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ORIGINAL ARTICLE

Pre-treatment with FK506 reduces hepatic ischemia-reperfusion injury in rats

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KEYWORDS
FK506;
Hypoxia-inducible factor-1α;
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Hepatic ischemia-reperfusion injury;
Liver protection

Summary
Aim: The study is aimed to investigate the protective effects and possible mechanism of tacrolimus (FK506) pre-treatment in hepatic ischemia-reperfusion injury in rats.
Methods: The rats were randomly assigned into four groups, which were S, IR, L and H group, and then all groups were subjected to 60 min of 70% partial warm liver ischemia, except S group. Rats in the L and H group were pre-treated with two different doses FK506 at 60 min before ischemia. The rats of the IR group received an identical volume of normal saline. All animals were sacrificed after 6 h of reperfusion. Transaminases were measured by biochemistry analyzer. Elisa kit was used to detect TNF-α, IL-6 and IL-1β levels in serum. Liver specimens were stained with hematoxylin and eosin (HE) to assess the pathologic changes. The expressions of heme oxygenase-1 (HO-1), hypoxia-inducible factor-1α (HIF-1α), nuclear factor of activated T cells (NFAT3) were measured by real-time quantitative PCR and western blotting and the Bcl-2 and the Bax protein were tested by western blotting.
Results: In rats pre-treated with FK506, the levels of transaminases, TNF-α and IL-1β were reduced significantly and also liver damage was dramatically mitigated compared to those without FK506 pre-treatment. Moreover, the expression of HO-1 at the level of both transcription and translation increased clearly and the activation of the HIF-1α was found in FK506 pre-treated livers. Moreover, NFAT3 protein transportation to the nucleus was reduced and Bax protein expression was decreased, but the expression of Bcl-2 protein was markedly increased after FK506 pre-treatment.
Conclusion: FK506 pre-treatment could lessen hepatic ischemia-reperfusion injury through up-regulating the expression of HIF-1α and HO-1, and inhibiting nuclear translocation of NFAT3 in liver tissues.

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Introduction

Ischemia-reperfusion (IR) injury is regarded as a complex phenomenon of pathophysiology, which is a dynamic process including localized ischemic insult and inflammation-mediated reperfusion injury [1]. In other word, it can cause cellular damage to an organ, which is exposed to hypoxia in the ischemic period; the restoration of oxygen application can aggravate cellular damage after reperfusion [2—4].

Hypoxia-inducible factor-1 (HIF-1), as an oxygen-sensitive transcription factor and a master regulator of the cellular response to oxygen homeostasis, plays a vital role in hepatic IR injury. HIF-1 is a heterodimeric complex consisting of two subunits: HIF-1α and HIF-1β [5]. HIF-1α is extremely unstable under normoxia and will be degraded soon in cytoplasm [6]. In contrast, HIF-1β is stable and translocates from cytoplasm to nucleus where dimerizes with the constitutive HIF-1α. Subsequently, the heterodimer binds to hypoxia respone element of their promoter region of the target genes and initiates the regulation of related genes expression [6,7].

FK506 is an immunosuppressant extracted from *Streptomyces tsukubaensis* and widely used in solid organ transplant [8]. It reduces the transplant rejection and the IR injury [9]. Although some studies have elucidated the regulated ability of HIF-1 or its target transcriptional genes in IR injury, there has been no relative study published on the role of FK506 in the expression of HIF-1α or its target genes.

Nuclear factor of activated T cells (NFAT) is a family of transcription factors initially discovered in T cells [10]. Their activation depends on the calcium/calmodulin-signalling pathway [11]. When the cells receive stimulus, the calcium concentration can increase and then combines with calmodulin. The calcium/calmodulin can activate calcineurin. Activated calcineurin dephosphorylates NFAT, leading to its nuclear translocation and enhancing its binding to consensus DNA sites. Subsequently, NFAT initiates the regulation of related genes expression [12]. The NFAT signalling is involved in the regulation of cell growth and development in a variety of different tissues and cells [13,14]. NFAT3 is one of the five known isoforms in this family, which is known to be involved in gene expression, linking surface receptor stimulation to nuclear events, and altering the fate of the cell. Some studies have found that NFAT3 is expressed in non-immune tissues, like heart, brain and breast [15,16]. There are a few studies involving NFAT3 in liver, but the specific role of NFAT3 signalling in hepatic IR injury was not reported so far.

Therefore, we intend to discuss the effect of FK506 pre-treatment on hepatic ischemia-reperfusion injury in this study. And we also discuss the relationship between FK506 pre-treatment and the expression of HIF-1α and NFAT3 in order to explore the possible mechanism of FK506-mediated liver protection in rats following hepatic IR.

Materials and methods

Animals and experimental groups

Male Sprague-Dawley rats (220—250 g) were purchased from HFK Bioscience Co. Ltd in this study. Rats had access to water and a standard diet ad libitum and were randomly divided into four groups:

- sham-operated group (S, $n = 8$), the abdomen was only opened and closed;
- ischemia-reperfusion group (IR, $n = 8$), only normal saline was given via dorsal penile vein at 60 min before setting up the IR model;
- low-dose FK506 + ischemia-reperfusion group (L, $n = 8$), preoperative treatment with FK506 (0.3 mg/kg) [17] was administered via dorsal penile vein at 60 min before inducing the IR model;
- high-dose FK506 + ischemia-reperfusion group (H, $n = 8$), the dorsal penile vein injection of FK506 (1.0 mg/kg) [18] was administered at 60 min before making the IR model.

Surgical procedure

A rat model of 70% partial warm liver IR was used. The rat was anesthetized by abdominal injection of pentobarbital. The abdomen was opened at the median line. The ligament attachments connecting the liver, diaphragm, abdominal wall and neighbouring organs were divided. The hepatic artery and portal vein should be found and separated. Using a vascular microclamp interrupted the portal venous and arterial hepatic blood supply to the cephalad three lobes of the liver. The reperfusion was initiated after 60 min of ischemia by removal of the clamp. After complete reperfusion and return of the reddish colour of the liver surface, the abdominal cavity was meticulously inspected for bleeding. Finally, the abdominal cavity was closed. After 6 h of reperfusion, blood samples were taken and the serum transaminases levels were determined. Subsequently, the liver tissues were harvested for further examination, and all rats were sacrificed after operation.

Determining the sampling time

Undergoing 60 min of warm ischemia and 3, 6, 12 and 24 h of reperfusion, we detected the levels of serum ALT and AST as indicators of hepatocellular injury and made the sampling time.

Hepatocellular damage

The extent of hepatocellular damage was assessed by the serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) using a biochemistry analyzer (Cobas-Mira Plus, Roche, Manheim, Germany).

Detection of serum TNF-α, IL-6 and IL-1β

TNF-α, IL-6 and IL-1β levels in serum were measured using Elisa kit (R&D system Inc, MN, USA) as described in the manufacturer's instructions.

Histology

Liver tissues were fixed in 10% paraformaldehyde, embedded in paraffin, sectioned at 4 μm, and then stained with

Table 1 Suzuki's histological criteria for the assessment of hepatic damage.

Score	Congestion	Vacuolization	Necrosis
0	None	None	None
1	Minimal (0—10%)	Minimal (0—10%)	Minimal (0—10%)
2	Mild (11—30%)	Mild (11—30%)	Mild (11—30%)
3	Moderate (31—60%)	Moderate (31—60%)	Moderate (31—60%)
4	Severe (> 60%)	Severe (> 60%)	Severe (> 60%)

haematoxylin and eosin (HE). The degree of hepatic damage after IR was assessed by Suzuki's histological criteria (Table 1).

RT-q PCR analysis

Total RNA was extracted from the frozen liver tissues with Trizol reagent (Takara Bio Inc., Tokyo, Japan). One μg of RNA was reverse-transcribed into cDNA using oligo-dT primers with a Superscript III First-Strand Synthesis System (Invitrogen). RT-qPCR was performed with iCycler IQ system (Bio-Rad, Hercules, CA). The primer sequences were as follows:

- HO-1: sense primer 5′-ACGCATATACCCGCTACCTG-3′;
- antisense primer 5′-TGCTGATCTGGGATTTTCCT-3′,
- HIF-1α: sense primer 5′-GTCGGACAGCCTCACCAAACAG-3′;
- antisense primer 5′-TAGGTAGTGAGCCACCAGT-3′,
- NFAT3: sense primer 5′-TCTTCAGGACCTCTGCCCTA-3′;
- antisense primer 5′-AGCCTAGGAGCTTGACCACA-3′;
- β-actin: sense 5′- CGGAACCGCTCATTGCC-3′;
- antisense 5′- ACCCACACTGTGCCCATCTA-3′.

The cycling conditions comprised 5 min polymerase activation at 95°C, 40 cycles of 95°C for 5 s and 60°C for 30 s and a single fluorescence measurement. Each expression gene was normalized with β-actin mRNA using a Delta-Delta CT method.

Western blotting

The proteins were extracted from the frozen liver tissues and quantified using a protein assay (Bio-Rad Laboratories, CA). The protein samples (80 μg) were fractionated by SDS-PAGE and transferred to a nitrocellulose membrane. The membrane was blocked in 5% non-fat dry milk for 1 h. Immunoblotting was conducted overnight at 4°C using antibodies against HIF-1α (Novus Bio, USA), NFAT3 (Santa Cruz Biotechnology, Inc., USA), HO-1 (Abcam, NV, USA), Bax and Bcl-2 (Cell Signaling Technology Inc., USA). Using the IgG-HRP secondary antibody (ZSGB Bio, Beijing, China) incubated the membrane for 1 h at room temperature. Each protein expression was visualized via the ECL detection system (Pierce ECL Substrate Western blot detection system, Thermo Scientific, IL).

Statistical analysis

All data are presented as mean ± SD. Statistical analysis was performed using analysis of variance and then the data were analyzed for significance via Student's *t*-tests using Graph Pad Prism software (Version 5.0; Graph Pad, San Diego, CA). $P < 0.05$ was considered to be statistically significant.

Results

Detecting serum ALT and AST and determining the sampling time

After 60 min of warm ischemia, we detected the levels of serum ALT and AST in different reperfusion time. The results showed that the serum ALT and AST began to rise after 3 h of reperfusion, reached the peak at 6 h, and reduced gradually at 12 h and 24 h. The levels of ALT and AST were significantly higher at 6 h of reperfusion than them at 3 h, 12 h and 24 h of reperfusion ($P < 0.05$; Fig. 1).

Effect of FK506 on liver damage

After 1 h of warm ischemia and 6 h of reperfusion, the levels of serum ALT and AST in the IR group were significantly higher compared with the S group. Pre-treatment with FK506 at a concentration of 0.3 mg/kg and 1.0 mg/kg caused significant reduction of ALT and AST when compared to the IR group ($P < 0.01$; Fig. 2). There was no significant difference in transaminase levels between the L group and the H group.

Effect of FK506 on inflammatory cytokines

Inflammatory cytokines play a vital role in the pathophysiology of the hepatic IR injury. We used Elisa kit to measure the levels of TNF-α, IL-6 and IL-1β in the serum. TNF-α and IL-1β were markedly lower in the L group and the H group, as compared to the IR group after 6 h of reperfusion ($P < 0.05$;

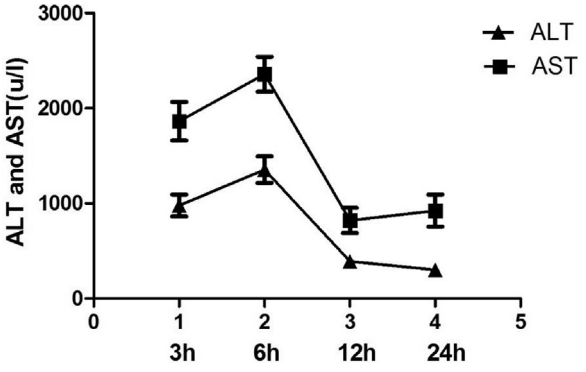


Figure 1 The levels of ALT and AST in different reperfusion time after 60 min of warm ischemia. The levels of ALT and AST were detected at 3 h, 6 h, 12 h and 24 h of reperfusion. The serum ALT and AST began to rise after 3 h of reperfusion, reached the peak at 6 h, and reduced gradually at 12 h and 24 h. Compared with 6 h of reperfusion, the levels of ALT and AST were significantly lower after 12 h and 24 h of reperfusion ($P < 0.05$).

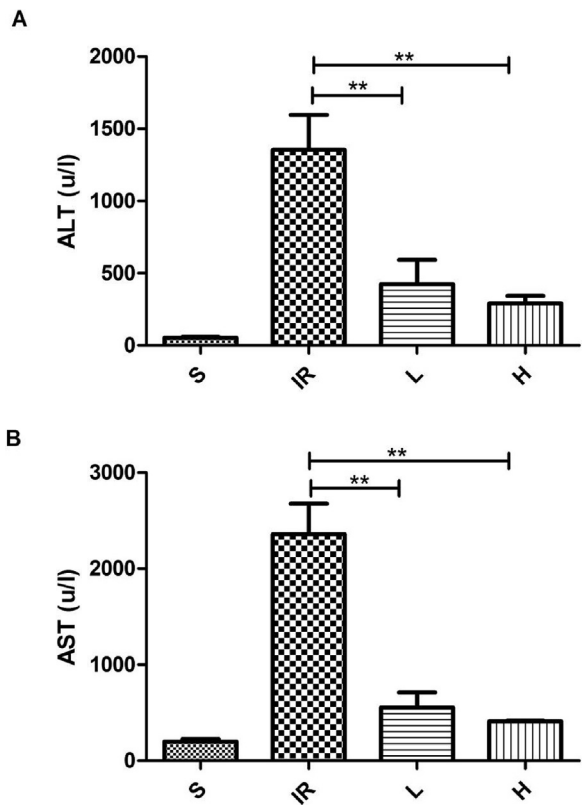


Figure 2 Alteration of serum levels of ALT and AST after the IR. The levels of ALT and AST in the L group and the H group were lower than the IR group after reperfusion. There was no significant difference between the L group and the H group in ALT and AST levels after reperfusion (**$P < 0.01$).

Fig. 3A, 3C). IL-6 was only decreased slightly in the L group ($P > 0.05$; Fig. 3B), but it was significantly inhibited in the H group ($P < 0.05$; Fig. 3B). The difference of each factor was not significant in the L group and the H group.

Effect of FK506 on liver histology

In the S group, the hepatic tissue structure was normal. The liver in the IR group showed hepatic sinusoids congestion, neutrophil infiltration and necrosis. Many hepatocytes were swollen and vacuolated. In contrast, tissue damage of the L group and the H group following FK506 treatment was significantly decreased and necrosis was less detected (Fig. 4). The degree of IR injury was quantified using Suzuki's histological criteria. The results showed that significant differences were noted in congestion, vacuolization and necrosis between the IR group and the L and H groups ($P < 0.05$; Table 2).

Effects of FK506 on HIF-1α, HO-1 and NFAT3 mRNA expression

When the rats underwent IR, quantitative analysis of the liver HIF-1α, HO-1 and NFAT3 mRNA was performed by real-time RT-PCR. The HIF-1α mRNA was not detected. The expression of HO-1 mRNA was greatly increased in the L group compared with the IR group after 6 h of reperfusion

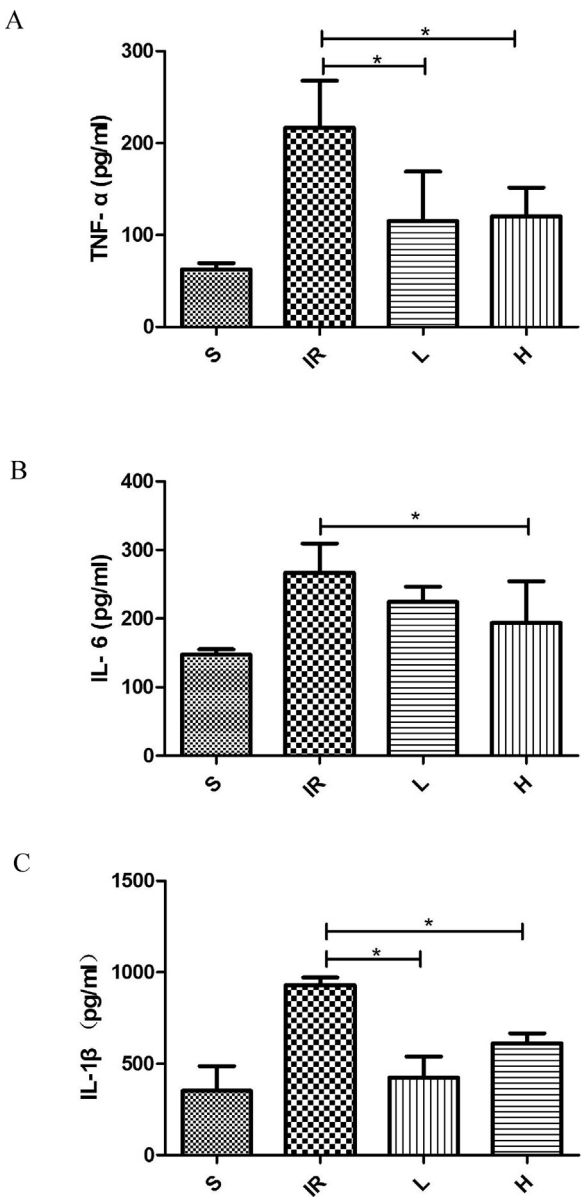


Figure 3 Alteration of serum levels of TNF-α, IL-6 and IL-β after the IR. A. The levels of TNF-α in the L group and the H group were lower than the IR group after reperfusion (*$P < 0.05$). B. IL-6 was only decreased slightly in the L group ($P > 0.05$), but its expression was significantly inhibited in the H group (*$P < 0.05$). C. The levels of IL-1β were significantly decreased in the L group and the H group compared with the IR group (*$P < 0.05$).

($P < 0.05$; Fig. 5) and widely inhibited in the H group. The level of NFAT3 transcript in the IR group was lower than in the L group and the H group ($P < 0.05$; Fig. 6). The difference was not significant between the L group and the H group.

Effects of FK506 on HIF-1α, HO-1, NFAT3, Bax and Bcl-2 protein

The activation of the HIF-1α, HO-1, NFAT3, Bax and Bcl-2 was measured using western blotting analysis. In the

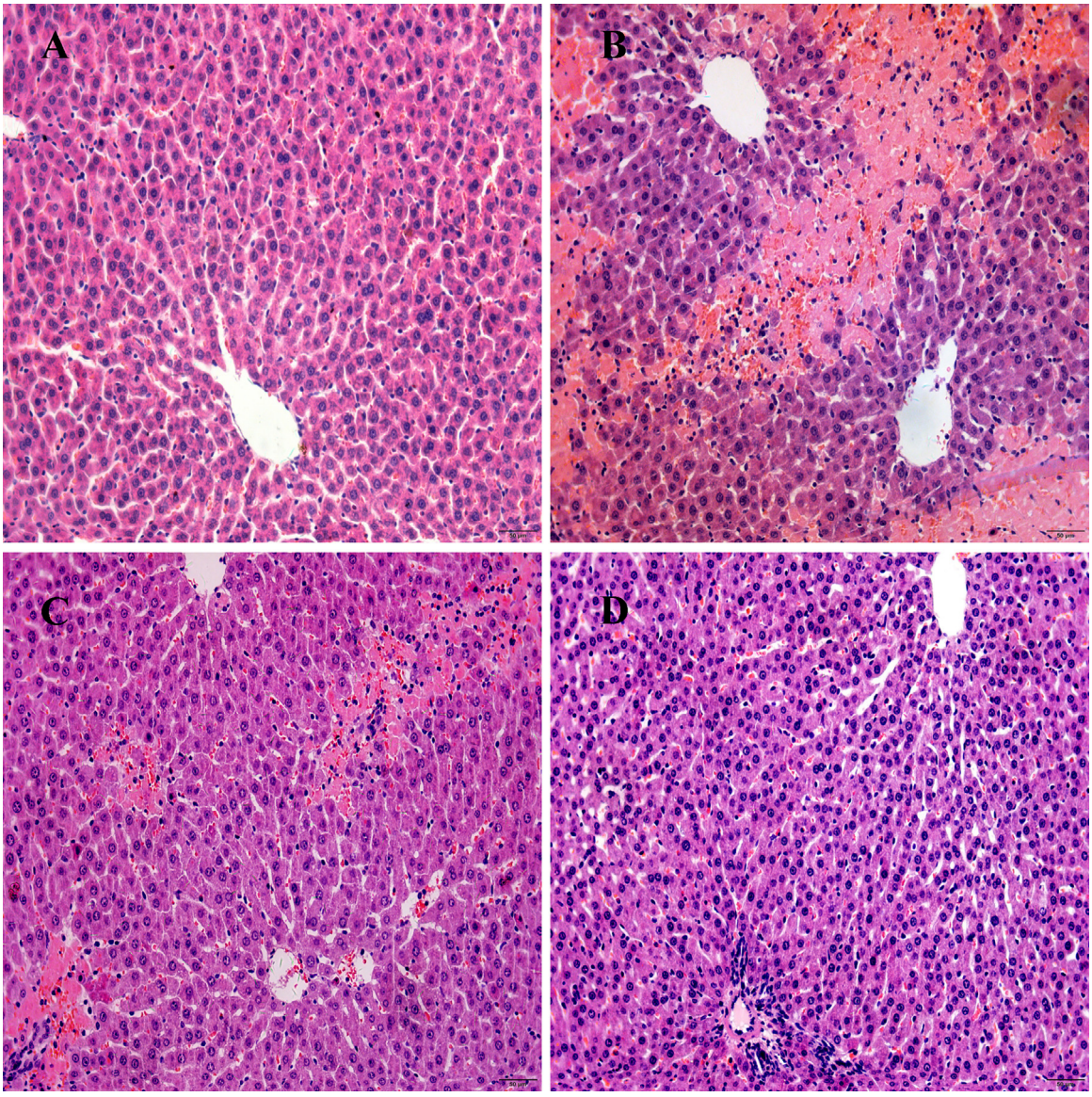


Figure 4 Representative hematoxylin and eosin staining of the livers after the IR (original magnification × 200). An example of microphotograph from (A) the S group, (B) the IR group, (C) the L group, (D) the H group.

Table 2 Suzuki's of each group (mean ± SD).

Group	N	Congestion	Vacuolization	Necrosis	Aggregate score
S	8	0	0	0	0
IR	8	2.00 ± 0.38	2.38 ± 0.42	3.25 ± 0.25	7.25 ± 0.56
L	8	1.00 ± 0.27[a]	1.13 ± 0.35[a]	1.38 ± 0.18[a]	3.5 ± 0.27[a]
H	8	0.75 ± 0.25[a]	0.88 ± 0.23[a]	1.25 ± 0.16[a]	2.88 ± 0.52[a]

[a] Compared with IR group, $P < 0.05$.

S group, the expression of HIF-1α was very little. After 6 h of reperfusion, the activation of HIF-1α was slightly increased in the IR group. In the L group, pre-treatment with low-dose FK506 clearly enhanced the expression of HIF-1α. However, there was little HIF-1α protein in the H group (Fig. 7A). The expression of HO-1 was significantly increased in the L group compared with the IR group after 1 h of warm ischemia and 6 h of reperfusion. In contrast, the levels of HO-1 were not markedly changed between the L group and the H group (Fig. 7B). To investigate the role of NFAT3 signalling in hepatic ischemia-reperfusion injury, we detected the levels of NFAT3, Bax and Bcl-2 in IR model. The results showed that the activation of NFAT3 and Bcl-2 underwent IR were inhibited partially compared with the

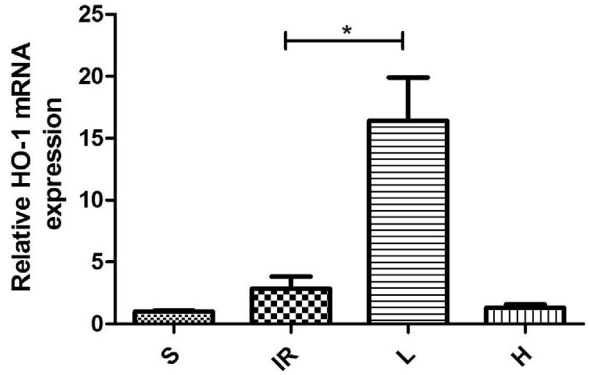


Figure 5 Alteration of the relative expression of HO-1 mRNA after the IR. The expression of HO-1 mRNA was greatly increased in the L group compared with the IR group after 6 h of reperfusion (*$P < 0.05$). The high-dose FK506 couldn't improve the expression of HO-1 mRNA in the H group after reperfusion.

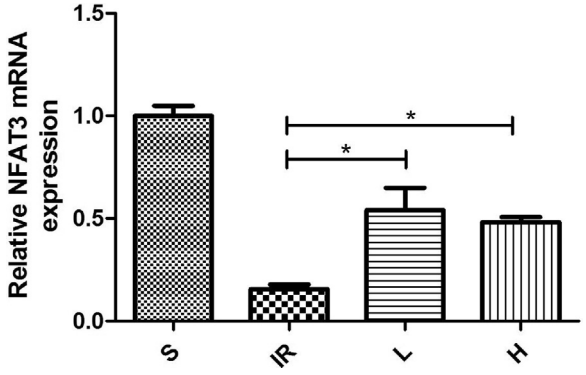
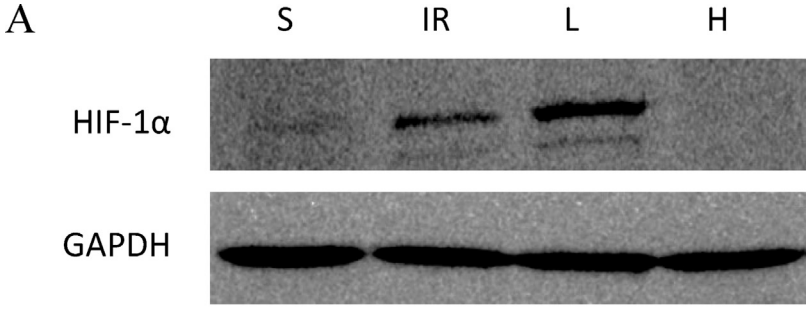


Figure 6 Alteration of the relative expression of NFAT3 mRNA after the IR. The expression of NFAT3 mRNA was significantly increased in the L group and the H group compared with the IR group after 6 h of reperfusion (*$P < 0.05$). However, the difference was not significant in the L group and the H group ($P > 0.05$).

S group. Pre-treatment with FK506 significantly enhanced the expression of NFAT3 and Bcl-2 (Fig. 8A, 8B). For Bax, the trend was just the opposite compared with the Bcl-2 (Fig. 8B).

Discussion

The hepatic IR injury is a common tissue and organ damage, which is a vital indicator that affects the liver function after



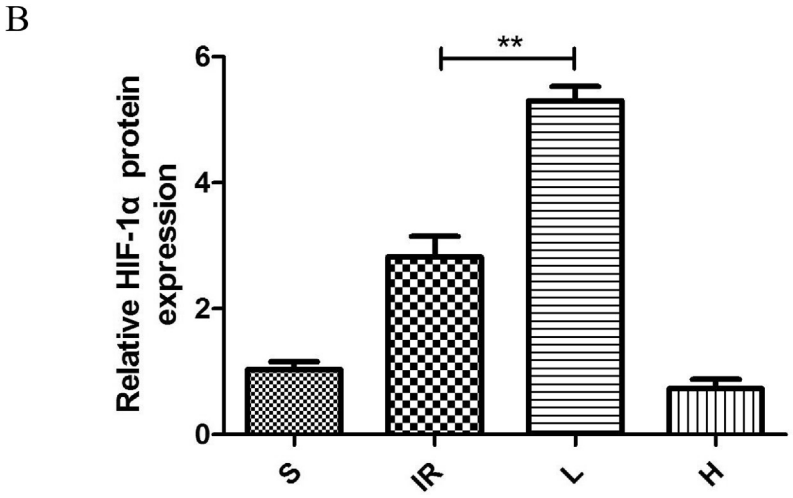


Figure 7 Alteration of the expression of HIF-1α and HO-1 protein after the IR. After 6 h of reperfusion, the expression of HIF-1α protein was slightly increased in the IR group and clearly enhanced by FK506 pre-treatment in the L group. However, there was little HIF-1α protein in the H group. The expression of HO-1 protein was significantly increased in the L group compared with the IR group after 6 h of reperfusion. In contrast, the levels of HO-1 were not markedly changed between the L group and the H group.

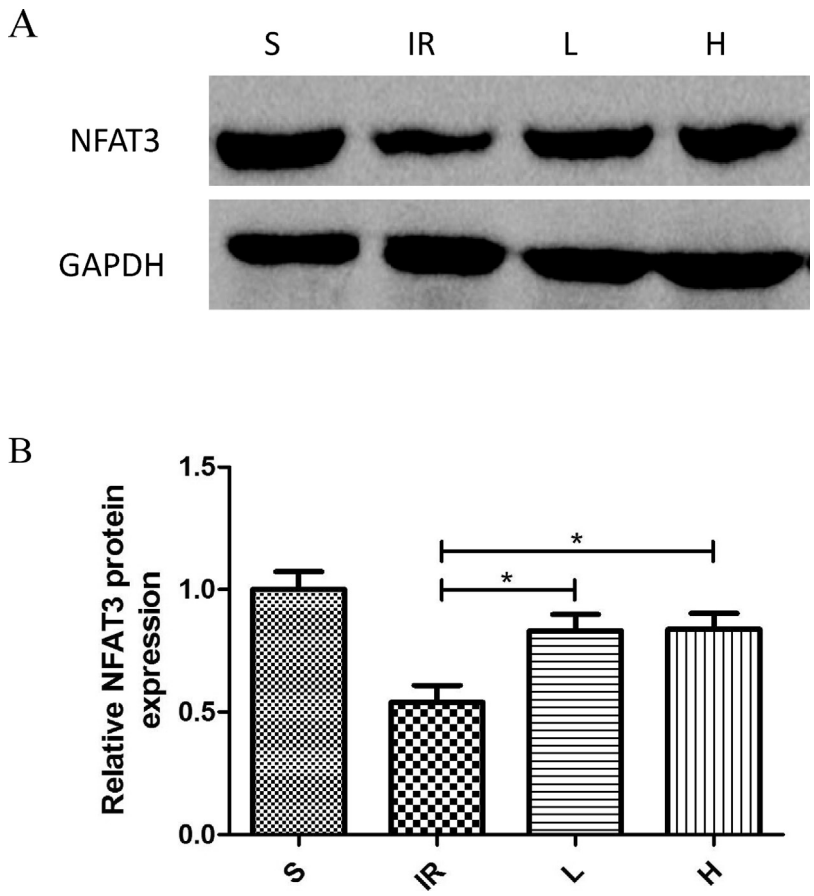


Figure 8 Alteration of the expression of NFAT3, Bcl-2 and Bax protein after the IR. The activation of NFAT3 and Bcl-2 underwent IR were inhibited partially compared with the S group. Pre-treatment with FK506 significantly enhanced the expression of NFAT3 and Bcl-2 after 6 h of reperfusion. For Bax, the trend was just the opposite compared with the NFAT3 and Bcl-2.

liver transplantation or hepatectomy [1,19,20]. The hepatic IR injury can decrease the liver detoxication, increase the microcirculation power and even lead to liver failure, which directly influences the prognosis of disease, success rate of surgery, and patient survival [21,22]. Therefore, how to protect ischemic hepatocytes and reduce reperfusion injury has become a clinical subject that needs to be solved now.

During the ischemic period, the reduction of blood flow for the liver exposes the tissue or cell to hypoxia. At this time, the liver is adapted to hypoxia through a variety of physiological response and then initiates the transcription of related genes to maintain oxygen supply. HIF-1α is an important component of the major hypoxia-sensing mechanism in eukaryotic cells and its expression can adapt cell to hypoxia and thus play a protective role. Although there are a few studies concerning the role of HIF-1α in hepatic IR injury [23—25], including anti-inflammatory and anti-necrosis, the correlation between FK506 pre-treatment and HIF-1α during the IR is unknown.

Studies have demonstrated that FK506 could attenuate hepatic injury caused by IR. Treatment with FK506 prior to 1 h of warm ischemia dramatically decreased the serum ALT and AST levels following 6 h of reperfusion. In this study, we also got the same results that FK506 pre-treatment decreased the serum ALT and AST expression and thus reduced hepatocellular damage in hepatic IR injury. Furthermore, some evidences have revealed that the inflammatory response is a major cause for liver damages after IR [26,27]. Our present study demonstrated that the levels of TNF-α, IL-6 and IL-1β were clearly decreased in FK506-treated group compared with those in IR group, which suggested that the protective effects of FK506 were realized by the inhibition of inflammatory response. Consistently, the liver damages, such as hepatic sinusoids congestion, neutrophil infiltration and laminar necrosis, were markedly alleviated in animal livers with FK506 pre-treatment after IR.

Ben et al. have proved that the activation of HIF-1α signalling could protect liver from IR injury by decreasing mitochondrial damage, lipid peroxydation and apoptosis [28]. Recent studies also demonstrated that the activation of HIF-1α signalling decreased hepatic oxidative stress, inflammation, apoptosis and necrosis and maintained hepatocyte survival in rats accompanied by IR [25,29]. In clinical trial, Amador et al. found that the survival of graft liver was associated with markedly upregulated HIF-1α level [30]. Kim et al. revealed that FK506 could preserve HIF-1 expression and transcriptional activity, and the cellular adaptation to hypoxia. Thus, FK506 could be considered as a good immunosuppressive agent that would not interfere with organs survival under hypoxic conditions [31,32]. However, there is no study demonstrating whether the effect of FK506

on hepatocyte is associated with HIF-1 expression in hepatic IR model. So in order to explore the effect of FK506 pre-treatment on HIF-1α expression in rat after IR injury, we detected the expression of HIF-1α through real-time quantitative PCR and western blotting analysis. The HIF-1α mRNA was not detected, which the reason may be that HIF-1α is extremely unstable under normoxia condition and will be degraded soon in cytoplasm [6]. Although there was no valid data presented in mRNA, HIF-1α protein was significantly upregulated following reperfusion in low-dose FK506-treated group compared with the untreated group. Combined with the literatures reported, we thought that FK506 could preserve HIF-1α-mediated hypoxic adaptation in hepatic IR. It's a pity that the high-dose FK506 didn't play a promoting role in HIF-1α expression.

Heme oxygenase-1 (HO-1), a target gene of the HIF-1, has been previously indicated to have the protective property for liver against IR injury [33,34]. The over-expression of HO-1 can improve liver function and decrease the inflammatory cell infiltration. Likewise, the hepatic IR injury subjected to extended cold ischemia could be also decreased via over-expression of HO-1 [35]. Wang et al. found that induction of HO-1 in vivo could improve liver tolerance to IR including anti-inflammatory, antioxidant and maintenance of the microcirculation [36]. Zhong et al. found that the upregulation of HIF-1α and HO-1 protein dramatically decreased cell necrosis and prevented liver damage after hepatic IR [24]. Another study also indicated that HIF-1α accumulation increased the expression of HO-1, and improved the liver protection against IR injury [37]. In addition, HO-1 plays an obvious role in promoting the regeneration of impaired liver tissue [38]. In our study, the level of HO-1 mRNA in the low-dose FK506-treated group was higher than in the IR group. But high-dose FK506 treatment didn't show promoted effect on HO-1 mRNA. Western blotting analysis indicated that the expression of HO-1 protein was increased in rats with FK506 pre-treatment compared with untreated groups, but it was slightly elevated in the H group. Previous studies discovered HO-1 over-expression could inhibit the release of TNF-α and IL-6, and reduce inflammatory cells infiltration, which ultimately reduced the liver damage [39,18,40]. Combining with the expression of TNF-α and IL-6 we tested and histological findings, we speculated that FK506 played protective roles by increasing expression of HO-1.

The major side effects of FK506 in the clinical patients were toxicity for the liver, kidneys, heart and pancreas, seriously leading to life-threatening episodes [18,41]. Because the therapeutic window of FK506 was particularly narrow in clinic, the dose of using FK506 was in as low as possible in order to reduce its toxicity. In our experiment, FK506 at doses of 0.3 and 1.0 mg/kg can all achieve therapeutic effect, but the expressions of HIF-1α and HO-1 were not improved further in rats with high-dose FK506 treatment, which maybe they didn't have dose-dependence on FK506, and related to the toxicity of FK506 for liver.

Previous studies have found that FK506 could protect hepatocytes from injury induced by IR through induction of infiltrating T-lymphocyte apoptosis or directly making hepatocytes resist to damage [42—44]. In addition, the calcineurin inhibitor, FK506, has been shown to inactivate calcineurin, repress NFAT translocation, and suppress T cell activation [45]. So in this study, we used FK506 as an inhibitor of NFAT3 translocation to explore the role of NFAT3 signalling in IR injury. The mRNA analysis of NFAT3 displayed a significant decrease in the IR group compared with the S group. However, the expression of NFAT3 mRNA was markedly increased in FK506-treated rats after reperfusion. Consistently, western blotting analysis showed that the expression of NFAT3 protein in the cytoplasm of IR group was less than that in the S group. Pre-treatment with FK506 significantly enhanced the expression of NFAT3 protein in the cytoplasm after reperfusion. These results indicated that FK506 pre-treatment blocked NFAT3 into nucleus, leading to NFAT3 accumulation in cytoplasm. And the inhibition of nuclear translocation of NFAT3 might block the transcription of its target gene. Rao et al. have demonstrated that NFAT proteins binding to promoter regions could upregulate the expression of some factors, such as TNF-α [11]. In our study, the TNF-α expression in FK506-treated rats was obviously decreased compared with FK506-untreated rats. Studies have showed that the level of TNF-α could reflect the IR associated damage. Thus, we anticipated that NFAT3 represented a putative injury suppressing mechanism and the inhibition of nuclear translocation of NFAT3 reversed the impaired mechanism from IR because of the application of FK506.

Recently, studies have revealed that NFAT3 have an inhibitory effect on the activation of Bcl-2/Bax in renal tubular cell damage [46], and FK506 maintain the level of Bcl-2 after liver transplantation [44]. Based on these evidences, we detected the expression of Bcl-2 and Bax protein after reperfusion. We found that Bcl-2/Bax activation was dramatically decreased in liver subjected to IR. But the application of FK506 reversed Bcl-2/Bax activation. In this regard, we inferred that the expression of Bcl-2, Bax and NFAT3 might be correlated, which might be the reduction of nuclear translocation of NFAT3 increased the expression of Bcl-2 and inhibited the expression of Bax. Therefore, FK506 pre-treatment inhibited the nuclear translocation of NFAT3, and improved Bcl-2/Bax activation and ultimately reduced liver injury.

Conclusion

FK506 pre-treatment significantly reduced inflammatory response and hepatocellular damage after IR by the upregulation of HIF-1α and HO-1, and the inhibition of nuclear translocation of NFAT3 in rats. Thus, FK506 might be a useful medicine to decrease or eliminate injury caused by IR in liver transplantation and surgery.

Disclosure of interest

The authors declare that they have no competing interest.

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References

[1] Papadopoulos D, Siempis T, Theodorakou E, Tsoulfas G. Hepatic ischemia and reperfusion injury and trauma: current concepts. Arch Trauma Res 2013;2:63—70.

[2] Massip-Salcedo M, Roselló-Catafau J, Prieto J, Avíla MA, Peralta C. The response of the hepatocyte to ischemia. Liver Int 2007;27:6—16.

[3] de Groot H, Rauen U. Ischemia-reperfusion injury: processes in pathogenetic networks: a review. Transplant Proc 2007;39:481—4.

[4] Jaeschke H. Molecular mechanisms of hepatic ischemia-reperfusion injury and preconditioning. Am J Physiol Gastrointest Liver Physiol 2003;284:G15—26.

[5] Schofield CJ, Ratcliffe PJ. Oxygen sensing by HIF hydroxylases. Nat Rev Mol Cell Biol 2004;5:343—54.

[6] Kaelin WG. Proline hydroxylation and gene expression. Ann Rev Biochem 2005;74:115—28.

[7] Depping R, Steinhoff A, Schindler SG, Friedrich B, Fagerlund R, Metzen E, et al. Nuclear translocation of hypoxia-inducible factors (HIFs): involvement of the classical importin alpha/beta pathway. Biochim Biophys Acta 2008;1783:394—404.

[8] Clipstone NA, Crabtree GR. Identification of calcineurin as a key signalling enzyme in T-lymphocyte activation. Nature 1992;357:695—7.

[9] St PS, Moss AA, Mulligan DC. Effects of tacrolimus on ischemia-reperfusion injury. Liver Transpl 2003;9:105—16.

[10] Shaw JP, Utz PJ, Durand DB, Toole JJ, Emmel EA, Crabtree GR. Identification of a putative regulator of early T cell activation genes. Science 1988;241:202—5.

[11] Rao A, Luo C, Hogan PG. Transcription factors of the NFAT family: regulation and function. Ann Rev Immunol 1997;15:707—47.

[12] Crabtree GR, Schreiber SL. SnapShot: Ca2 + -calcineurin-NFAT signaling. Cell 2009;138:210—1.

[13] Hogan PG, Chen L, Nardone J, Rao A. Transcriptional regulation by calcium, calcineurin, and NFAT. Genes Dev 2003;17:2205—32.

[14] Horsley V, Pavlath GK. NFAT: ubiquitous regulator of cell differentiation and adaptation. J Cell Biol 2002;156:771—4.

[15] Hoey T, Sun YL, Williamson K, Xu X. Isolation of two new members of the NF-AT gene family and functional characterization of the NF-AT proteins. Immunity 1995;2:461—72.

[16] Lyakh L, Ghosh P, Rice NR. Expression of NFAT-family proteins in normal human T cells. Mol Cell Biol 1997;17:2475—84.

[17] Sakr MF, Zetti GM, Hassanein TI, Farghali H, Nalesnik MA, Gavaler JS, et al. FK 506 ameliorates the hepatic injury associated with ischemia and reperfusion in rats. Hepatology 1991;13:947—51.

[18] Tamura A, Li XK, Funeshima N, Enosawa S, Amemiya H, Kitajima M, et al. Immunosuppressive therapy using FTY720 combined with tacrolimus in rat liver transplantation. Surgery 2000;127:47—54.

[19] Tashiro H, Kuroda S, Mikuriya Y, Ohdan H. Ischemia-reperfusion injury in patients with fatty liver and the clinical impact of steatotic liver on hepatic surgery. Surg Today 2014;44:1611—25.

[20] Gehrau RC, Mas VR, Dumur CI, Ladie DE, Suh JL, Luebbert S, et al. Regulation of molecular pathways in ischemia-reperfusion injury after liver transplantation. Transplantation 2013;96:926—34.

[21] Elias-Miro M, Jimenez-Castro MB, Rodes J, Peralta C. Current knowledge on oxidative stress in hepatic ischemia/reperfusion. Free Radic Res 2013;47:555—68.

[22] Zhai Y, Petrowsky H, Hong JC, Busuttil RW, Kupiec-Weglinski JW. Ischaemia-reperfusion injury in liver transplantation — from bench to bedside. Nat Rev Gastroenterol Hepatol 2013;10:79—89.

[23] Alchera E, Tacchini L, Imarisio C, Dal Ponte C, De Ponti C, Gammella E, et al. Adenosine-dependent activation of hypoxia-inducible factor-1 induces late preconditioning in liver cells. Hepatology 2008;48:230—9.

[24] Zhong Z, Ramshesh VK, Rehman H, Currin RT, Sridharan V, Theruvath TP, et al. Activation of the oxygen-sensing signal cascade prevents mitochondrial injury after mouse liver ischemia-reperfusion. Am J Physiol Gastrointest Liver Physiol 2008;295:G823—32.

[25] Lehwald N, Tao GZ, Jang KY, Sorkin M, Knoefel WT, Sylvester KG. Wnt-beta-catenin signaling protects against hepatic ischemia and reperfusion injury in mice. Gastroenterology 2011;141:707—18 [711—18].

[26] Soares MP, Brouard S, Smith RN, Bach FH. Heme oxygenase-1, a protective gene that prevents the rejection of transplanted organs. Immunol Rev 2001;184:275—85.

[27] Wagener FA, Volk HD, Willis D, Abraham NG, Soares MP, Adema GJ, et al. Different faces of the heme-heme oxygenase system in inflammation. Pharmacol Rev 2003;55:551—71.

[28] Ben MI, Mouchel Y, Pajaud J, Ribault C, Lucas C, Laurent A, et al. Pretreatment with mangafodipir improves liver graft tolerance to ischemia/reperfusion injury in rat. PLoS One 2012;7:e50235.

[29] Yang YY, Lee PC, Huang YT, Lee WP, Kuo YJ, Lee KC, et al. Involvement of the HIF-1α and Wnt/β-catenin pathways in the protective effects of losartan on fatty liver graft with ischaemia/reperfusion injury. Clin Sci 2014;126:163—74.

[30] Amador A, Grande L, Martí J, Deulofeu R, Miquel R, Solá A, et al. Ischemic pre-conditioning in deceased donor liver transplantation: a prospective randomized clinical trial. Am J Transplant 2007;7:2180—9.

[31] Kim KE, Jung YJ, Yeo EJ, Chun YS, Park JW. FK506: An immunosuppressive agent preserving HIF-1 activity. Immunopharmacol Immunotoxicol 2006;28:609—20.

[32] Kim KE, Jung YJ, Li SH, Chun YS, Ahn C, Park JW. Cloning of miniature pig HIF-1alpha and its responses to immunosuppressive agents. Immunopharmacol Immunotoxicol 2008;30:105—15.

[33] Amersi F, Buelow R, Kato H, Ke B, Coito AJ, Shen XD, et al. Upregulation of heme oxygenase-1 protects genetically fat Zucker rat livers from ischemia/reperfusion injury. J Clin Invest 1999;104:1631—9.

[34] Redaelli CA, Tian YH, Schaffner T, Ledermann M, Baer HU, Dufour JF. Extended preservation of rat liver graft by induction of heme oxygenase-1. Hepatology 2002;35:1082—92.

[35] Kato H, Amersi F, Buelow R, Melinek J, Coito AJ, Ke B, et al. Heme oxygenase-1 overexpression protects rat livers from ischemia/reperfusion injury with extended cold preservation. Am J Transplant 2001;1:121—8.

[36] Wang C, Wang Z, Tao S, Ding J, Sun L, Li J, et al. Preconditioning donor liver with Nodosin perfusion lessens rat ischemia reperfusion injury via heme oxygenase-1 upregulation. J Gastroenterol Hepatol 2012;27:832—40.

[37] Zaouali MA. Hypoxia inducible factor-1α accumulation in steatotic liver preservation: role of nitric oxide. World J Gastroenterol 2010;16:3499.

[38] Luo YH, Li ZD, Liu LX, Dong GH. Pretreatment with erythropoietin reduces hepatic ischemia-reperfusion injury. Hepatobiliary Pancreat Dis Int 2009;8:294—9.

[39] Zeng Z, Huang HF, He F, Wu LX, Lin J, Chen MQ. Diazoxide attenuates ischemia/reperfusion injury via upregulation of heme oxygenase-1 after liver transplantation in rats. World J Gastroenterol 2012;18:1765—72.

[40] Sun L, Shi T, Qiao H, Jiang X, Jiang H, Krissansen GW, et al. Hepatic overexpression of heme oxygenase-1 improves liver allograft survival by expanding T regulatory cells. J Surg Res 2011;166:e187—94.
[41] Pirsch JD, Miller J, Deierhoi MH, Vincenti F, Filo RS. A comparison of tacrolimus (FK506) and cyclosporine for immunosuppression after cadaveric renal transplantation. FK506 Kidney Transplant Study Group. Transplantation 1997;63:977—83.
[42] Crenesse D, Laurens M, Heurteaux C, Cursio R, Saint-Paul MC, Schmid-Alliana A, et al. Rat liver ischemia-reperfusion-induced apoptosis and necrosis are decreased by FK506 pretreatment. Eur J Pharmacol 2003;473:177—84.
[43] Laurens M, Scozzari G, Patrono D, St-Paul MC, Gugenheim J, Huet PM, et al. Warm ischemia-reperfusion injury is decreased by tacrolimus in steatotic rat liver. Liver Transpl 2006;12:217—25.
[44] Moriuchi H, Kamohara Y, Eguchi S, Gu W, Fujioka H, Yamamoto T, et al. Diverse effects of FK506 on the apoptosis of hepatocytes and infiltrating lymphocytes in an allografted rat liver. J Surg Res 2011;167:131—9.
[45] Taylor AL, Watson CJ, Bradley JA. Immunosuppressive agents in solid organ transplantation: mechanisms of action and therapeutic efficacy. Crit Rev Oncol Hematol 2005;56: 23—46.
[46] Lin H, Sue YM, Chou Y, Cheng CF, Chang CC, Li HF, et al. Activation of a nuclear factor of activated T-lymphocyte-3 (NFAT3) by oxidative stress in carboplatin-mediated renal apoptosis. Br J Pharmacol 2010;161:1661—76.