publications [18,19]. The cytochrome P450 system depends on the NADPH:cytochrome b_5 reductase as cooperating enzyme. Both heme enzymes were shown to release ROS under certain pathophysiological conditions, which involve the reductive activation of xenobiotics [22,29,30]. In this study we have shown that LPS challenge results in a decrease in the tissue levels of both proteins (Table 1), indicating endoplasmic reticulum dysfunction. Therefore, the source of increased ROS generation (mitochondria), and the target of ROS (presumably endoplasmic reticulum and plasma membrane) do not belong to the same intracellular compartment. The fact that mitochondria produce

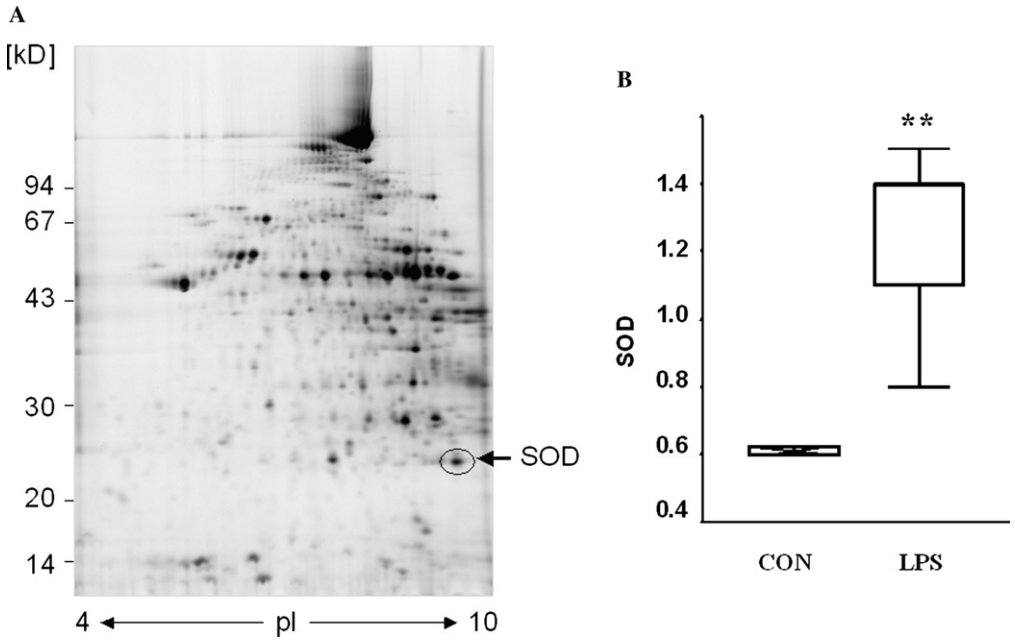

Fig. 4. Effect of LPS challenge on the expression of mitochondrial SOD in liver mitochondria. (A) A typical 2D-electrophoresis of rat liver mitochondria. (B) Comparison of mitochondrial SOD expression (in Arbitrary Units) control and LPS-treated rats. $^{**}p < 0.005$.

increased levels of ROS without damaging themselves can be explained by the existence of a mechanism protecting mitochondria toward their own ROS. It is commonly accepted that the primary ROS species produced by mitochondria is $O_2^{\cdot-}$, which can be dismutated in vivo by SOD. Therefore, the increased expression of SOD observed in this study (Fig. 4) is a possible explanation for the resistance of mitochondria from LPS-treated rats to their own ROS. It has been shown that increase in SOD activity significantly diminishes damaging consequences of oxidative stress [31–34]. Based on the data presented here and in the literature we suggest the following hypothesis of how intracellular ROS can lead to liver necrosis under endotoxic shock. It starts with an adaptive upregulation of mitochondrial function in liver cells, probably, via expression of ATP-synthase [26]. This results in an improved coupling of oxidative phosphorylation, which in turn increases mitochondrial ROS production as a side product of respiration. Increased ROS production is due to increased membrane potential of hyperactive mitochondria as it has been suggested in a number of publications [35,36]. Increased production of mitochondrial ROS does not damage mitochondria because they are protected by upregulated mitochondrial SOD. This fact has been already reported as an increase in SOD activity [28,37]. We have shown upregulation of SOD expression on the protein level. We suppose that plasma membrane and endoplasmic reticulum are not as strongly protected against ROS as mitochondria. Therefore, mitochondrial ROS damage plasma membrane and endoplasmic reticulum. Further studies will be required to accept or reject this mechanism.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2006.10.180.

References

[1] V. Ben-Shaul, L. Lomnitski, A. Nyska, Y. Zurovsky, M. Bergman, S. Grossman, The effect of natural antioxidants, NAO and apocynin, on oxidative stress in the rat heart following LPS challenge, Toxicol. Lett. 123 (2001) 1–10.
[2] V.M. Victor, M. Rocha, M. De la Fuente, Immune cells: free radicals and antioxidants in sepsis, Int. Immunopharmacol. 4 (2004) 327–347.
[3] A.Y. Andreyev, Y.E. Kushnareva, A.A. Starkov, Mitochondrial metabolism of reactive oxygen species, Biochemistry (Mosc.) 70 (2005) 200–214.
[4] D.E. Taylor, C.A. Piantadosi, Oxidative metabolism in sepsis and sepsis syndrome, J. Crit. Care 10 (1995) 122–135.
[5] R.W. Samsel, P.T. Schumacker, Oxygen delivery to tissues, Eur. Respir. J. 4 (1991) 1258–1267.
[6] B.A. Houtchens, D.R. Westenskow, Oxygen consumption in septic shock: collective review, Circ. Shock 13 (1984) 361–384.

[7] E.D. Crouser, M.W. Julian, D.V. Blaho, D.R. Pfeiffer, Endotoxin-induced mitochondrial damage correlates with impaired respiratory activity, Crit. Care Med. 30 (2002) 276–284.
[8] E.R. Geller, S. Jankauskas, J. Kirkpatrick, Mitochondrial death in sepsis: a failed concept, J. Surg. Res. 40 (1986) 514–517.
[9] L. Mela-Riker, D. Bartos, A.A. Vlessis, L. Widener, P. Muller, D.D. Trunkey, Chronic hyperdynamic sepsis in the rat. II. Characterization of liver and muscle energy metabolism, Circ. Shock 36 (1992) 83–92.
[10] D.E. Taylor, A.J. Ghio, C.A. Piantadosi, Reactive oxygen species produced by liver mitochondria of rats in sepsis, Arch. Biochem. Biophys. 316 (1995) 70–76.
[11] L. Mela, L.V. Bacalzo Jr., L.D. Miller, Defective oxidative metabolism of rat liver mitochondria in hemorrhagic and endotoxin shock, Am. J. Physiol. 220 (1971) 571–577.
[12] S.P. Kantrow, D.E. Taylor, M.S. Carraway, C.A. Piantadosi, Oxidative metabolism in rat hepatocytes and mitochondria during sepsis, Arch. Biochem. Biophys. 345 (1997) 278–288.
[13] E.D. Crouser, M.W. Julian, J.E. Huff, M.S. Joshi, J.A. Bauer, M.E. Gadd, M.D. Wewers, D.R. Pfeiffer, Abnormal permeability of inner and outer mitochondrial membranes contributes independently to mitochondrial dysfunction in the liver during acute endotoxemia, Crit. Care Med. 32 (2004) 478–488.
[14] J. Tanaka, Y. Kono, Y. Shimahara, T. Sato, R.T. Jones, R.A. Cowley, B.F. Trump, A study of oxidative phosphorylative activity and calcium-induced respiration of rat liver mitochondria following living *Escherichia coli* injection, Adv. Shock Res. 7 (1982) 77–90.
[15] D.E. Taylor, S.P. Kantrow, C.A. Piantadosi, Mitochondrial respiration after sepsis and prolonged hypoxia, Am. J. Physiol. 275 (1998) L139–L144.
[16] S.M. Lu, S.M. Song, J.C. Liu, H.M. Yang, P. Li, Z.G. Wang, Changes of proton transportation across the inner mitochondrial membrane and H^+-ATPase in endotoxic shock rats, Chin. J. Traumatol. 6 (2003) 292–296.
[17] A.V. Kozlov, K. Staniek, S. Haindl, C. Piskernik, W. Öhlinger, L. Gille, H. Nohl, S. Bahrami, H. Redl, Different effects of endotoxic shock on the respiratory function of liver and heart mitochondria in rats, Am. J. Physiol. 290 (2006) G543–G549.
[18] S.H. Lee, S.M. Lee, Suppression of hepatic cytochrome P450-mediated drug metabolism during the late stage of sepsis in rats, Shock 23 (2005) 144–149.
[19] J.H. Crawford, S. Yang, M. Zhou, H.H. Simms, P. Wang, Down-regulation of hepatic CYP1A2 plays an important role in inflammatory responses in sepsis, Crit. Care Med. 32 (2004) 502–508.
[20] F. Fitzal, H. Redl, W. Strohmaier, E.R. Werner, S. Bahrami, A 4-amino analogue of tetrahydrobiopterin attenuates endotoxin-induced hemodynamic alterations and organ injury in rats, Shock 18 (2002) 158–162.
[21] K. Staniek, H. Nohl, H_2O_2 detection from intact mitochondria as a measure for one-electron reduction of dioxygen requires a non-invasive assay system, Biochim. Biophys. Acta 1413 (1999) 70–80.
[22] E. Perez-Reyes, B. Kalyanaraman, R.P. Mason, The reductive metabolism of metronidazole and ronidazole by aerobic liver microsomes, Mol. Pharmacol. 17 (1980) 239–244.
[23] R.A. Prough, M.D. Burke, R.T. Mayer, Direct fluorometric methods for measuring mixed function oxidase activity, Methods Enzymol. 52 (1978) 372–377.
[24] T. Omura, R. Sato, The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature, J. Biol. Chem. 239 (1964) 2370–2378.
[25] A.V. Kozlov, L. Szalay, F. Umar, K. Kropik, K. Staniek, H. Niedermuller, S. Bahrami, H. Nohl, Skeletal muscles, heart, and lung are the main sources of oxygen radicals in old rats, Biochim. Biophys. Acta 1740 (2005) 382–389.
[26] I. Miller, M. Gemeiner, B. Gesslbauer, A. Kungl, C. Piskernik, S. Haindl, S. Nurnberger, S. Bahrami, H. Redl, A.V. Kozlov, Proteome analysis of rat liver mitochondria reveals a possible compensatory response to endotoxic shock, FEBS Lett. 580 (2006) 1257–1262.
[27] P.K. Gonzalez, J. Zhuang, S.R. Doctrow, B. Malfroy, P.F. Benson, M.J. Menconi, M.P. Fink, Role of oxidant stress in the adult respiratory distress syndrome: evaluation of a novel antioxidant strategy in a porcine model of endotoxin-induced acute lung injury, Shock 6 (Suppl. 1) (1996) S23–S26.
[28] M. Leach, S. Frank, A. Olbrich, J. Pfeilschifter, C. Thiemermann, Decline in the expression of copper/zinc superoxide dismutase in the kidney of rats with endotoxic shock: effects of the superoxide anion radical scavenger, tempol, on organ injury, Br. J. Pharmacol. 125 (1998) 817–825.
[29] Y. Minamiyama, S. Takemura, S. Toyokuni, S. Imaoka, Y. Funae, K. Hirohashi, T. Yoshikawa, S. Okada, CYP3A induction aggravates endotoxemic liver injury via reactive oxygen species in male rats, Free Radic. Biol. Med. 37 (2004) 703–712.
[30] H. Ischiropoulos, T. Kumae, Y. Kikkawa, Effect of interferon inducers on superoxide anion generation from rat liver microsomes detected by lucigenin chemiluminescence, Biochem. Biophys. Res. Commun. 161 (1989) 1042–1048.
[31] T.G. Lehmann, T. Luedde, R.F. Schwabe, H. Bunzendahl, R.J. Samulski, J.J. Lemasters, D.A. Brenner, Minimizing oxidative stress by gene delivery of superoxide dismutase accelerates regeneration after transplantation of reduced-size livers in the rat, Liver Transpl. 12 (2006) 550–559.
[32] I. Vouldoukis, M. Conti, P. Krauss, C. Kamate, S. Blazquez, M. Tefit, D. Mazier, A. Calenda, B. Dugas, Supplementation with gliadin-combined plant superoxide dismutase extract promotes antioxidant defences and protects against oxidative stress, Phytother. Res. 18 (2004) 957–962.
[33] W. Ibrahim, U.S. Lee, H.C. Yen, D.K. St. Clair, C.K. Chow, Antioxidant and oxidative status in tissues of manganese superoxide dismutase transgenic mice, Free Radic. Biol. Med. 28 (2000) 397–402.
[34] Z. Radak, K. Asano, M. Inoue, T. Kizaki, S. Oh-Ishi, K. Suzuki, N. Taniguchi, H. Ohno, Superoxide dismutase derivative prevents oxidative damage in liver and kidney of rats induced by exhausting exercise, Eur. J. Appl. Physiol. Occup. Physiol. 72 (1996) 189–194.
[35] I. Lee, E. Bender, S. Arnold, B. Kadenbach, New control of mitochondrial membrane potential and ROS formation—a hypothesis, Biol. Chem. 382 (2001) 1629–1636.
[36] V.P. Skulachev, Bioenergetic aspects of apoptosis, necrosis and mitoptosis, Apoptosis 11 (2006) 473–485.
[37] D.M. Guidot, Endotoxin pretreatment in vivo increases the mitochondrial respiratory capacity in rat hepatocytes, Arch. Biochem. Biophys. 354 (1998) 9–17.