Received: 2018.03.15
Accepted: 2018.08.15
Published: 2018.11.23

Protective Effects of Apoptosis of Kupffer Cells Induced by Zoledronate Liposomes Following Hepatic Ischemia-Reperfusion Injury

Authors' Contribution:

Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

AG 1 Qiao-Hong Zhao
AG 2 Feng Han
B 3 Kun Wu
C 3 Jie Zhang
C 3 Tian-Fang Xia
D 3 Jian Chen
D 3 Zhen-Shen Qing
EF 3 Li-Qun Pang

1 Department of Nursing, Jiangsu College of Nursing, Huai'an, Jiangsu, P.R. China
2 Department of General Surgery, Lian'shui County People's Hospital, Lian'shui, Jiangsu, P.R. China
3 Department of General Surgery, The Affiliated Huaian No.1 People's Hospital of Nanjing Medical University, Huai'an, Jiangsu, P.R. China

Corresponding Author: Li-Qun Pang, e-mail: cnliqunpang@163.com

Source of support: This study was supported by Huai'an Science and Technology Bureau International Cooperation Project (HG201112)

Background: The goal of this study was to observe the effect of the apoptosis of Kupffer cells (KCs) selectively induced by zoledronate liposomes following the hepatic ischemia-reperfusion injury (IRI) in the rat liver transplantation model and to explore its mechanisms.

Material/Methods: The rat liver transplantation model was established using the improved Kamada method. Male Sprague Dawley rats were randomly divided into 3 groups: no liver transplantation or drug treatment (Group A); donor rats were injected with 1 mL normal saline through the tail vein for 3 continuous days before transplantation, and the donor liver was preserved in cold for 2 hours (Group B); donor rats were injected with 1 mL zoledronate liposomes (0.001 mg/mL) through the tail vein for 3 continuous days before transplantation, and the donor liver was preserved in cold for 2 hours (Group C). At 24 hours after transplantation, the receiving rats were sacrificed for sampling.

Results: Compared with Group C and Group A, the bile secretion flow was dramatically decreased in Group B, whereas the serum liver function index [alanine aminotransferase (ALT), glutamate aminotransferase (AST), and γ -glutamyl transpeptidase (γ -GT)] was significantly increased ($P < 0.01$), and the pathological injury area was obviously increased. Compared with Group B, the levels of serum interleukin1 (IL-1), tumor necrosis factor- α (TNF- α), and the apoptotic index in Group C were significantly decreased ($P < 0.05$), and Suzuki scores of congestion, vacuolar degeneration, and necrosis were all reduced ($P < 0.05$).

Conclusions: The apoptosis of KCs selectively induced by zoledronate liposomes inhibited the inflammatory cascade reaction induced by KC activation and reduced the release of cytokines and decreased the extent of IRI in the liver transplantation in animal model.

MeSH Keywords: Kupffer Cells • Liver Transplantation • Reperfusion Injury

Full-text PDF: <https://www.annalsoftransplantation.com/abstract/index/idArt/909982>

2474

3

2

29

Background

Ischemia-reperfusion injury (IRI) is an important cause of liver damage during surgical procedures, such as hepatic resection and liver transplantation, and represents the main cause of graft dysfunction post-transplantation [1], severe shock, and other pathological processes [2]. The process of IRI always results in adenosine triphosphatase (ATP) depletion, the production of reactive oxygen species (ROS), and progressive tissue destruction, and the process is accelerated on reperfusion in the recipient [3]. Effective prevention and treatment for the IRI in the grafted liver can improve the function of the grafted liver and increase the success rate of liver transplantation. Thus, it is currently one of the hot topics in the field of liver surgery [4–6]. A large number of studies have shown that ischemic preconditioning, which refers to one or more transient IR episodes induced in body tissues or organs that confers a state of endogenous protection of body tissue and cells against subsequent long-term ischemic injuries [7], and drug pretreatment, including more than 8 kinds of drugs [8], can be helpful in the prevention and treatment of the grafted liver IRI [9,10]. In the early stage of IRI, the activation of Kupffer cells (KCs) and the over-expression of inflammatory factors, such as tumor necrosis factor- α (TNF- α), are the main initiating factors leading to the persistent imbalance of the self-stabilizing mechanism between pro-inflammatory and anti-inflammatory reactions *in vivo* [11]. Effective regulation of KC function may be an effective way to prevent and treat the IRI of the grafted liver. In a systematic review, KC inactivator was surely one of the most widely used drugs for donor animals [8].

Zoledronate is a nitrogen-containing bisphosphonate that selectively induces apoptosis in monocyte-macrophage cells [12]. In this study, we successfully established an IRI rat model of liver transplantation, and then zoledronate liposome was used to pretreat the donor liver to induce KC apoptosis in the donor liver, to explore its effects on the grafted liver IRI, and to provide a theoretical basis for the protection of the grafted liver IRI.

Material and Methods

Animals and grouping

A total of 40 healthy male Sprague-Dawley rats (clean grade), weighing 220–260 g, were purchased from the Laboratory Animal Center, Soochow University, China. The body weight of the recipient rats was equal to or slightly heavier than the donor rats.

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use

protocol has been reviewed and approved by the Institutional Animal Care and Use Committee of Nanjing Medical University.

After temporary rearing, 40 rats were selected and randomly divided into 3 groups. After food fasting for 12 hours and liquid fasting for 4 hours, rats underwent intraperitoneal anesthesia by ketamine (80 mg/kg). The *in situ* liver transplantation was performed by the “improved 2-cuff technique” [13,14]. A sham-operation (Group A, n=8) was conducted in which rats underwent laparotomy without liver transplantation. A saline control group (Group B, n=16) included the donor rats that received the tail vein injection of 1 mL saline once per day for 3 consecutive days. Then, the donor liver was taken out and preserved in cold for 2 hours before it was transplanted into the recipient rats. The zoledronate pretreatment group (Group C, n=16) consisted of donor rats that received a tail vein injection of 1 mL zoledronate liposome (0.001 mg/mL, $C_5H_{10}N_2O_7P_2H_2O$ with molecular weight=290.11; 4 mg/bottle; batch number: H20041953; Jiangsu Hengrui Pharmaceutical Co., Ltd., China) once per day for 3 consecutive days. Then, the donor liver was taken out and preserved in cold for 2 hours before it was transplanted into the recipient rats.

Sampling and determination of indicators

The recipient rats were anesthetized at 24 hours after the transplantation to determine the bile secretion flow and collect blood samples. Then, the rats were sacrificed for additional sampling.

Determination of bile secretion flow

A catheter for epidural anesthesia was inserted into the common bile duct from the proximal end. According to the length of the bile in the catheter, bile secretion flow per minute was estimated and then divided by the weight of the donor liver, which was defined as the bile secretion flow per gram of liver per minute ($\mu\text{L}/\text{min}\cdot\text{g}$ liver). The capacity of the catheter was calculated by injecting 50 μL colored liquid into the catheter with a microinjector and measuring the length a total of 6 times. As a result, 1 mm=0.65 $\pm$ 0.26 μL .

Blood collection and liver tests

For each rat, 5 mL blood was drawn from the inferior vena cava and centrifuged at 2500 r/min for 15 minutes. The serum was collected in EP tubes and stored for the detection of alanine aminotransferase (ALT), glutamate aminotransferase (AST), interleukin (IL-1), LDH (lactate dehydrogenase), γ -GT (γ -glutamyl transpeptidase), and TNF- α using enzyme linked immunosorbent assay (ELISA) kits (Santa Cruz).

Table 1. Bile secretion, hepatic function and IL-1/TNF- α of rats in the three groups after 24h of transplantation.

Group	Bile secretion ($\mu\text{L}/\text{min}\cdot\text{g liver}$)	ALT (U/L)	AST (U/L)	AKP (U/L)	$\gamma\text{-GT}$ (U/L)	Interleukin-1 (pg/ml)	TNF- α (ng/ml)
A	12.10 $\pm$ 1.64	185.25 $\pm$ 16.91	129.63 $\pm$ 13.92	135.31 $\pm$ 10.9	130.92 $\pm$ 10.12	17.56 $\pm$ 2.77	4.23 $\pm$ 0.68
B	2.73 $\pm$ 0.63*	2653.75 $\pm$ 141.17*	3865.68 $\pm$ 236.87*	2411.5 $\pm$ 49.82*	1245.53 $\pm$ 123.72*	1098.76 $\pm$ 78.42*	985.77 $\pm$ 62.54*
C	9.62 $\pm$ 1.38 [#]	232.32 $\pm$ 18.76 [#]	218.67 $\pm$ 15.49 [#]	229.96 $\pm$ 12.51 [#]	242.64 $\pm$ 19.91 [#]	378.20 $\pm$ 43.23**	307.34 $\pm$ 37.22**

A – Sham-operation; B – Saline; C – Zoledronate-liposomes. Data are expressed as mean $\pm$ SD. * $P < 0.01$, compare with Group A; [#] $P < 0.01$, compare with Group B. ALT – alanine transaminase; AST – glutamic-oxaloacetic transaminase; AKP – alkaline phosphatase; $\gamma\text{-GT}$ – γ -glutamyl transpeptidase; TNF- α – tumor necrosis factor α .

Liver tissues sampling

KCs are located in the hepatic sinus of the liver and have with irregular shapes. Most parts of the cell protrude into or completely mobilizes into the sinusoids. Because they extend into the sinusoidal space and come into direct contact with the hepatocytes. The grafted liver specimens in the recipient rats were sampled and fixed in 10% formalin and embedded in paraffin. Serial sections of 4 μm were stained using hematoxylin-eosin methods and observed under a light microscope. The sections were all used for determining the numbers of KCs by immunohistochemistry (IHC) using antibody CD68 and detecting *in situ* hepatocyte apoptosis using a TUNEL Kit (Roche, USA).

Apoptosis index and immunohistochemical quantitative analysis

The slices were observed at 400 $\times$ magnification under the microscope. The images were taken and input into Image-Pro^{Plus} 10.0 image software to select and measure the positive staining area and visual field of view. Five fields were randomly selected in each slice for calculating the “positive area,” which was defined as: the mean proportion (%) of the positive area of TNF- α in the visual area.

The apoptotic index (AI) was expressed as a percentage. Five fields at 400 $\times$ magnification were randomly selected in each slice for calculating AI following the formula: AI=(the number of apoptotic cells/100 cells) $\times$ 100%.

Neutrophil infiltration is an important factor in liver IRI, and Suzuki semi-quantitative scoring was used to estimate congestion, vacuolar degeneration, and necrosis in different groups [15].

Statistical analysis

The SPSS15.0 statistical software package was employed for the statistical analysis of the data. All data are expressed as

mean $\pm$ standard deviation (SD). Pairwise comparisons of the measurement data among the groups were performed using a one-way ANOVA, whereas the ranked data were compared using the Rank sum test (Kruskal-Wallis method). The difference at the level of $P < 0.05$ was regarded as significant.

Results

Bile secretion flow

At 24 hours after the liver transplantation, the bile secretion flow was measured, which was significantly decreased in Group B (Saline group, 2.73 $\pm$ 0.63 $\mu\text{L}/\text{min}\cdot\text{g liver}$; $P < 0.01$) as compared with that in Group A (Sham-operation group, 12.10 $\pm$ 1.64 $\mu\text{L}/\text{min}\cdot\text{g liver}$) or Group C (zoledronate liposome group, 9.62 $\pm$ 1.38 $\mu\text{L}/\text{min}\cdot\text{g liver}$). There was no significant difference in the amount of bile secretion between Group C and Group A ($P > 0.05$, Table 1).

Serum levels of ALT, AST, LDH, and $\gamma\text{-GT}$

The serum levels of ALT, AST, LDH, and $\gamma\text{-GT}$ in Group B were all significantly higher than those in Groups A or C ($P < 0.01$), whereas there was no significant difference in the 4 indicators between Groups A and C ($P > 0.05$, Table 1).

Serum levels of IL-1 and TNF- α

Compared with Group A, the serum levels of IL-1 and TNF- α in Group B and Group C were significantly increased ($P < 0.01$). However, as compared with Group B, the levels of IL-1 and TNF- α in Group C were notably decreased ($P < 0.01$, Table 1).

Hematoxylin-eosin staining

There was no obvious abnormal change observed in the liver sections in Group A (Figure 1A–1C), but most hepatocytes

Figure 1. Differences of livers in 3 groups after 24 hours of operation (200×). (A–C) Pathological changes; (D–F) Expression of TNF-α; (G–I) Cell apoptosis.

Table 2. The Suzuki's scores of the hepatocyte and AI/of TNF-α in each group.

Group	Congestion	Vacuolar degeneration	Necrosis	Total	Apoptosis index (AI)	Tumor necrosis factor-α
A	0	0	0	0	2.62±0.43	1.34±0.25
B	4.60±1.15*	5.71±1.45*	7.27±1.72*	17.58±4.32*	27.96±5.21*	34.66±7.49*
C	1.14±0.52*##	1.76±0.71*##	0.65±0.31*##	3.55±1.54*##	13.65±3.76*#	12.32±2.09*#

A – Sham-operation; B – Saline; C – Zoledronate-liposomes. * $P<0.01$, compare with Group A; ## $P<0.01$, compare with Group B; # $P<0.05$, compare with Group B.

were swollen and cloudy, with cytoplasmic balloon degeneration, hepatocellular sheeted degeneration and necrosis, moderate and severe dilatation in the liver sinusoid, and obvious congestion (Figure 1D–1F). In contrast, hepatocytes in Group C revealed mild swelling, cytoplasmic balloon degeneration, and a small amount of sinusoidal congestion, but without a significant necrosis area (Figure 1G–1I).

The Suzuki scores of congestion, vacuolar degeneration, and necrosis of Group B were remarkably higher than those in Group A ($P<0.05$). In contrast, the Suzuki scores for the aforementioned 3 indexes in Group C were prominently lower than those in Group B ($P<0.05$, Table 2).

Figure 2. Expression of CD68 (KCs) in the liver tissues after 24 hours of operation by IHC (200×). (A) Group A; (B) Group B; (C) Group C; (D) In the donor liver at 24 hours after Zoledronate-liposome preconditioning.

Expression of TNF- α

IHC staining revealed that few hepatocytes in Group A expressed TNF- α (1.34 ± 0.25 , Figure 1D). Conversely, a large number of hepatocytes and sinus endothelial cells in Group B were stained brown (34.66 ± 7.49 , Figure 1E), whereas in Group C, weakly positive cells with weak brownish-yellow stain were observed in only a few local hepatocytes (12.32 ± 2.09 , Figure 1F). Compared with that in Group A, the positive percentages of TNF- α in Groups B and C were both significantly elevated ($P < 0.01$ and $P < 0.05$, respectively; Figure 1D–1F).

The apoptosis index (AI)

The TUNEL assay revealed that only a very small number of apoptotic cells was found in Group A (Figure 1G). Compared with that in Group A (2.62 ± 0.43), the AIs in Group B and Group C were both enhanced (27.96 ± 5.21 and 13.65 ± 3.76 , respectively; $P < 0.01$, Figure 1H, 1I). Compared with Group B, the AI in Group C was significantly decreased ($P < 0.05$, Table 2).

Table 3. Expression of CD68 (Kupffer cells, KCs) in each group.

Group	Number of KCs
A	7.6 ± 1.5
B	$28.6 \pm 9.5^{**}$
C	$19.3 \pm 5.1^{\#}$
D	$2.8 \pm 0.6^{*}$

A – Sham-operation; B – saline; C – zoledronate-liposomes; D – donorliver. $^{**} P < 0.01$, compare with Group A; $^{*} P < 0.05$, compare with Group A; $^{\#} P < 0.01$, compare with Group B.

Kupffer cells (KCs)

The number of KCs (positive for CD68) was 7.6 ± 1.5 for Group A, 28.6 ± 9.5 for Group B, and 19.3 ± 5.1 for Group C. There was significant difference among the 3 groups ($P < 0.01$). The number of KCs (positive for CD68) in the donor liver was markedly reduced by the zoledronate liposomes pretreatment (2.8 ± 0.6), as compared with that in Group A ($P < 0.05$, Figure 2, Table 3).

Discussion

Liver transplantation has become the primary treatment for the end-stage liver disease. During the transplantation, IRI is an unavoidable pathological process in the donor liver and is closely related to the primary non-function of the grafted liver [2]. Existing research [6,11] shows that liver IRI is divided into 2 stages: early injury and late injury, with very complicated mechanisms. Early injury on hepatocytes is directly induced by the cytokines, such as TNF- α , IL-1, and IL-6 released by activated KCs and a large amount of ROS. During the cold preservation of the donor liver, the energy metabolism of the liver is impaired, and the synthesis of ATP is decreased, although decomposition is enhanced. In addition to the acidic environment of the tissues induced by anaerobic glycolysis and lactic acid accumulation and the increase in intracellular Ca²⁺ activation status, KCs are kept in a pre-activated state. During the transplantation surgery, the portal vein is blocked, which causes congestion and increased permeability into the intestinal canal, facilitating the entry and accumulation of bacterial endotoxins in the portal vein system. Approximately 15 minutes after the reperfusion of the grafted liver, the endotoxin accumulated in the portal blood flow can essentially activate all of the KCs [16]. The activated KCs have enhanced phagocytosis and produce a variety of biologically active substances, such as ROS and water-soluble enzymes, which can metabolize and detoxify the bacteria, endotoxins, and many toxic substances that enter the liver, and thus play a protective role in the grafted liver. However, at the same time, ROS and water-soluble enzymes, together with a large number of inflammatory cytokines, such as TNF- α , IL-1, PAF, and IL-6 secreted by KC activation, interact with CD4⁺ T cells and cause neutrophils to adhere to the sinusoidal endothelial cells (SLE) and infiltrate the hepatic parenchymal cells, forming an inflammatory cascade that further aggravates the reperfusion injury of the grafted liver [17,18].

The previous study shows that cell apoptosis in tissue is the main reason leading to organ damage and non-function [19]. Under the attack of ischemia-reperfusion injury, the transplanted liver is damaged not only from the direct role of ischemia-reperfusion injury, but also from the activation and enlargement of apoptosis. Animal experiments that study the liver ischemia-reperfusion injury had found that ischemia-reperfusion injury can cause apoptosis of rat hepatocytes, and the apoptosis mainly occurs during early stage post-reperfusion [20]. Apoptosis is also found in the renal tubular epithelial cells in renal transplantation [21] and heart tissue in cardiac allografts [22], suggesting that apoptosis plays an important role in graft ischemia-reperfusion injury.

Therefore, in the early stage of graft reperfusion, the activation of KC and the over-expression of inflammatory factors, such as TNF- α and IL-1, are the main initiating factors of the

pathological process that causes the persistent imbalances of the proinflammatory/anti-inflammatory homeostasis *in vivo* and further amplify the inflammatory cascade; thus, leading to IRI and liver failure of the grafted liver. Effective regulation of the KC function may be an effective way to prevent and treat the IRI in the grafted liver [23].

At present, the inhibition and regulation of KC function has become one of the hot topics in research regarding protecting the transplanted liver from IRI. The existing studies are mainly focusing on suppressing the phagocytosis and secretion of KCs by taurine, carbon monoxide, and hemoxygenase-1 (HO-1), or inducing KCs apoptosis by GdCl₃, so as to reduce IRI in the grafted liver [24–28]. Although they have achieved some protective effects, the reagents cannot be commercialized, and no related clinical research has been reported.

Zoledronate, a nitrogen-containing bisphosphonate, has the effect that selectively induces apoptosis on mononuclear macrophages. There was a study that found that zoledronate liposomes treatment could significantly increase the apoptosis in the liver of rats [29]. Liposome is an ideal drug carrier, which is circulated in the blood system and mainly phagocytized by the reticuloendothelial system of the liver and spleen. Regarding this, we constructed a zoledronate liposome to pretreat the donor liver to study the protective roles of its selective induction of the apoptosis of KCs on the transplanted liver during the IRI.

In the present study, we found that KCs in the transplanted rat liver were significantly decreased and the levels of serum TNF- α and IL-1 were also prominently reduced after pretreatment with zoledronate liposomes ($P < 0.05$). Moreover, the apoptosis index of hepatocytes was notably decreased by pretreatment with zoledronate liposomes ($P < 0.05$). Liver histopathological examination revealed that the degeneration and necrosis of hepatocytes were significantly improved by zoledronate liposomes. However, in the control group treated with saline, the structure of hepatic cords in hepatic lobules was unclear, the hepatocytes were obviously swollen, and some hepatocytes were necrotic. Our results showed that zoledronate liposomes can inhibit the inflammatory cascade and reduce the release of cytokines (such as TNF- α and IL-1) by inducing apoptosis of KCs, and thereby reducing the extent of IRI in liver transplantation.

Conclusions

Kupffer cells are one of the important factors that lead to liver damage during the IRI process of liver transplantation. Zoledronate effectively protects the liver grafts from ischemia-reperfusion injury by inducing KCs apoptosis and reducing the release of cytokines.

Conflicts of interest

None.

References:

- Gracia-Sancho J, Casillas-Ramírez A, Peralta C: Molecular pathways in protecting the liver from ischaemia/reperfusion injury: A 2015 update. *Clin Sci (Lond)*, 2015; 129: 345–62
- Saidi RF, Kenari SK: Liver ischemia/reperfusion injury: An overview. *J Invest Surg*, 2014; 27: 366–79
- Akhtar MZ, Henderson T, Sutherland A et al: Novel approaches to preventing ischemia-reperfusion injury during liver transplantation. *Transplant Proc*, 2013; 45: 2083–92
- Jegatheeswaran S, Siriwardena AK: Experimental and clinical evidence for modification of hepatic ischaemia-reperfusion injury by N-acetylcysteine during major liver surgery. *HPB (Oxford)*, 2011; 13: 71–78
- Zhang EW, Fang T, Arnold PB et al: The effect of activated protein C on attenuation of ischemia-reperfusion injury in a rat muscle flap model. *Ann Plast Surg*, 2015; 75: 448–54
- Junnarkar SP, Tapuria N, Dutt N et al: Bucillamine improves hepatic microcirculation and reduces hepatocellular injury after liver warm ischaemia-reperfusion injury. *HPB (Oxford)*, 2009; 11: 264–73
- Qing ZS, Zhang XS, Gao CC et al: Protective effect of ischemia preconditioning on ischemia-reperfusion injury in rat liver transplantation. *Genet Mol Res*, 2015; 14: 3018–25
- Yamanaka K, Houben P, Bruns H et al: A systematic review of pharmacological treatment options used to reduce ischemia reperfusion injury in rat liver transplantation. *PLoS One*, 2015; 10: e0122214
- Andreani P, Hoti E, de la Serna S et al: Ischaemic preconditioning of the graft in adult living related right lobe liver transplantation: Impact on ischaemia-reperfusion injury and clinical relevance. *HPB (Oxford)*, 2010; 12: 439–46
- Martins PN, Chang S, Mahadevapa B et al: Liver grafts from selected older donors do not have significantly more ischaemia reperfusion injury. *HPB (Oxford)*, 2011; 13: 212–20
- Xiao ZY, Banan B, Jia J et al: CD47 blockade reduces ischemia reperfusion injury and improves survival in a rat liver transplantation model. *Liver Transpl*, 2015; 21: 468–77
- Fukushima A, Ozaki A, Ishida W et al: Suppression of macrophage infiltration into the conjunctiva by clodronate liposomes in experimental immune-mediated blepharoconjunctivitis. *Cell Biol Int*, 2005; 29: 277–86
- Peng Y, Gong JP, Yan LN et al: Improved two-cuff technique for orthotopic liver transplantation in rat. *Hepatobiliary Pancreat Dis Int*, 2004; 3: 33–37
- Oldani G, Lacotte S, Morel P et al: Orthotopic liver transplantation in rats. *J Vis Exp*, 2012; (65): e4143
- Suzuki S, Toledo-Pereyra LH, Rodriguez FJ, Cejalvo D: Neutrophil infiltration as an important factor in liver ischemia and reperfusion injury. *Transplantation*, 1993; 55: 1265–72
- Guthrie G, Tackett B, Stoll B et al: Phytosterols synergize with endotoxin to augment inflammation in Kupffer cells but alone have limited direct effect on hepatocytes. *J Parenter Enteral Nutr*, 2017 [Epub ahead of print]
- Harada H, Wakabayashi G, Takayanagi A et al: Transfer of the interleukin-1 receptor antagonist gene into rat liver abrogates hepatic ischemia-reperfusion injury. *Transplantation*, 2002; 74: 1434–41
- Tay SS, Wong YC, Roediger B et al: Intrahepatic activation of naive CD4+ T cells by liver-resident phagocytic cells. *J Immunol*, 2014; 193: 2087–95
- Song GH, Huang FB, Gao JP et al: Effects of fluoride on DNA damage and caspase-mediated apoptosis in the liver of rats. *Biol Trace Elem Res*, 2015; 166: 173–82
- Tao T, Chen F, Bo L et al: Ginsenoside Rg1 protects mouse liver against ischemia-reperfusion injury through anti-inflammatory and anti-apoptosis properties. *J Surg Res*, 2014; 191: 231–38
- Pérez EC, Shulzhenko N, Morgun A et al: Expression of Fas, FasL, and soluble Fas mRNA in endomyocardial biopsies of human cardiac allografts. *Hum Immunol*, 2006; 67: 22–26
- Carstens J, Markussen N, Madsen M: The granule exocytosis and Fas/FasLigand pathways at the time of transplantation and during borderline and acute rejection of human renal allografts. *Transplant Proc*, 2005; 37: 3294–97
- van Rooijen N, van Kesteren-Hendriks E: Clodronate liposomes: Perspectives in research and therapeutics. *J Liposome Res*, 2002; 12: 81–94
- Kincius M, Liang R, Nickkholgh A et al: Taurine protects from liver injury after warm ischemia in rats: The role of Kupffer cells. *Eur Surg Res*, 2007; 39: 275–83
- Tomiyama K, Ikeda A, Ueki S et al: Inhibition of Kupffer cell-mediated early proinflammatory response with carbon monoxide in transplant-induced hepatic ischemia/reperfusion injury in rats. *Hepatology*, 2008; 48: 1608–20
- Zeng Z, Huang HF, Chen MQ et al: Heme oxygenase-1 protects donor livers from ischemia/reperfusion injury: The role of Kupffer cells. *World J Gastroenterol*, 2010; 16: 1285–92
- Jahnke C, Mehrabi A, Golling M et al: Evaluation of microperfusion disturbances in the transplanted liver after Kupffer cell destruction using GdCl₃: An experimental porcine study. *Transplant Proc*, 2006; 38: 1588–95
- Li JY, Gu X, Zhang WH et al: GdCl₃ abates hepatic ischemia-reperfusion injury by inhibiting apoptosis in rats. *Hepatobiliary Pancreat Dis Int*, 2009; 8: 518–23
- Aydogan A, Kocer G, Ozmen O et al: Immunohistochemical expression of caspase-3, caspase-5, caspase-7 and apoptotic protease-activating factor-1 (APAF-1) in the liver and kidney of rats exposed to zoledronic acid (ZOL) and basic fibroblast growth factor (bFGF). *Vet Q*, 2014; 34: 137–42