

## Original Article

# 17 $\beta$ -Estradiol protects the liver against warm ischemia-reperfusion injury through the Akt/GSK-3 $\beta$ pathway

Longjiang Shao\*, Ding Sun\*, Yueyu Liu, Weigang Zhang, Haixin Qian, Lei Qin, Xiaohua Yang

Department of General Surgery, The First Affiliated Hospital of Soochow University, Suzhou 215006, China. \*Equal contributors.

Received October 30, 2017; Accepted September 10, 2018; Epub January 15, 2019; Published January 30, 2019

**Abstract:** Objective: To investigate the mechanism of the protective effect of 17 $\beta$ -Estradiol (E2) on warm ischemia-reperfusion (IR) injury in rat model. Methods: 24 Sprague-Dawley (SD) male and 12 SD female rats were randomized into six groups. Sham group (Male, Female, Male+17 $\beta$ -E2), IR group (Male, Female, Male+17 $\beta$ -E2), 6 per group. E2 (4 mg/kg) was used by peritoneal injection in Male+17 $\beta$ -E2 groups 1 h before IR injury. Male and female group received same quantity of saline. The rats were sacrificed at 6 h after I/R, concentration of serum alanine aminotransferase (ALT), histomorphology of liver tissue, apoptotic ratio of hepatic cells, expression level of p-Akt and p-glycogen synthase kinase-3 $\beta$  (p-GSK-3 $\beta$ ) were detected. Results: (1) 6 h after IR injury, the concentration of ALT and apoptotic ratio of hepatic cells in Female and Male+17 $\beta$ -E2 group was significantly lower than in Male group. (2) The degree of hepatic injury in Female and Male+17 $\beta$ -E2 group was lower than in Male group. (3) The activation of p-Akt and p-GSK-3 $\beta$  in Female and Male+17 $\beta$ -E2 group was higher than in Male group. Conclusion: E2 protects the liver against warm IR injury through the Akt/GSK-3 $\beta$  kinase pathway. Estrogen therapy might be a "promising treatment" in clinical settings associated with warm IR injury during hepatectomy.

**Keywords:** 17 $\beta$ -Estradiol, ischemia-reperfusion injury, apoptosis, akt, glycogen synthase kinase-3 $\beta$ , Bcl-2-associated death promoter

## Introduction

Ischemia-reperfusion (IR) injury is one of the common clinical pathophysiology processes, which also occurs during trauma, hemorrhagic shock, solid organ transplantation, liver resection, etc [1, 2]. The mechanism of IR injury directly linked to liver cells' injury that was induced by aseptic inflammation and Toll-like receptor's activations. In the previous study, we discovered that females recovered better than males when suffered clinical hemorrhagic shock, liver surgery and organ transplantation. It was firmly proved that male rats had better cardiac function after IR injury by using estrogen in experiments [3, 4]. In our study, we tried to investigate the role of 17 $\beta$ -Estradiol (E2) in the protection of hepatic IR injury in rats and explore its possible mechanism.

Akt is a serine/threonine protein kinase, as well as an critical signal transduction enzyme in-

involved in cell survival and apoptosis [5-7], which can inactivate specific molecules, such as glycogen synthase kinase-3 $\beta$  (GSK-3 $\beta$ ) and Bcl-2-associated death promoter (BAD) to improved cell survival rate [8, 9]. Activation of Akt inhibits cell necrosis and apoptosis by regulating its various downstream molecules such as GSK-3 $\beta$  and BAD [10-15].

GSK-3 $\beta$ , a serine/threonine protein kinase and the downstream protein of Akt, is a multifunctional kinase that inhibits multiple signaling pathways by its activity [16]. It can promote systemic inflammatory response and increase release of pro-inflammatory, inducing apoptosis, and cell proliferation [17-19]. It has recently been reported that GSK-3 $\beta$  involved many diseases, including type 2 diabetes, Alzheimer's disease, cancer, ect [20-23].

BAD, one of the BH3 domain protein, is a member of the proapoptotic Bcl-2 family with BH3-

only. It is capable of inactivating antiapoptotic Bcl-2 proteins by linking the upstream cell survival signaling pathways and downstream pathways [24]. BAD plays a role as a mediator between the Bcl-2 family proteins and a variety of different kinase signaling pathways [25]. Some present study have shown that Akt kinase pathway activation play a important role in the protection against IR injury [26, 27]. To sum up, this study was to investigate the role of E2 in protection of warm IR injury in rat liver and search its relationship with Akt/GSK-3 $\beta$  signaling pathway.

### Materials and methods

#### *Rats and reagents*

In this study, 24 Sprague-Dawley (SD) male rats and 12 SD female rats were selected according to body weight of 200-220 g. Rats were fed in cage in an air-conditioned room with controlled 12 h light and dark cycles. And they were allowed free access to food and water. We conducted this experiment in accordance with the Guide for the Care and Use of Laboratory Animals. The in situ cell death detection kits were purchased from the Roche Chemical, Indianapolis, IN. The polyvinylidene difluoride membranes were purchased from the Amersham Pharmacia, Piscataway, NJ. The horseradish peroxidase-conjugated second antibodies were purchased from the Santa Cruz Biotechnology, Santa Cruz, CA. The SuperSignal West Dura Extended Duration substrate was purchased from Pierce Biotechnology, Rockford, IL. And the E2 was purchased from the Sigma Chemical, St Louis, MO.

#### *Experimental design and operation*

24 male SD rats were randomly divided into four groups. 12 female SD rats were randomly divided into two groups. Thus, every group contained 6 SD rats. Sham group (Male group, Female group, Male+17 $\beta$ -E2 group), IR group (Male group, Female group, Male+17 $\beta$ -E2 group). E2 (4 mg/kg) was used by intraperitoneal injection in Male+17 $\beta$ -E2 group's rats 1 h before IR injury. Male and female group received same quantity of saline. Before operation, the rats were fasted for 12 h and were only allowed free access to water. The rats were anesthetized with an intraperitoneal injection of ketamine at 100 mg/kg. The epigastrium

was opened by a midline incision. Then, the first porta hepatis was fully exposed. Hepatic warm ischemia was induced by occlusions (using the non-invasive vascular clamps) of the hepatic hilar vessels connected to the left, middle and caudate lobe of liver, guaranting the model of hepatic warm IR injury at 70%. After 60 min, the non invasive vascular clamps were released. Saline (1 ml) was used by injection from the vena dorsalis penis after IR injury. In the sham group, the rats were laparotomized and the wound closed without IR injury. Rats were sacrificed at 6 h after reperfusion, and samples were taken.

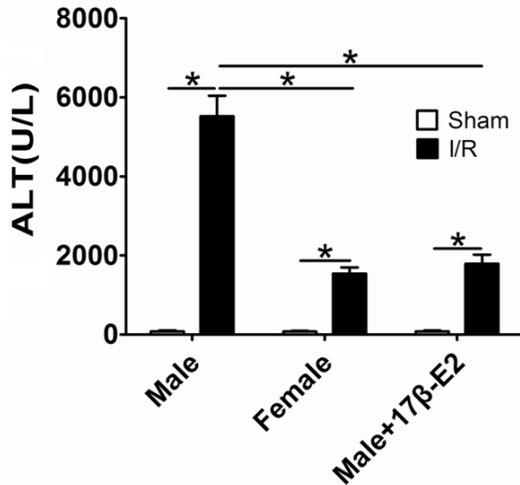
#### *Testing index*

*Testing hepatic functions:* Blood samples were drawn from the IVC of the rats. Each rat was drawn about 3-4 ml blood, which was centrifuged and serum was extracted. Then, serum alanine aminotransferase (ALT) levels were measured using an automatic biochemical analyzer.

*Hematoxylin-eosin (H/E) staining:* Liver tissues were gotten from the liver's left lobe. The liver tissues were fixed in 10% formalin solution, dehydrated through graded alcohol series and embedded in paraffin. The tissues were observed under the microscope once we stained them with hematoxylin and eosin solutions.

*Detection of apoptosis with terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay:* We detected of apoptosis with TUNEL assay using the in situ cell death detection kit (Roche Diagnostics, Indianapolis, IN). The paraffin-embedded sections were deparaffinized and then incubated to proteinase K working solution (20 mg/mL) for 30 min at room temperature. The liver sections were incubated with terminal deoxynucleotidyl transferase buffer at 37°C for 1 h and then transferred to stop buffer for 5 min at room temperature. The tissue sections were incubated in antifluorescein antibody conjugated with alkaline phosphatase and visualized with substrate solution after being washed in phosphate-buffered saline. The tissue sections were counterstained with hematoxylin and observed under light microscopy. The percentage of apoptotic cells was calculated by counting approximated 500 cells at least five randomly chosen microscopic fields. The apoptotic index was defined as the

## Estrogen against ischemia-reperfusion injury



**Figure 1.** Changes in liver function. 6 h after IR injury, the serum ALT levels in the Female and Male+17β-E2 group were significantly lower than those in the Male group. There was no statistically significant compared the Male+17β-E2 group with the Female group. (\* $P < 0.05$ , Male group vs Female group and Male+17β-E2 group.  $P > 0.05$ , Female group vs Male+17β-E2 group.)

percentage of the number of apoptotic cells to the total number of cells. The paraffin-embedded liver sections were deparaffinized, hydrated and permeated. In brief, the liver sections were incubated with terminal deoxynucleotidyl transferase by TUNEL assay. Then, we counterstained the liver sections with hematoxylin, observed and calculated the apoptotic cells under light microscopy.

### Western blot analysis

Protein expression of p-Akt and p-GSK-3β in rat livers was investigated by Western blot analysis. The protein of the hepatic tissue was extracted by lysis buffer and then separated by sodium dodecylsulfate polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride membranes (Amersham Pharmacia, Piscataway, NJ). The polyvinylidene difluoride membrane was incubated at room temperature in a blocking solution composed of 5% skim milk powder dissolved in 1× Tris-buffered saline-Tween 20 (10 mM Tris, pH 8.0, 140 mM NaCl, and 0.1% Tween 20) for 60 min. The membrane was then incubated with the following primary antibodies: anti-Akt, anti-phospho-Akt, anti-GSK-3b, anti-phospho-GSK-3b, anti-total caspase-3, anti-cleaved caspase-3 (diluted 1:1000; Cell Signaling Technology, Beverly, MA) overnight at 4°C. After washing three times

with Tris-buffered saline-Tween 20 for 15 min, the membrane was incubated for 60 min with horseradish peroxidase-conjugated second antibody (1:5000; Santa Cruz Biotechnology, Santa Cruz, CA). The membrane was washed again three times with Tris-buffered saline-Tween 20 before being exposed to the SuperSignal West Dura Extended Duration substrate (Pierce Biotechnology, Rockford, IL). The band intensity was quantified by densitometric analysis using a densitometer.

### Statistical analysis

Data are expressed as the mean  $\pm$  standard ( $\bar{x} \pm s$ ), using one-way analysis of variance by the statistical software SPSS 11.5.  $P$  value less than 0.05 was considered statistically significant.

## Results

### Change in liver function

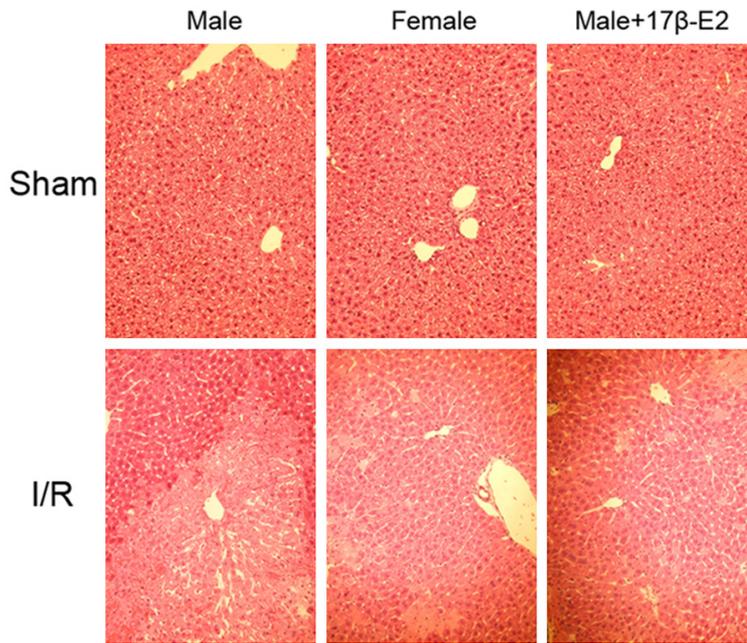
ALT is hepatocyte enzyme that is regarded as a mark of hepatocellular injury. The serum ALT levels in the Female and Male+17β-E2 group were significantly lower than those in the Male group at 6 h after IR.  $P$  value less than 0.05 was considered statistically significant. What's more, we compared the Male+17β-E2 group with the Female group, there was no statistically significant (Figure 1).

### Histological changes in liver tissue

The liver histopathology change happen during trauma, shock, organ transplant, liver surgery, etc. The HE staining of liver in the male group showed a large number of hepatocellular necrosis at 6 h after IR and the hepatic cords had disintegrated, the sinusoids were filled with erythrocytes, the hepatic lobules and portal areas were infiltrated by neutrophils. In the female group and the Male+17β-E2 group, the severity of liver cells injury decreased compared with the Male group. They showed a small amount of hepatocellular necrosis, the hepatic cords were basically normal, only a little sinusoids were filled with erythrocytes (Figure 2).

### Impact of IR injury on liver apoptosis

The TUNEL assay was used to detect liver's apoptosis caused by IR injury. There was a significant difference between the Male group, the



**Figure 2.** Histological changes in liver tissue ( $\times 200$ ). 6 h after IR injury, optical microscopy analysis after the HE staining demonstrated that the liver cells were injured seriously in Male group. On the contrary, in the Female group and the Male+17 $\beta$ -E2 group, the severity of liver cells injury was decreased.

Female group and the Male+17 $\beta$ -E2 group. 6 h after IR injury, TUNEL showed a large number of hepatocyte apoptosis in Male group. However, the apoptotic ratios of hepatic cells in Female and Male+17 $\beta$ -E2 group were significantly lower than in Male group. *P* value less than 0.05 was considered statistically significant. Then, we compared the Male+17 $\beta$ -E2 group with the Female group, there was no statistically significant (**Figure 3**).

*Expression of p-Akt and p-GSK-3 $\beta$  after IR injury in rats's hepatocytes*

Western blot was used to detect the expression of p-Akt and p-GSK-3 $\beta$  in hepatocytes. The expression of p-Akt in Sham group was lower after IR injury. The activity of p-Akt in Female group and Male+17 $\beta$ -E2 group was significantly higher than that in Male group after IR injury (**Figure 4A**). Moreover, the activity of p-GSK-3 $\beta$  in Female group and Male+17 $\beta$ -E2 group was significantly higher than that in Male group after IR injury (**Figure 4B**).

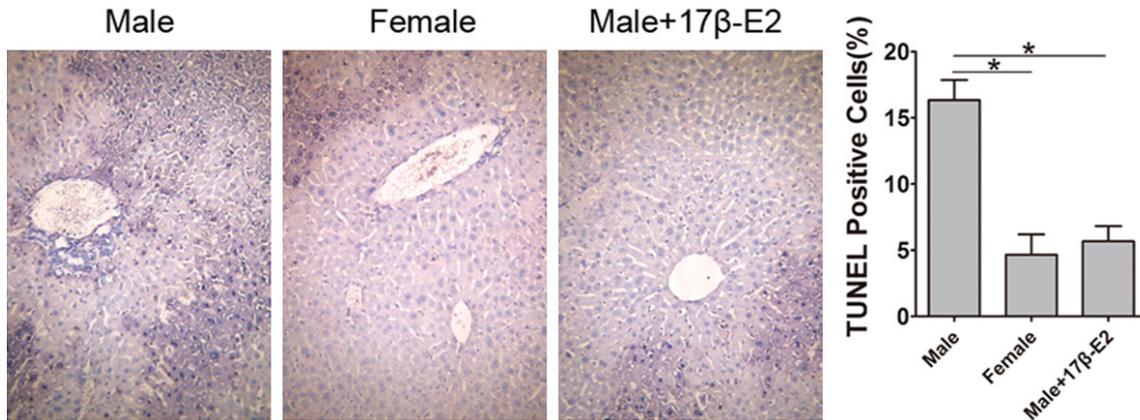
**Discussion**

IR injury is a serious clinical problem. However, the mechanisms have not been fully elucidated

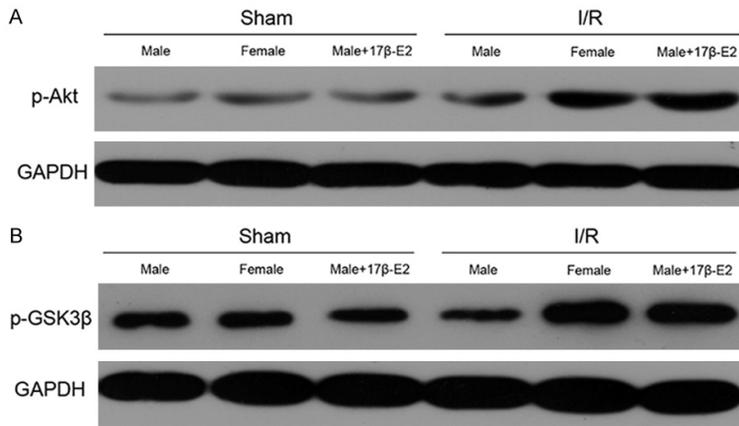
yet by us. There are some methods to prevent and treat it, such as ischemic preconditioning and the use of various drugs, but the efficacy is not effective [28]. We discovered that female patients recover better than males after surgery. Many studies reported that estrogen is capable of fighting against IR injury when its specific mechanisms are not clear [29-34]. The results of this study showed that the level of serum ALT in female rats was significantly lower than that in male rats after IR injury, and the damage of liver tissue, as same as the apoptosis of liver cells, was lighter than that of male rats. In addition. The level of serum ALT, liver injury and hepatocyte apoptosis were greatly decreased after operation when the male rats were pretreated with E2 although a small

amounts of liver injury and hepatocyte apoptosis were observed. This suggested that estrogen has a protective effect on IR injury in rats liver.

Some previous studies reveal the mechanism about the protective effect of estrogen. Arturo reported that estrogen can reduce the IR injury of the heart by activating the Protein Kinase C (PKC) and Akt signaling pathways. Akt, also known as Protein Kinase B (PKB), is a kind of serine/threonine protein kinase. The Akt signaling pathway plays a important role in cell survival, proliferation and apoptosis [35]. When the specific ligand activates the cell surface receptor, Akt is recruited into the inner surface of the cell membrane. Activated by phosphorylation of pyruvate dehydrogenase kinase 1 (PDK1), Akt leave the cell membrane into the cytoplasm to play a role. Activation of Akt is able to inhibit cell apoptosis through a variety of ways, such as phosphorylating Bcl-2 family members BAD and inhibiting GSK-3 $\beta$ 's activity. Activated Akt not only can inhibit cell apoptosis by inactivating BAD or GSK-3 $\beta$  and preventing the release of Mitochondrial cytochrome C [36], but also directly affect the transcription factor



**Figure 3.** Impact of IR injury on liver apoptosis ( $\times 200$ ). 6 h after IR injury, TUNEL showed a large number of hepatocyte apoptosis in Male group. However, the apoptotic ratios of hepatic cells in Female and Male+17 $\beta$ -E2 group were significantly lower than in Male group. What's more, compared the Male+17 $\beta$ -E2 group with the Female group, there was no statistically significant. \* $P < 0.05$ , Male group vs Female group and Male+17 $\beta$ -E2 group.  $P > 0.05$ , Female group vs Male+17 $\beta$ -E2 group.



**Figure 4.** Expression of p-Akt after IR injury in rats's hepatocytes. Western blot was used to detect the expression of p-Akt in hepatocytes. The expression of p-Akt in Sham group was low after IR injury. The activity of p-Akt in Female group and Male+17 $\beta$ -E2 group was significantly higher than that in Male group after IR injury. What's more, the activity of p-GSK-3 $\beta$  in Female group and Male+17 $\beta$ -E2 group was significantly higher than that in Male group after IR injury.

phenomenon indicated that E2 could exert its ability to protect hepatic IR injury by activating Akt and inactivating GSK-3 $\beta$ .

In summary, in this study, we found that estrogen play a protective role in rats liver warm IR injury by regulate Akt/GSK-3 $\beta$  signaling pathway. Estrogen therapy might be a "promising treatment" in clinical settings associated with warm IR injury during hepatectomy.

**Acknowledgements**

This work was supported by KeJiaoXingWei (KJXW20120-09).

**Disclosure of conflict of interest**

None.

**Address correspondence to:** Drs. Lei Qin and Xiaohua Yang, Department of General Surgery, The First Affiliated Hospital of Soochow University, 188# Shizi Road, Suzhou 215006, China. E-mail: 13584800496@163.com (LQ); Tel: +86135848-00496; E-mail: yangxiaohua@suda.edu.cn (XHY)

**References**

[1] Chae SU, Ha KC, Piao CS, Chae SW, Chae HJ. Estrogen attenuates cardiac ischemia-reperfu-

to promote cell survival rate [37, 38]. GSK-3 $\beta$  is a downstream signaling molecule of Akt. It is a kind of serine/threonine protein kinase which is a vital influence factor in cell proliferation, cell differentiation and cell apoptosis. What's more, GSK-3 $\beta$  is a continuously activated protein kinase which can promote cell apoptosis. Once Akt is activated, it will cause GSK-3 $\beta$  to be phosphorylated followed by inactivation of GSK-3 $\beta$ . In this trial, we observed that the activity of p-Akt and p-GSK-3 $\beta$  in the Female and Male+17 $\beta$ -E2 group was significantly higher than that in the Male group after IR injury. This

## Estrogen against ischemia-reperfusion injury

- sion injury via inhibition of calpain-mediated bid cleavage. *Arch Pharm Res* 2007; 30: 1225-1225.
- [2] Eltzschig HK, Eckle T. Ischemia and reperfusion—from mechanism to translation. *Nat Med* 2011; 17: 1391-1401.
- [3] Shen SQ, Zhang Y and Xiong CL. The protective effects of 17beta-estradiol on hepatic ischemia-reperfusion injury in rat model, associated with regulation of heat-shock protein expression. *J Surg Res* 2007; 140: 67-76.
- [4] Sivasinprasasn S, Shinlapawittayatorn K, Chattipakorn SC and Chattipakorn N. Estrogenic impact on cardiac ischemic/reperfusion injury. *J Cardiovasc Transl Res* 2016; 9: 23-39.
- [5] Pei B, Yang M, Qi X, Shen X, Chen X and Zhang F. Quercetin ameliorates ischemia/reperfusion-induced cognitive deficits by inhibiting ASK1/JNK3/caspase-3 by enhancing the Akt signaling pathway. *Biochem Biophys Res Commun* 2016; 478: 199-205.
- [6] Zhang W, Zhang JQ, Meng FM and Xue FS. Dexmedetomidine protects against lung ischemia-reperfusion injury by the PI3K/Akt/HIF-1alpha signaling pathway. *J Anesth* 2016; 30: 826-833.
- [7] Ding Q, He X, Hsu JM, Xia W, Chen CT, Li LY, Lee DF, Liu JC, Zhong Q, Wang X and Hung MC. Degradation of Mcl-1 by beta-TrCP mediates glycogen synthase kinase 3-induced tumor suppression and chemosensitization. *Mol Cell Biol* 2007; 27: 4006-4017.
- [8] Ge XH, Zhu GJ, Geng DQ, Zhang HZ, He JM, Guo AZ, Ma LL, Yu DH. Metformin protects the brain against ischemia/reperfusion injury through PI3K/Akt1/JNK3 signaling pathways in rats. *Physiol Behav* 2017; 170: 115-123.
- [9] Carini R, Grazia De Cesaris M, Splendore R, Baldanzi G, Nitti MP, Alchera E, Filigheddu N, Domenicotti C, Pronzato MA, Graziani A, Albano E. Role of phosphatidylinositol 3-kinase in the development of hepatocyte preconditioning. *Gastroenterology* 2004; 127: 914-923.
- [10] Izuishi K, Tsung A, Hossain MA, Fujiwara M, Wakabayashi H, Masaki T, Billiar TR, Maeta H. Ischemic preconditioning of the murine liver protects through the Akt kinase pathway. *Hepatology* 2006; 44: 573-580.
- [11] Meijer L, Flajolet M and Greengard P. Pharmacological inhibitors of glycogen synthase kinase 3. *Trends Pharmacol Sci* 2004; 25: 471-480.
- [12] Uehara T, Bennett B, Sakata ST, Satoh Y, Bilter GK, Westwick JK, Brenner DA. JNK mediates hepatic ischemia reperfusion injury. *J Hepatol* 2005; 42: 850-859.
- [13] Yin H, Chao L and Chao J. Adrenomedullin protects against myocardial apoptosis after ischemia/reperfusion through activation of Akt-GSK signaling. *Hypertension* 2004; 43: 109-116.
- [14] Zhang R, Zhang L, Manaenko A, Ye Z, Liu W, Sun X. Helium preconditioning protects mouse liver against ischemia and reperfusion injury through the PI3K/Akt pathway. *J Hepatol* 2014; 61: 1048-1055.
- [15] Ye Z, Xia P, Cheng ZG and Guo Q. Neuroprotection induced by sevoflurane-delayed post-conditioning is attributable to increased phosphorylation of mitochondrial GSK-3beta through the PI3K/Akt survival pathway. *J Neurol Sci* 2015; 348: 216-225.
- [16] Obligado SH, Ibraghimov-Beskrovnyaya O, Zuk A, Meijer L, Nelson PJ. CDK/GSK-3 inhibitors as therapeutic agents for parenchymal renal diseases. *Kidney Int* 2008; 73: 684-690.
- [17] Wang Y and Tong K. Glycogen synthase kinase-3beta inhibitor ameliorates imbalance of connexin 43 in an acute kidney injury model. *Toxicol Rep* 2015; 2: 1391-1395.
- [18] Yan Y, Li G, Tian X, Ye Y, Gao Z, Yao J, Zhang F, Wang S. Ischemic preconditioning increases GSK-3beta/beta-catenin levels and ameliorates liver ischemia/reperfusion injury in rats. *Int J Mol Med* 2015; 35: 1625-1632.
- [19] Hu S, Begum AN, Jones MR, Oh MS, Beech WK, Beech BH, Yang F, Chen P, Ubeda OJ, Kim PC, Davies P, Ma Q, Cole GM, Frautschy SA. GSK3 inhibitors show benefits in an Alzheimer's disease (AD) model of neurodegeneration but adverse effects in control animals. *Neurobiol Dis* 2009; 33: 193-206.
- [20] Liberman Z and Eldar-Finkelman H. Serine 332 phosphorylation of insulin receptor substrate-1 by glycogen synthase kinase-3 attenuates insulin signaling. *J Biol Chem* 2005; 280: 4422-4428.
- [21] Wang Z, Smith KS, Murphy M, Piloto O, Somerville TC, Cleary ML. Glycogen synthase kinase 3 in MLL leukaemia maintenance and targeted therapy. *Nature* 2008; 455: 1205-1209.
- [22] Zhao G, Ma H, Shen X, Xu GF, Zhu YL, Chen B, Tie R, Qu P, Lv Y, Zhang H, Yu J. Role of glycogen synthase kinase 3beta in protective effect of propofol against hepatic ischemia-reperfusion injury. *J Surg Res* 2013; 185: 388-398.
- [23] Wang XT, Pei DS, Xu J, Guan QH, Sun YF, Liu XM, Zhang GY. Opposing effects of Bad phosphorylation at two distinct sites by Akt1 and JNK1/2 on ischemic brain injury. *Cell Signal* 2007; 19: 1844-1856.
- [24] Fan J, Xu G, Nagel DJ, Hua Z, Zhang N, Yin G. A model of ischemia and reperfusion increases JNK activity, inhibits the association of BAD and 14-3-3, and induces apoptosis of rabbit spinal neurocytes. *Neurosci Lett* 2010; 473: 196-201.

## Estrogen against ischemia-reperfusion injury

- [25] Li H, Chen O, Ye Z, Zhang R, Hu H, Zhang N, Huang J, Liu W, Sun X. Inhalation of high concentrations of hydrogen ameliorates liver ischemia/reperfusion injury through A2A receptor mediated PI3K-Akt pathway. *Biochem Pharmacol* 2017; 130: 83-92.
- [26] Müller C, Dünschede F, Koch E, Vollmar AM, Kiemer AK. Alpha-lipoic acid preconditioning reduces ischemia-reperfusion injury of the rat liver via the PI3-kinase/Akt pathway. *Am J Physiol Gastrointest Liver Physiol* 2003; 285: 769-769.
- [27] Koh PO. Melatonin prevents hepatic injury-induced decrease in Akt downstream targets phosphorylations. *J Pineal Res* 2011; 51: 214-219.
- [28] Breithaupt-Faloppa AC, Thais Fantozzi E, Romero DC, Rodrigues Ada S, de Sousa PT, Lino Dos Santos Franco A, Oliveira-Filho RM, Boris Vargaftig B, Tavares de Lima W. Acute effects of estradiol on lung inflammation due to intestinal ischemic insult in male rats. *Shock* 2014; 41: 208-213.
- [29] Cai M, Ma YL, Qin P, Li Y, Zhang LX, Nie H, Peng Z, Dong H, Dong HL, Hou WG, Xiong LZ. The loss of estrogen efficacy against cerebral ischemia in aged postmenopausal female mice. *Neurosci Lett* 2014; 558: 115-119.
- [30] Fantozzi ET, Breithaupt-Faloppa AC, Ricardo-da-Silva FY, Rodrigues-Garbin S, Romero DC, da Silva Rodrigues A, Riffo-Vasquez Y, Tavares-de-Lima W. Estradiol mediates the long-lasting lung inflammation induced by intestinal ischemia and reperfusion. *J Surg Res* 2018; 221: 1-7.
- [31] Iran-Nejad A, Nematbakhsh M, Eshraghi-Jazi F, Talebi A. Preventive role of estradiol on kidney injury induced by renal ischemia-reperfusion in male and female rats. *Int J Prev Med* 2015; 6: 22-22.
- [32] Ricardo-da-Silva FY, Fantozzi ET, Rodrigues-Garbin S, Oliveira-Filho RM, Vargaftig BB, Breithaupt-Faloppa AC, Tavares de Lima W. Estradiol modulates local gut injury induced by intestinal ischemia-reperfusion in male rats. *Shock* 2017; 48: 477-483.
- [33] Wu CC, Chang CY, Chang ST, Chen SH. 17beta-Estradiol accelerated renal tubule regeneration in male rats after ischemia/reperfusion-induced acute kidney injury. *Shock* 2016; 46: 158-163.
- [34] Osaki M, Oshimura M, Ito H. PI3K-Akt pathway: its functions and alterations in human cancer. *Apoptosis* 2004; 9: 667-676.
- [35] Hetman M, Cavanaugh JE, Kimelman D, Xia Z. Role of glycogen synthase kinase-3beta in neuronal apoptosis induced by trophic withdrawal. *J Neurosci* 2000; 20: 2567-2574.
- [36] Sun X, Zhang Y, Wang J, Wei L, Li H, Hanley G, Zhao M, Li Y, Yin D. Beta-arrestin 2 modulates resveratrol-induced apoptosis and regulation of Akt/GSK3ss pathways. *Biochim Biophys Acta* 2010; 180: 912-918.
- [37] Yin D, Woodruff M, Zhang Y, Whaley S, Miao J, Ferslew K, Zhao J, Stuart C. Morphine promotes Jurkat cell apoptosis through pro-apoptotic FADD/P53 and anti-apoptotic PI3K/Akt/NF-kappaB pathways. *J Neuroimmunol* 2006; 174: 101-107.
- [38] Hernández-Reséndiz S, Palma-Flores C, De Los Santos S, Román-Anguiano NG, Flores M, de la Peña A, Flores PL, Fernández-G JM, Coral-Vázquez RM, Zazueta C. Reduction of no-reflow and reperfusion injury with the synthetic 17beta-aminoestrogen compound Prolame is associated with PI3K/Akt/eNOS signaling cascade. *Basic Res Cardiol* 2015; 110: 1-1.