Suppression of mitochondrial ATPase inhibitor protein (IF1) in the liver of late septic rats

Li-Ju Huang a, Chin Hsu a, Tsen-Ni Tsai a, Shu-Jung Wang a, Rei-Cheng Yang b,c,⁎

a Department of Physiology, Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung 807, Taiwan
b Graduate Institute of Physiology and Molecular Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung 807, Taiwan
c Department of Pediatrics, Kaohsiung Medical University Hospital, Kaohsiung 807, Taiwan

Received 7 September 2006; received in revised form 21 March 2007; accepted 27 March 2007
Available online 3 April 2007

Abstract

Sepsis and ensuing multiple organ failure continue to be the most leading cause of death in critically ill patients. Despite hepatocyte-related dysfunctions such as necrosis, apoptosis as well as mitochondrial damage are observed in the process of sepsis, the molecular mechanism of pathogenesis remains uncertain. We recently identified one of the differentially expressed genes, mitochondrial ATPase inhibitor protein (IF1) which is down-regulated in late septic liver. Hence, we further hypothesized that the variation of IF1 protein may be one of the causal events of the hepatic dysfunction during late sepsis. The results showed that the elevated mitochondrial F0F1-ATPase activity is concomitant with the decline of intramitochondrial ATP concentration in late septic liver. In addition, the key finding of this study showed that the mRNA and the mitochondrial content of IF1 were decreased in late sepsis while no detectable IF1 was found in cytoplasm. When analyzed by immunoprecipitation, it seems reasonable to imply that the association capability of IF1 with F1-ATPase β-subunit is not affected. These results confirm the first evidence showing that the suppression of IF1 expression and subsequent elevated mitochondrial F0F1-ATPase activity might contribute to the bioenergetic failure in the liver during late sepsis.

© 2007 Elsevier B.V. All rights reserved.

Keywords: Sepsis; Mitochondrial F0F1-ATPase; IF1; Liver

1. Introduction

Sepsis is a systemic response to an infection and is still the most common cause of death in intensive care units (ICUs) among adults and children [1,2]. In septic patients, severe inflammation damages the heart, kidneys, lungs, liver, central nervous system and coagulation system, leading to multiple organ dysfunctions. The liver plays a pivotal role in modulating the systemic response to sepsis because of its central contribution in metabolism and host defense mechanism. It has been demonstrated that the mortality rate in patients with hepatic dysfunction is greater than those without [3]. Furthermore, the sepsis-induced liver dysfunction manifested by disseminated intravascular coagulation (DIC), reduced hepatic lactate and amino acid clearances, decreased gluconeogenesis, and glyco- genolysis with subsequent hypoglycemia, is typically considered to be a complication of late sepsis [4].

Hepatic dysfunction associated with necrosis, apoptosis as well as mitochondrial damage of hepatocytes were observed during sepsis [5–7], while the underlying molecular mechanism remained uncertain. Several lines of evidence indicate that cellular energy is deranged in sepsis, some by inadequate tissue perfusion but mostly by impaired mitochondrial respiration [8]. Under aerobic conditions, most of the ATP necessary to supply organs and tissues is generated by the mitochondrial oxidative phosphorylation mechanism. Cell functions can continue only by uninterrupted mitochondrial energy production. Therefore, dysfunctional mitochondrial respiratory chain reaction can affect all organs and tissues and cause a wide variety of disorders [9]. Many studies of impaired NADH availability, mitochondrial ultrastructure and enzymatic activities of complex I and IV have been revealed to closely correlate with decreased
ATP content during late sepsis [9–11]. However, the level of ATP declination is more prominent than that of enzyme dysfunctions, therefore, it is unable to fully define the low ATP content during sepsis. In addition, Brealey and colleagues [12] demonstrated that the hepatic ATP concentrations were significantly lower in the septic rats together with the concurrent rise in ADP and AMP, suggesting the presence of increased ATP hydrolysis.

We recently identified differentially expressed genes (DEGs) in the late septic liver using a PCR-based suppression subtractive hybridization (SSH) method. Nine DEGs were isolated and five of them were not reported to be linked to sepsis [13]. Among the 9 DEGs, mitochondrial ATPase inhibitor protein (IF1) was found to be a novel gene in mitochondrial dysfunction during sepsis. In rat liver, IF1 is a small protein containing 82 amino acid residues and reacts reversibly with F0F1-ATPase and inhibits up to 90% of its ATPase activity [14]. Papa et al. have shown that the synthetic peptide with the Leu 42–Lys 58 sequence is equally effective as IF1 in inhibiting the ATPase activity of both the F0F1 complex in the membrane and soluble F1 [15]. Accordingly, we hypothesized that IF1 may be involved in the hepatic dysfunction during late sepsis. The current study was designed to evaluate the expression of IF1 and mitochondrial F0F1-ATPase activity in the liver of sham-operation and late sepsis, and its mechanism was discussed.

2. Materials and methods

2.1. Induction of septic animals

Male Sprague–Dawley rats weighing 300–350 g were purchased from the National Experimental Animal Center (Nan-Kang, Taipei, Taiwan) and were bred in the animal center of Kaohsiung Medical University. Animals were divided randomly into sham-operation and experimental groups and were fasted prior to the operation procedure. After the animals were deeply anesthetized with pentobarbital (60 mg/kg, intraperitoneally), sepsis was induced by cecal ligation and puncture (CLP) according to the method of Wichterman et al. [16]. Among the 9 DEGs, mitochondrial ATPase inhibitor protein (IF1) was found to be a novel gene in mitochondrial dysfunction during sepsis. In rat liver, IF1 is a small protein containing 82 amino acid residues and reacts reversibly with F0F1-ATPase and inhibits up to 90% of its ATPase activity [14]. Papa et al. have shown that the synthetic peptide with the Leu 42–Lys 58 sequence is equally effective as IF1 in inhibiting the ATPase activity of both the F0F1 complex in the membrane and soluble F1 [15]. Accordingly, we hypothesized that IF1 may be involved in the hepatic dysfunction during late sepsis. The current study was designed to evaluate the expression of IF1 and mitochondrial F0F1-ATPase activity in the liver of sham-operation and late sepsis, and its mechanism was discussed.

2.5. Measurement of mitochondrial ATP content

Mitochondrial ATP content was determined by using an ATP determination kit (Molecular Probes, Eugene, USA) with some modification, as described by Drew and Leeuwenburgh [21]. ATP standards were prepared using known concentrations of ATP (10, 20, 100, 200, 500 and 1000 μM, respectively). In the beginning, the background of the reaction mixture (190 μl) was recorded using an automatic luminometer (Lucy 1, Anthos Labtech Instruments, Salzburg, Austria) before the mitochondria was added. The background value associated with the reaction mixture could be subtracted. Then, 10 μl freshly isolated mitochondria (1 μg) was added to the reaction mixture to detect the ATP content immediately.

2.6. Estimation of ATP synthase activity by coupled enzyme assay

ATP synthase activity was measured by monitoring the increase in absorbance at 340 nm using an NADP⁺ -linked, ADP-regenerating system, as previously described [22]. Equal amount of fresh MgATP-SMPs (final protein concentration was 0.1 mg/ml) was added to the reaction mixture containing 10 mM KPO4, 10 mM succinate, 50 mM Tris-acetate (pH 7.5), 1 mM glucose, 1 mM NADP⁺, 2 mM MgCl₂, 2 mg/ml bovine albumin, 200 mM sucrose, 20 IU hexokinase (Roche, Penzberg, Germany) and 0.25 IU glucose-6-P dehydrogenase (Roche, Penzberg, Germany). The total volume in the cuvette was 1 ml. The blank cuvette contained air. The reaction was assayed at 25 °C and started by the addition of 1 mM ADP using a Hitachi U-2000 spectrophotometer (Ibaraki, Japan) equipped with 340 nm filter.

2.7. Reconstitution of synthetic IF1 peptide with MgATP-SMPs

IF1 reconstitution was performed as described by Zanotti et al. [23]. MgATP-SMPs (0.3 mg/ml) was incubated in a reconstitution buffer (200 mM sucrose, 10 mM Tris/Acetate pH 6.7, 1 mM EDTA, 6 mM MgCl₂, and 1 mM Mg-ATP) with 2.4 μM IF1 synthetic peptide containing the binding segment
(Leu 42–Lys 58) peptide was obtained from M MDBio, Inc.) for 10 min at 21 °C. Then, the suspension was centrifuged at 100,000 × g for 20 min and the reconstituted particles were resuspended in reconstitution buffer. An equal amount of protein was used to measure the ATP hydrolytic activity as described in the following method.

2.8. Measurement of mitochondrial ATP hydrolytic activity by coupled enzyme assay

The mitochondrial ATP hydrolytic activity was measured using a coupled assay to follow the oxidation of NADH to NAD⁺ as described by Zanotti et al. [23]. The MgATP-SMPs (final protein concentration was 0.1 mg/ml) was added to the reaction mixture containing 200 mM sucrose, 50 mM KCl, 5 mM MgCl₂, 10 IU lactate dehydrogenase (Roche, Penzberg, Germany), 4 IU pyruvate kinase (Roche, Penzberg, Germany), 1 mM phosphonoxyphosphate, 0.1 mM NADH, 20 mM Tris/HCl, pH 7.4. The total volume was 1 ml. The blank cuvette contained air. The reaction was started by the addition of 1 mM ATP and run using a Hitachi U-2000 spectrophotometer (Ibaraki, Japan) equipped with 340 nm filter. The linear reaction was followed for 3 min at 340 nm and 25 °C.

2.9. RNA preparation and Northern blot analysis

Total RNA was isolated from fresh liver tissue using TRizol reagent (Molecular Research Center Inc., Cincinnati, USA). For Northern blot analysis, the RNA samples (25 µg/lane) were fractionated by electrophoresis on a 1% agarose formaldehyde gel and transferred to N+-Hybond membrane (Amersham Pharmacia Inc., New Territories, Hong Kong). Input loading was normalized to 18S rRNA. The cDNA (5 µg) of IF1 was labeled with [α-32P]-dCTP using a High Prime reaction mix according to the manufacturer’s instructions (Roche, Mannheim, Germany). Hybridization was performed at 68 °C overnight in hybridization buffer (Clontect Laboratories, Inc., Palo Alto, USA). After hybridization, the membrane was washed three times and then wrapped in a plastic wrap to keep it from drying out and immediately exposed to film for autoradiography. Relative density of each band was measured by computer-assisted densitometry and Bio-1D V.97 software (Vilber lourmat, France).

2.10. Western blot analysis

Equal amount of protein extract (5 µg/lane) were separated by 12% SDS-PAGE (for individual IF1, CoO IV and GAPDH protein detection) or 8% SDS-PAGE (for individual F₁-ATPase α-, β-subunit protein detection), then transferred to polyvinylidene difluoride membranes (NEN Life Science Products, Boston, USA). The membranes were blocked in TBS-t and supplemented with 5% nonfat dry milk powder on a shaker at 4 °C overnight. Then the membranes were incubated with primary mouse IF1 (1:1000), F₁-ATPase α-(1:1000)(Molecular Probes, Eugene, USA) and β-subunit antibodies (1:1000) (Abcam Inc., Cambridge, UK) for 1 h at room temperature, respectively. After that, incubation with goat antimouse secondary antibody conjugated with peroxidase (1:10,000 in TBS-t/5% milk) (Santa Cruz, California, USA) was performed for 1 h at room temperature. Detection was by ECL (Pharmacia Biotech, Välinge, Sweden) according to the manufacturer’s instructions, and subsequently exposed to X-ray film, respectively. Relative density of each band was measured by computer-assisted densitometry and Bio-1D V.97 software (Vilber lourmat, France).

2.11. Immunoprecipitation

Immunoprecipitation was used to detect protein–protein interaction between IF1 and F₁-ATPase α- and β-subunit. For immunoprecipitation, 200 µg of sub mitochondrial particles were mixed with 20 µg of F₁-ATPase β-subunit monoclonal antibody (Abcam Inc., Cambridge, UK) and incubated with gentle rocking at 4 °C overnight. An equal amount of Protein A/G-agarose (Calbiochem, Darmstadt, Germany) was added and rocked for additional 1 h at 4 °C. Precipitated complexes were centrifuged and washed four times in immunoprecipitation binding buffer [50 mM Tris-base, 150 mM NaCl, 5 mM EDTA, pH 7.0, 0.1% NP-40, 1-fold protease-inhibitory-cocktail (Roche, Mannheim, Germany)], and then boiled in 2-fold SDS-sample buffer for 5 min. The precipitated complexes were centrifuged again and then the fractions of the supernatant were analyzed by 12% SDS-PAGE (for IF1 protein detection) or 8% SDS-PAGE (for individual F₁-ATPase α-, β-subunit protein detection), and then immunoblotted with indicated antibodies. The subunits of the F₁-ATPase were analyzed using Coomassie Brilliant Blue R-250 staining. Because only immunoprecipitated protein was added to each lane, no other proteins could be reliably detected on the gels. Submitochondrial particles from sham-operation and late septic liver containing identical amounts of proteins (as measured) were used for the immunoprecipitation. It was important to confirm that these submitochondrial particles were equivalent. To achieve that, we performed Western blotting of submitochondrial particles from sham-operation and late septic liver used for F₁-ATPase β-subunit immunoprecipitation. For this Western blotting, an anti-cytochrome c oxidase subunit IV (CoO IV) antibody was used. This control, then, provided evidence that equal amounts of CoO IV were present when a fixed amount of submitochondrial particles (as measured) were sampled for F₁-ATPase β-subunit immunoprecipitation. Relative density of each band was measured by computer-assisted densitometry and Bio-1D V.97 software (Vilber lourmat, France).

2.12. Statistical analysis

To obtain reaction rates, the linear range of the absorbance change with respect to time was used for both ATP synthase and ATP hydrolytic activity. A molar extinction coefficient of 6.22 × 10³ M cm⁻¹ was used for NADH to calculate the activity in μmolos ATP hydrolyzed or synthesized per min per mg protein. All data are expressed as mean ± SD. Statistical analysis was performed using Student’s t test. A value of p < 0.05 was considered significant difference.

3. Results

3.1. Ultrastructure abnormality of hepatic mitochondria in late sepsis

The hepatic mitochondrial ultrastructure was observed by transmission electron microscope. The quality of isolated mitochondria from rat liver was pure according to our method (Fig. 1A). In the sham-operated control group, the mitochondria was smooth with distinct cristae and complete membranes (Fig. 1C). At 18 h after CLP induction, mitochondrial swelling was accompanied by a disruption of outer membrane integrity and disarranged cristae (Fig. 1B, D). As observed previously [11], these changes were typical of mitochondrial injury of liver during sepsis.

3.2. Decrease of mitochondrial ATP content in septic liver

To determine mitochondrial dysfunction in late sepsis, intra-mitochondrial ATP content and ATP synthase activity of complex V were measured. The result showed that mitochondrial ATP content decreased significantly in late septic liver (Fig. 2A). While, no significant difference of ATP synthesis activity was observed between sham-operated and late septic livers (Fig. 2B).

3.3. IF1 inhibited the activity of ATP hydrolase in submitochondrial particles of septic liver

In order to elucidate the role of IF1 in inhibiting the activity of mitochondrial ATP hydrolase during sepsis, the ATP hydrolysis of septic liver MgATP-SMPs was determined without or with reconstitution with IF1–(Leu 42–Lys 58) synthetic peptide (Fig. 3). The result showed that the ATP hydrolysis in MgATP-
SMPs prepared from septic liver was significantly increased by 112% after CLP. Whereas, reconstitution of MgATP-SMPs with IF1 synthetic peptide reversed the increase of ATP hydrolysis caused by septic condition. It implies that the elevated ATP hydrolysis may contribute to the decrease of mitochondrial ATP content under septic condition. Furthermore, loss of IF1 inhibition on the ATP hydrolase activity of MgATP-SMPs from septic liver may contribute to the decrease of ATP content during sepsis.

3.4. IF1 mRNA expression

In order to confirm the previous result from differential expressed genes after suppression subtractive hybridization (SSH), steady state mRNA levels of IF1 in sham and late septic groups were compared by Northern blot analysis using 18S as a loading control (Fig. 4). The result showed that the mRNA expression of IF1 was significantly decreased by 46% in the late septic liver compared to the sham-operated group ($p<0.01$).

3.5. Declination of IF1 protein content in mitochondria at late sepsis

To investigate the precise level of IF1 in the mitochondria, the cytosolic and mitochondrial fractions were isolated and the purity of the two fractions was confirmed by cross-reacting with monoclonal antibodies directed against GAPDH and cytochrome $c$ oxidase subunit IV. The results showed that GAPDH and the cytochrome $c$ oxidase subunit IV protein were highly enriched in the cytosolic and mitochondrial fractions, respectively (Fig. 5). The presence of IF1 protein was determined in the mitochondria of both sham-operated and late septic livers.
The results shown in Fig. 5 reveal that the IF1 protein level significantly decreased in the late septic condition by 57% compared to the sham group \( (p < 0.05) \). Cytochrome c oxidase subunit IV (CcO IV) was used as a mitochondrial marker protein to normalize the levels of IF1. Under all treatment conditions, CcO IV protein expression was unaltered. To investigate the possible transporting of IF1, cytosolic and mitochondrial fractions were detected using Western blot analysis. In the upper panel of Fig. 5, it is shown, regardless of which fraction underwent sepsis, that the IF1 was only present in the mitochondria but not in the cytosolic fractions.

3.6. No change of \( \alpha \) and \( \beta \) subunit proteins of F\(_1\)-ATPase during sepsis

To explore the role of subunit proteins of F\(_1\)-ATPase contributing to the mitochondrial dysfunction, the F\(_1\)-ATPase \( \alpha \)- or \( \beta \)-subunits were quantified by Western blot analysis. The results showed that neither \( \alpha \)- nor \( \beta \)-subunit of F\(_1\)-ATPase was changed in the liver at late stage of sepsis (Fig. 6A and B).

3.7. Interaction of IF1 and F\(_1\)-ATPase \( \alpha/\beta \) subunit protein in liver of late sepsis

For further understanding of the interaction of IF1 with F\(_1\)-ATPase under septic conditions, submitochondrial particles were immunoprecipitated by using an antibody specific for F\(_1\)-ATPase \( \alpha \)- or \( \beta \)-subunits.
ATPase β-subunit. Then, the association of IF1 on F1-ATPase was identified by Western blotting analysis using antibodies of IF1 and α-, β-subunit proteins. Additionally, another Western blotting of submitochondrial particles with antibody against cytochrome c oxidase subunit IV (CcO IV) was performed, which used as a loading control in immunoprecipitation study (Fig. 7C). The subunit profile of F1F0 and other SMP proteins in this immunoprecipitation procedure is shown in Fig. 7A. The α, β, and δ subunits were individually detected as a single band except γ and ε subunits.

The result showed that the association of IF1 with F1-ATPase-β subunit was significantly decreased by 40.7% (p < 0.01) during late sepsis (Fig. 7B). Neither α- nor β-subunit of precipitated F1-ATPase changed significantly in the septic liver.

4. Discussion

The most common cause of death in critical sepsis is the dysfunction of multiple organs. Among the organ systems, hepatic failure is usually thought to be a late complication following pulmonary and renal failure in the cascade of sepsis [24]. The liver plays roles in four key areas of metabolism: fuel management, nitrogen excretion, the regulation of water distribution between the blood and tissues, and the detoxification of foreign substances. Besides, it is a major organ of synthesis of functional proteins, such as clotting factors, albumin. So far, the study of underlying pathophysiology of the sepsis-induced hepatic dysfunction is not conclusive. Many reports suggest that mitochondrial dysfunction with a progressive energy deficiency should be the reason for hepatic dysfunction [25,26]. Other studies have also discovered that intramitochondrial production of the hydroxyl radical results in local damage to lipid, DNA or...
protein components of the mitochondria in sepsis [27,28]. Furthermore, disruption of mitochondrial membrane has been reported to be an early and irreversible event leading to apoptosis, which may be important in human sepsis with systemic organ dysfunction [29]. From our previous and current studies, we demonstrated ATP content declined significantly in both total liver tissues and mitochondria. Besides, we also observed that mitochondrial ultrastructure was obviously damaged. It is clear that energy deficiency is present during sepsis that is highly associated with mitochondrial dysfunction.

The mitochondrial respiratory chain is located in the inner mitochondrial membrane and is composed of four individual enzyme complexes (I to IV). Electrons derived from oxidation of glucose or fatty acids are transferred through the complex I, III, and IV to carry protons from the mitochondrial matrix to the intermembrane space. An electrochemical proton gradient is then generated, which is utilized by the fifth enzyme complex (ATP synthase) to produce ATP from adenosine diphosphate (ADP) and inorganic phosphate. In fact, a number of sepsis-induced alterations in mitochondrial respiratory activity have been described, including complex I, II, III, and IV [10-12]. However, there are few studies of the role of complex V in the septic liver. ATP synthase consists of two main parts; a hydrophilic F₁-part carrying catalytic sites (F₁-ATPase) and a hydrophobic F₀-part that is involved in proton translocation. ATP synthase is responsible for the majority of ATP synthesis (~95% of energy needed) during normoxic conditions, but it is prone to carry out ATP hydrolysis under an ischemic situation, also termed as F₀F₁-ATPase.

Experimental evidence suggests that F₀F₁-ATPase is an important consumer of ATP during ischemia [12,14]. When oxygen deprivation collapses the mitochondrial electrochemical gradient, F₀F₁-ATPase subsequently switches from ATP synthesis to ATP hydrolysis. Oligomycin, a non-selective F₀F₁-ATPase inhibitor, is revealed to decrease the rate of ATP depletion in globally ischemic dog heart ex vivo [14]. Other studies further showed that the selective F₀F₁-ATPase hydrolase inhibitor, BMS-199264, reduced ATP declination during ischemia and exerted significantly cardioprotective effects without affecting ATP synthesis in normal or reperfused tissue [30]. It is clear that an increase of F₀F₁-ATPase activity plays a role in decreasing ATP concentration during certain pathological conditions. The current study obviously showed that the mitochondrial F₀F₁-ATPase activity is notably elevated in late septic liver, while the synthetic activity of ATP is not different between sham-operated and late septic rats. Eventually, the intramitochondrial ATP concentration reduced significantly in late septic liver. These results indicate that mitochondrial F₀F₁-ATPase participates in hepatic energy insufficiency in sepsis.

The activity of the proton-pumping F₀F₁-ATPase is regulated in vivo by different specific regulatory elements, such as ADP, a proton motive force (ΔμH⁺) generated by electrochemical gradient and IF1 inhibitor protein [31]. Among them, the IF1 plays a prominent role. IF1 was first isolated by Pullman and Monroy in 1963 as a powerful inhibitor of hydrolysis, and binds to F₀F₁-ATPase in a 1:1 stoichiometry [32]. Studies from Yamada and Huzel [33] showed that IF1 activity could not be measured in the GM28 fibroblasts cultured from the skeletal muscle of the patients of Luft’s disease, a rare human mitochondrial myopathy. Besides, Banerjee et al. made the important observation that inhibition of F₀F₁-ATPase and preservation of ATP is dependent on the concentration of IF1 under paracetamol-induced hepatotoxicity in rats [34]. Accordingly, we assume that the IF1 expression is probably a crucial factor in modulating the F₀F₁-ATPase activity during septic conditions. In our result, it ob-
viously revealed the addition of IF1-(Leu 42–Lys 58) synthetic peptide exerted a marked inhibitory effect on the rate of ATP hydrolysis in MgATP-SMPs of late septic liver. This result reflects the expression failure of IF1 might highly correlate with the elevated F0F1-ATPase activity in the liver of late sepsis. As expected, the key finding of this study showed that the mRNA as well as the mitochondrial protein content of IF1 decreased significantly pending late sepsis.

IF1 gene is a nuclear gene and the presequence is required for transport into the mitochondria [35]. Several reports have shown that IF1 binds at the β-subunit of the enzyme or at the interface between α- and β-subunits of F0F1-ATPase [36,37], and also in close proximity of the rotor subunits γ and ε [38], thus blocking catalytic conformational changes and rotation of the central stalk to inhibit futile ATP hydrolysis. In this study, we observed that there is no detectable IF1 protein in the cytosolic fraction regardless of sham or septic group. It implies that sepsis-associated declination of IF1 in the mitochondria is not due to the transporting failure. We further observed the association activity of mitochondrial IF1 with β-subunit significantly decreased in late sepsis, while the protein level of α- and β-subunits is not altered. However, compared with the IF1 content in the mitochondria and immunoprecipitated F1-ATPase complex, it seems reasonable to conclude that the association activity of IF1 protein is not affected. Therefore, downregulation of IF1 expression seems to be the key mechanism in energy deficiency of septic liver.

In conclusion, we suggest for the first time that the suppression of IF1 expression and subsequent elevated mitochondrial F0F1-ATPase activity might contribute to the bioenergetic failure in the liver during late sepsis. It is important to point out that the opposite effect of IF1 has been demonstrated during its overexpression in rat hepatoma as compared to rat liver. When IF1 is 2-fold overexpressed in hepatoma mitochondria, it is associated more efficiently with the mitochondrial ATP synthase, thus reducing the futile hydrolysis of mitochondrial and glycolytic ATP [39]. In summary, it seems that down-regulation or over-expression of IF1 controls the ATP content of cells in an opposite fashion and according to cellular ATP requirements. We hope that this novel result might highlight an original therapeutic aspect of energy preservation in managing sepsis.

Acknowledgements

This work was supported by a grant from the National Science Council (NSC 94-2321-B-037-004), Taiwan. We also thank Steve Tredrea [Foreign Language Training Center, National Sun Yat-Sen University, Kaohsiung, Taiwan] for his editing services.

References


