



International Journal of Pharmacology

ISSN 1811-7775

science
alert

ansinet
Asian Network for Scientific Information

A Preliminary Study of Dexamethasone Against Ischemia/Reperfusion Liver Injury in Rats

¹Amr A. Fouad, ²Mahmoud H. El-Bidawy, ²Arif Mohy Uddin and ³Mohamed T. Yacoubi

¹Division of Pharmacology,

²Division of Physiology,

³Division of Pathology, Department of Biomedical Sciences, College of Medicine,
Al-Ahsa, King Faisal University, Saudi Arabia

Abstract: The hepatoprotective effect of dexamethasone was investigated in rats exposed to ischemia/reperfusion liver injury. Ischemia was induced by clamping the pedicle of the left hepatic lobe for 1 h followed by 3 h of reperfusion. Dexamethasone was administered 24 h before the ischemic insult in two i.p., doses (10 mg kg⁻¹, each) with 12 h interval. Dexamethasone significantly attenuated the ischemia/reperfusion-induced elevations in serum aminotransferase and hepatic levels of tumor necrosis factor- α and nitric oxide. Dexamethasone also significantly compensated deficits in hepatic antioxidant defense mechanisms (reduced glutathione and catalase and superoxide dismutase activities) and suppressed lipid peroxidation observed with liver hypoxia-reoxygenation. This was associated with significant restoration of the ischemia/reperfusion-induced increase in hepatic caspase-3 activity. Neutrophil infiltration and hepatocellular necrosis and apoptosis detected by histopathological examination of liver tissue were markedly ameliorated by pre-ischemic dexamethasone treatment. In conclusion, dexamethasone can be considered a potential therapeutic agent to protect against the major clinical challenge of liver injury resulting from ischemia/reperfusion.

Key words: Dexamethasone, liver, ischemia/reperfusion, inflammation, oxidative stress

INTRODUCTION

Liver ischemia/reperfusion is a major determinant of the outcome of many clinical conditions including liver resection and liver transplantation (Serracino-Ingloft *et al.*, 2001). Tissue injury occurs during the ischemic phase and much injury arises upon restoring the blood supply (reperfusion phase). Reperfusion of an ischemic tissue is associated with intense inflammatory reaction characterized by neutrophil infiltration with increased production of inflammatory mediators and toxic free radicals which aggravate tissue injury (Carden and Granger, 2000; Souza *et al.*, 2001; Wu *et al.*, 2009).

It is increasingly recognized that glucocorticoid effectively inhibit ischemia/reperfusion-induced tissue damage through their prominent anti-inflammatory and immunomodulatory actions. Dexamethasone, the synthetic glucocorticoid, significantly attenuated renal (Takahira *et al.*, 2001), testicular (Yazawa *et al.*, 2002), intestinal (Cavriani *et al.*, 2004; Deveci, 2008), myocardial (Varga *et al.*, 2004) and lung (Wagner *et al.*, 2008;

Sun *et al.*, 2009) injuries caused by ischemia/reperfusion. This can be attributed to the ability of dexamethasone to modulate enzyme systems and to inhibit nuclear factor-kappa B activation leading to reduction in neutrophil accumulation, vascular permeability, interstitial edema and inhibition of production of proinflammatory cytokines and arachidonic acid-derived inflammatory mediators (Cuzzocrea *et al.*, 2008; Sun *et al.*, 2009). However, to the best of this knowledge, no study had been performed to investigate the protective effect of dexamethasone in ischemia/reperfusion liver injury.

Therefore, the present study was conducted in order to evaluate the hepatoprotective effect of dexamethasone pretreatment, in two i.p., doses (10 mg kg⁻¹, each) with 12 h interval, in rats exposed to warm ischemia/reperfusion liver injury. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured. Hepatic levels of tumor necrosis factor- α (TNF- α) and Nitric Oxide (NO) were assessed. The oxidant/antioxidant status of the liver was evaluated by measuring hepatic malondialdehyde (MDA) and reduced glutathione (GSH) levels and catalase and superoxide dismutase (SOD)

activities. Also, caspase-3 activity (as an indicator of hepatocellular apoptosis) was measured in liver homogenate. In addition, histopathological examination of liver tissue was performed.

MATERIALS AND METHODS

Animals: Male Sprague-Dawley rats, weighing 200 ± 10 g were obtained from the Animal House, College of Medicine, Al-Ahsa, King Faisal University. The animals were housed at 25°C , $45 \pm 5\%$ humidity and 12 h light/12 h dark cycle. They were supplied with standard laboratory chow and water *ad libitum* and left to acclimatize for 1 week before the experiments. The experimental protocol was approved by the Local Animal Care Committee and all the experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals. This study was started in June 2008 and completed at January 2009. The study was performed at College of Medicine, Al-Ahsa, King Faisal University, Saudi Arabia.

Drugs: Dexamethasone sodium phosphate, white powder and urethane, white crystals (Sigma Chemical Company, USA). Both drugs were prepared in normal saline (0.9% NaCl). The used dose of dexamethasone was selected based on a previous studied by Mogilner *et al.* (2006).

Experimental protocol: The rats were randomly assigned to four groups. The first (control) group ($n = 6$) received i.p., normal saline, in two doses with 12 h interval and sham-operated 12 h following the last injection. The rats in the second and third groups ($n = 8$, each) received two i.p., injections of normal saline or dexamethasone (10 mg kg^{-1} , each), respectively, with 12 h interval and then subjected to warm ischemia/reperfusion liver injury 12 h after the last injection. The fourth group animals ($n = 8$) were treated with i.p. dexamethasone in two doses (10 mg kg^{-1} , each) with 12 h interval and sham-operated 12 h later.

Surgical procedure: The surgical technique was followed according to the method described by Kooij *et al.* (1994). Following urethane anesthesia (1.6 g kg^{-1} , i.p.), a mid-line abdominal incision was performed and the liver hilum was gently exposed. A fine atraumatic vascular clip was applied to the pedicle supplying the left hepatic lobe. This allowed for selective interruption of the blood supply of the left hepatic lobe, while preserving that of the right lobe. Thus, gastrointestinal congestion and hemodynamic

instability resulting from complete occlusion of the hepatic pedicle were avoided. Warm saline was injected into the abdomen which is temporarily closed. The blood flow to the left hepatic lobe was occluded for 1 h (ischemic phase), then the clip was removed after reopening the abdomen to begin the reperfusion phase which lasted for 3 h, after which the animal was sacrificed by exsanguination and sampling was done.

Sampling and biochemical analysis: Blood was aspirated with a syringe through a puncture in the right atrium. Blood samples were centrifuged at 5000 rpm for 10 min to obtain clear sera which were stored at -20°C . Subsequently, serum alanine aminotransferase and aspartate aminotransferase levels were measured using colorimetric assay kits according to the recommendations of the manufacturer (Randox Laboratories Ltd., UK).

The left hepatic lobe was resected, washed with cold saline and then kept at -80°C . Liver samples were subsequently homogenized in potassium phosphate buffer (0.05 M, pH 7.4). The homogenates were centrifuged at 5000 rpm for 10 min at 4°C and the resulting supernatant was used for analysis of biochemical parameters.

The level of TNF- α in liver homogenates was determined by enzyme-linked immunosorbent assay (ELISA) using rat tumor necrosis factor- α immunoassay kit according to the recommendations of the manufacturer (Biosource Europe, Belgium).

Also, the levels of NO, MDA and GSH and catalase and SOD activities were measured in liver homogenates using colorimetric assay kits as recommended by the manufacturer (Biodiagnostic, Egypt).

In addition, caspase-3 activity was measured using colorimetric assay kit according to the manufacturer's recommendations (Biosource Europe, Belgium). In brief, the chromophore p-nitroanilide (pNA) released from DEVD-pNA by caspase-3 was measured spectrophotometrically. The absorbance of pNA from samples of different groups was compared with that of sham-operated control and the fold increase in caspase-3 activity was determined.

Liver histopathological examination: Parts of the isolated hepatic tissue were fixed in 10% formalin solution and dehydrated in ascending grades of alcohol and embedded in paraffin. Five micron-thickness sections were taken, stained with hematoxylin and eosin and evaluated by light microscopy. Liver histopathological examination was performed by a pathologist unaware of the treatment protocol. The number of infiltrating neutrophils was

counted in 10 separate microscopic fields at a magnification of x400 and the mean number was calculated for each group. Also, hepatocellular necrosis and apoptosis induced by ischemia/reperfusion was assessed.

Statistics: The data are expressed as Mean±SEM. The results were analyzed by one-way Analysis of Variance (ANOVA) followed by Tukey test for multiple comparisons using SPSS for Windows (Version 11). Significance was considered at p<0.05.

RESULTS AND DISCUSSION

Effect of dexamethasone on serum aminotransferase: Rats exposed to liver ischemia/reperfusion showed significant increases in serum ALT and AST levels as compared to the control sham-operated animals. However, significant reductions in the levels of serum aminotransferase were observed with dexamethasone pretreatment (Fig. 1).

Effect of dexamethasone on tumor necrosis TNF-α and NO levels: Ischemia/reperfusion resulted in significant elevations of hepatic tumor necrosis factor-α and nitric oxide levels as compared to the corresponding values in the sham-operated control group. The animals received pre-ischemic dexamethasone treatment showed significantly lower levels of hepatic TNF-α (Fig. 2) and NO (Fig. 3) as compared to dexamethasone-untreated rats exposed to ischemia/reperfusion-liver injury.

Effect of dexamethasone on liver oxidant/antioxidant status: Pretreatment with dexamethasone significantly restored the hepatic antioxidant defense mechanisms exhausted during ischemia/reperfusion (GSH level and catalase and SOD activities). This was accompanied by a significant inhibition of ischemia/reperfusion-induced hepatic lipid peroxidation (Table 1).

Effect of dexamethasone on hepatocellular apoptosis: Ischemia/reperfusion caused four fold-increase in caspase-3 protease activity in rat liver homogenates, while dexamethasone-treated rats showed only one and half fold-increase in comparison to the sham-operated control animals (Fig. 4).

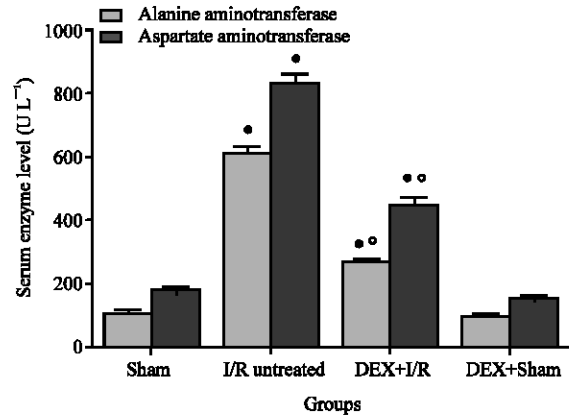


Fig. 1: Effect of pre-ischemic dexamethasone (DEX) treatment on serum alanine aminotransferase and aspartate aminotransferase levels in rats exposed to ischemia/reperfusion (I/R) liver injury. Data are expressed as Mean±SEM, *p<0.05 vs. sham-operated (sham) group, °p<0.05 vs. I/R untreated group

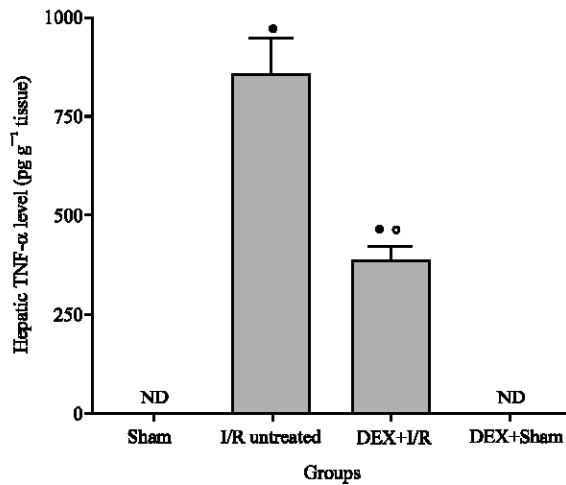


Fig. 2: Effect of pre-ischemic dexamethasone (DEX) treatment on hepatic level of tumor necrosis factor-α (TNF-α) in rats exposed to ischemia/reperfusion (I/R) liver injury. Data are expressed as Mean±SEM, ND: Non-detectable, *p<0.05 vs. sham-operated (sham) group, °p<0.05 vs. I/R untreated group

Table 1: Effects of pre-ischemic dexamethasone (DEX) treatment on hepatic malondialdehyde (MDA) and reduced glutathione (GSH) levels and catalase and superoxide dismutase (SOD) activities in rats exposed to ischemia/reperfusion (I/R) liver injury

Groups	Measured parameters			
	MDA (nmol g ⁻¹ tissue)	GSH (mmol g ⁻¹ tissue)	Catalase (U g ⁻¹ tissue)	SOD (U mg ⁻¹ protein)
Sham (n = 6)	101.13±4.22	11.34±1.54	10.78±0.66	82.45±4.11
I/R untreated (n = 8)	254.37±10.15*	4.17±0.24*	4.12±0.32*	46.44±2.34*
DEX+I/R (n = 8)	136.55±4.87*#	9.76±1.03#	7.52±0.51*#	73.16±3.11#
DEX+Sham (n = 8)	94.89±5.04	12.55±2.01	9.84±0.72	86.12±5.01

Data are expressed as Mean±SEM. *p<0.05 vs. sham-operated (sham) group, #p<0.05 vs. I/R untreated group

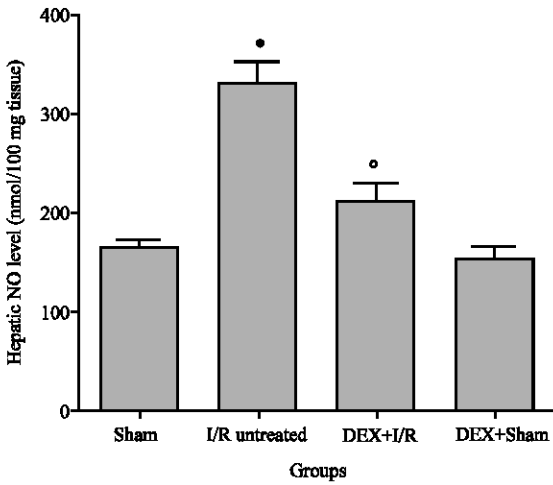


Fig. 3: Effect of pre-ischemic dexamethasone (DEX) treatment on hepatic Nitric Oxide (NO) level in rats exposed to ischemia/reperfusion (I/R) liver injury. Data are expressed as Mean±SEM, *p<0.05 vs. sham-operated (sham) group, °p<0.05 vs. I/R untreated group

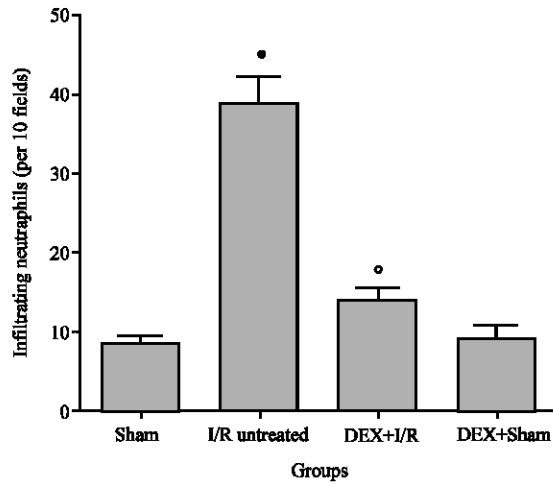


Fig. 5: The number of infiltrating neutrophils in liver tissue following ischemia/reperfusion (I/R) injury in rats treated and untreated with dexamethasone (DEX). Data are expressed as Mean±SEM, *p<0.05 vs. sham-operated (sham) group, °p<0.05 vs. I/R untreated group

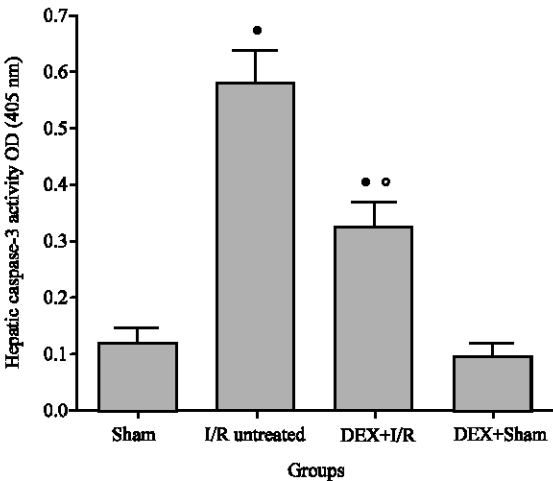


Fig. 4: Effect of pre-ischemic dexamethasone (DEX) treatment on hepatic caspase-3 activity in rats exposed to ischemia/reperfusion (I/R) liver injury. Data are expressed as Mean±SEM, *p<0.05 vs. sham-operated (sham) group, °p<0.05 vs. I/R untreated group

Effect of dexamethasone on liver histopathological examination: Hepatic ischemia/reperfusion resulted in a significant increase in the number of infiltrating neutrophils in the liver tissue. Pre-ischemic dexamethasone treatment significantly reduced the number of neutrophils infiltrating to the liver as a result of ischemia/reperfusion injury (Fig. 5).

Also, histological examination revealed marked hepatocellular damage induced by ischemia/reperfusion in the form of ballooning degeneration, cytoplasmic vacuolation, necrosis and apoptosis. The observed histopathological changes in liver tissue were markedly ameliorated as a result of pre-ischemic dexamethasone treatment (Fig. 6).

Hepatic pedicle clamping (Pringle's maneuver) is often used clinically during liver surgery to reduce intraoperative blood loss. However, the resulting ischemia/reperfusion injury is a major cause of hepatic failure contributing to postoperative morbidity and mortality (Belghiti *et al.*, 1999). Ischemia followed by reperfusion causes up-regulation of endothelial adhesion molecules facilitating the cascade processes of neutrophil infiltration. The release of inflammatory cytokines and reactive oxygen species from activated neutrophils is responsible for ischemia/reperfusion-induced tissue damage (Carden and Granger, 2000; Kim and Lee, 2008; Sehrlirli *et al.*, 2008).

In the present study, pre-ischemic dexamethasone treatment protected against ischemia/reperfusion liver injury in rats. Dexamethasone pretreatment significantly attenuated the ischemia/reperfusion-induced deterioration in the measured biochemical parameters and also mitigated neutrophil infiltration and hepatocellular damage observed by histopathological examination.

In agreement with the present results, dexamethasone pretreatment significantly reduced neutrophil accumulation and attenuated the increase in tissue

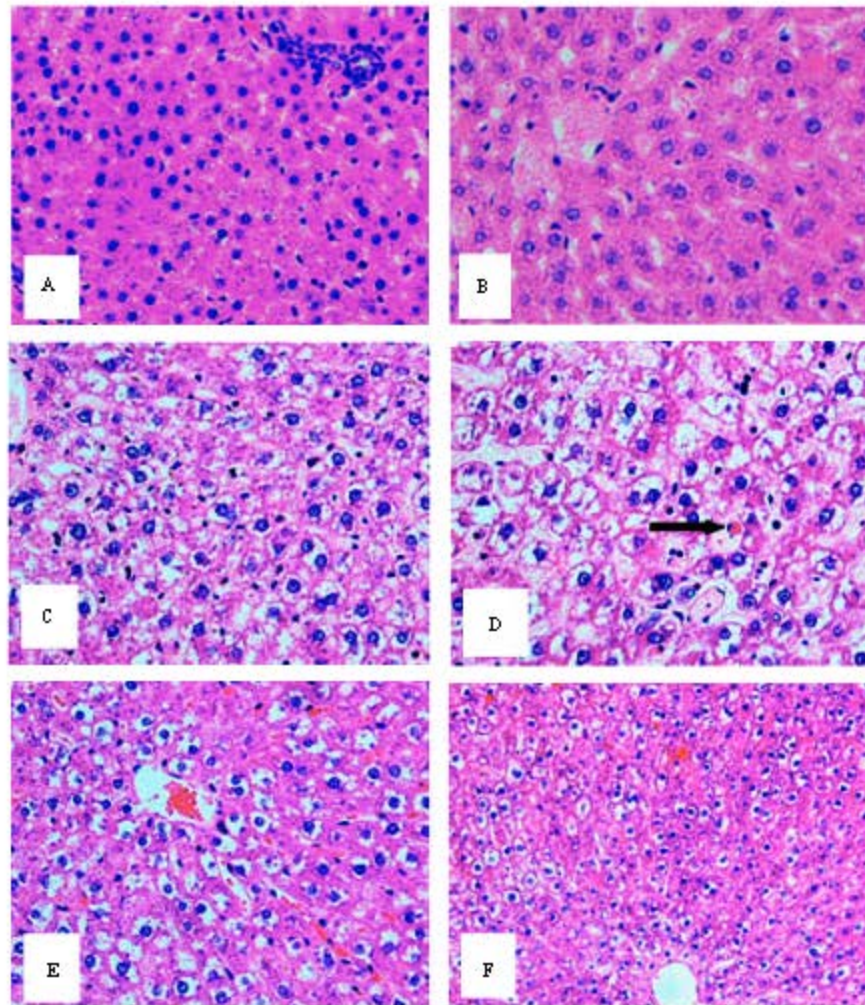


Fig. 6: Photomicrographs of rat liver (H and E, x400) from: (A) sham-operated control group, (B) dex amethasone sham-operated group showing normal hepatic architecture, (C and D) ischemia/reperfusion untreated group showing marked ballooning of hepatocytes, cytoplasmic vacuolation, cellular necrosis and apoptosis (black arrow), with excessive neutrophil infiltration and (E and F) pre-ischemic dex amethasone-treated group displaying remarkable improvement in the histological picture with marked amelioration of ischemia/reperfusion-induced hepatocellular damage and reduced number of infiltrating neutrophils

TNF- α level in different animal models of ischemia/reperfusion (Takahira *et al.*, 2001; Cavriani *et al.*, 2004; Vieira *et al.*, 2005).

In addition, TNF- α induces expression of cell adhesion molecules which causes neutrophils and platelets to adhere to sinusoidal lumen resulting in impaired microcirculation and hepatocellular damage (Essari *et al.*, 1997). Also, TNF- α causes up-regulation of inducible nitric oxide synthase (iNOS) with increased NO production (Morris and Billiar, 1994). Excess NO reacts with superoxide anion to generate peroxytrinitrite radical that causes further cellular damage and depletes

intracellular GSH increasing the susceptibility to oxidative stress (Liu *et al.*, 1997).

Earlier studies, in accordance with the present one, demonstrated that dexamethasone attenuated the elevation in NO level induced by various inflammatory conditions including ischemia/reperfusion. Dexamethasone inhibits nuclear factor-kappaB and prevents iNOS protein expression (Wang *et al.*, 2002; Cuzzocrea *et al.*, 2008).

The anti-inflammatory effect of dexamethasone is mainly due to activation of the glucocorticoid cytoplasmic receptors that control transcription of certain genes

encoding regulatory proteins. This results in reduction in neutrophil accumulation and inhibition of proinflammatory cytokine production (Romero *et al.*, 2003; Wallerath *et al.*, 2004).

In accordance with the present study, it was reported that glucocorticoid can prevent lipid peroxidation of biological membranes by scavenging toxic free radicals (Korompilias *et al.*, 1996; Marumo *et al.*, 1998). Glucocorticoid reduce the generation of reactive oxygen species (Komatsu *et al.*, 2007), increase antioxidant enzyme activity (Sadowska *et al.*, 2007; Chandrasekar *et al.*, 2008), decrease MDA level (Ozturk *et al.*, 2006) and elevate GSH level (Nagashima and Ogita, 2006) in tissues exposed to oxidative stress. Glucocorticoid may affect the antioxidant enzymes at the level of transcription or act by influencing trace element accumulation (Sadowska *et al.*, 2007). Also, the antioxidant effect of glucocorticoid may be secondary to inhibition of TNF- α and excess NO which are capable of inducing prolonged neutrophil activation with increased production of reactive oxygen species.

Recent studies showed that apoptosis is a significant contributor to cell death following ischemia/reperfusion. Both TNF- α and reactive oxygen species are implicated in ischemia/reperfusion-mediated cell apoptosis by activating the caspase family of protease (Faubion and Gores, 1999; Kim and Lee, 2008).

It was demonstrated that dexamethasone administration significantly reduced ischemia/reperfusion-induced apoptotic death in rat myocardium (Pearl *et al.*, 2002; Varga *et al.*, 2004) and testicular germ cells (Yazawa *et al.*, 2002; Mogilner *et al.*, 2006). The antiapoptotic effect of dexamethasone is attributed to its ability to reduce the generation of proinflammatory mediators and reactive oxygen species during ischemia/reperfusion. The present results, in agreement with the previous ones, showed that pre-ischemic dexamethasone treatment attenuated ischemia/reperfusion-induced hepatocellular apoptosis as evidenced by significant reduction in liver caspase-3 activity.

In conclusion, the present results indicate that pre-ischemic dexamethasone treatment effectively protected against warm ischemia/reperfusion liver injury. The anti-inflammatory and antioxidant activities of dexamethasone can be considered the main factors responsible for the observed hepatoprotective effect. Dexamethasone therefore represents a therapeutic option for clinical application to ameliorate ischemia/reperfusion-induced liver damage. However, this needs to be evaluated by further investigations.

REFERENCES

- Belghiti, J., R. Noun, R. Malafosse, P. Jagot and A. Sauvanet *et al.*, 1999. Continuous versus intermittent portal triad clamping for liver resection: A controlled study. *Ann. Surg.*, 229: 369-375.
- Carden, D.L. and J. Granger, 2000. Pathophysiology of ischemia-reperfusion injury. *J. Pathol.*, 190: 255-266.
- Cavriani, G., R.M. Oliveira-Filho, A.G. Trezena, Z. Lucia da Silva and H.V. Domingos *et al.*, 2004. Lung microvascular permeability and neutrophil recruitment are differently regulated by nitric oxide in a rat model of intestinal ischemia-reperfusion. *Eur. J. Pharmacol.*, 494: 241-249.
- Chandrasekar, I., A. Eis and G.G. Konduri, 2008. Betamethasone attenuates oxidant stress in endothelial cells from fetal lambs with persistent pulmonary hypertension. *Pediatr. Res.*, 63: 67-72.
- Cuzzocrea, S., S. Bruscoli, E. Mazzon, C. Crisafulli and V. Donato *et al.*, 2008. Peroxisome proliferator-activated receptor-alpha contributes to the anti-inflammatory activity of glucocorticoid. *Mol. Pharmacol.*, 73: 323-337.
- Deveci, E., 2008. Role of allopurinol, verapamil, dexamethasone and trifluoperazine as prophylactic agents in intestinal ischemia-reperfusion. *Anal. Quant. Cytol. Histol.*, 30: 99-104.
- Essani, N.A., M.L. Bajt, A. Farhood, S.L. Vonderfecht and H. Jaeschke, 1997. Transcriptional activation of vascular cell adhesion molecule-1 gene *in vivo* and its role in the pathophysiology of neutrophil-induced liver injury in murine endotoxin shock. *J. Immunol.*, 158: 5941-5948.
- Faubion, W.A. and G.J. Gores, 1999. Death receptors in liver biology and pathobiology. *Hepatology*, 29: 1-4.
- Kim, S.H. and S.M. Lee, 2008. Cytoprotective effect of melatonin against necrosis and apoptosis induced by ischemia/reperfusion injury in rat liver. *J. Pineal Res.*, 44: 165-171.
- Komatsu, F., H. Kudoh and Y. Kagawa, 2007. Evaluation of oxidative stress and effectiveness of low-dose glucocorticoid therapy on exacerbation of chronic obstructive pulmonary disease. *J. Gerontol. Ser. A: Biol. Sci. Med. Sci.*, 62: 459-464.
- Kooij, A., H.J. Schiller, M. Schijns, C.J. Van Noorden and W. Frederiks, 1994. Conversion of xanthine dehydrogenase into xanthine oxidase in rat liver and plasma at the onset of reperfusion after ischemia. *Hepatology*, 19: 1488-1495.
- Korompilias, A.V., L.E. Chen, A.V. Seaber and J.R. Urbaniak, 1996. Actions of glucocorticosteroids on ischemic-reperfused muscle and cutaneous tissue. *Microsurgery*, 17: 495-502.

- Liu, P., C.E. Hock, R. Nagele and P.Y. Wong, 1997. Formation of nitric oxide, superoxide and peroxynitrite in myocardial ischemia-reperfusion. *Am. J. Physiol.*, 272: 2327-2336.
- Marumo, T., V.B. Schini-Kerth, R.P. Brandes and R. Busse, 1998. Glucocorticoid inhibit superoxide anion production and p22 phox mRNA expression in human aortic smooth muscle cells. *Hypertension*, 32: 1083-1088.
- Mogilner, J.G., Y. Elenberg, M. Lurie, E. Shiloni, A.G. Coran and I. Sukhotnik, 2006. Effect of dexamethasone on germ cell apoptosis in the contralateral testis after testicular ischemia-reperfusion injury in the rat. *Fertil. Steril.*, 85: 1111-1117.
- Morris, S.M. Jr. and T.R. Billiar, 1994. New insights into the regulation of inducible nitric oxide synthesis. *Am. J. Physiol.*, 266: E829-E839.
- Nagashima, R. and K. Ogita, 2006. Enhanced biosynthesis of glutathione in the spiral ganglion of the cochlea after *in vitro* treatment with dexamethasone in mice. *Brain Res.*, 1117: 101-108.
- Ozturk, H., H. Eken, H. Ozturk and H. Buyukbayram, 2006. Effects of dexamethasone on small bowel and kidney oxidative stress and histological alterations in bile duct-ligated rats. *Pediatr. Surg. Int.*, 22: 709-718.
- Pearl, J.M., D.P. Nelson, S.M. Schwartz, C.J. Wagner, S.M. Bauer, E.A. Setser and J.Y. Duffy, 2002. Glucocorticoid reduce ischemia-reperfusion-induced myocardial apoptosis in immature hearts. *Ann. Thorac. Surg.*, 74: 830-836.
- Romero, I.A., K. Radewicz, E. Jubin, C.C. Michel, J. Greenwood, P.O. Couraud and P. Adamson, 2003. Changes in cytoskeletal and tight junctional proteins correlate with decreased permeability induced by dexamethasone in cultured rat brain endothelial cells. *Neurosci. Lett.*, 344: 112-116.
- Sadowska, A.M., B. Klebe, P. Germonpre and W.A. De Backer, 2007. Glucocorticosteroids as antioxidants in treatment of asthma and COPD. New application for an old medication. *Steroids*, 72: 1-6.
- Sehirli, O., Y. Ozel, E. Dulundu, U. Topaloglu, F. Ercan and G. Sener, 2008. Grape seed extract treatment reduces hepatic ischemia-reperfusion injury in rats. *Phytother. Res.*, 22: 43-48.
- Serracino-Inglott, F., N.A. Habib and R.T. Mathie, 2001. Hepatic ischemia-reperfusion injury. *Am. J. Surg.*, 181: 160-166.
- Souza, D.G., G.D. Cassali, S. Poole and M.M. Teixeira, 2001. Effects of inhibition of PDE4 and TNF-alpha on local and remote injuries following ischemia and reperfusion injury. *Br. J. Pharmacol.*, 134: 985-994.
- Sun, J., D. Yang, S. Li, Z. Xu, X. Wang and C. Bai, 2009. Effects of curcumin or dexamethasone on lung ischaemia-reperfusion injury in rats. *Eur. Respir. J.*, 33: 398-404.
- Takahira, R., K. Yonemura, Y. Fujise and A. Hishida, 2001. Dexamethasone attenuates neutrophil infiltration in the rat kidney in ischemia/reperfusion injury: The possible role of nitroxyl. *Free Radic. Biol. Med.*, 31: 809-815.
- Varga, E., N. Nagy, J. Lazar, G. Czifra, I. Bak, T. Biro and A. Tosaki, 2004. Inhibition of ischemia/reperfusion-induced damage by dexamethasone in isolated working rat hearts: The role of cytochrome c release. *Life Sci.*, 75: 2411-2423.
- Vieira, A.T., V. Pinho, L.B. Lepsch, C. Scavone and I.M. Ribeiro *et al.*, 2005. Mechanisms of the anti-inflammatory effects of the natural secosteroids physalins in a model of intestinal ischemia and reperfusion injury. *Br. J. Pharmacol.*, 146: 244-251.
- Wagner, E.M., J. Sánchez, J.Y. McClintock, J. Jenkins and A. Moldobaeva, 2008. Inflammation and ischemia-induced lung angiogenesis. *Am. J. Physiol. Lung Cell. Mol. Physiol.*, 294: L351-L357.
- Wallerath, T., A. Godecke, A. Molojavyi, H. Li, J. Schrader and U. Forstermann, 2004. Dexamethasone lacks effect on blood pressure in mice with disrupted endothelial NO synthase gene. *Nitric Oxide*, 10: 36-41.
- Wang, Y.P., C. Sato, K. Mizoguchi, Y. Yamashita, M. Oe and H. Maeta, 2002. Lipopolysaccharide triggers late preconditioning against myocardial infarction via, inducible nitric oxide synthase. *Cardiovasc. Res.*, 56: 33-42.
- Wu, Y.J., Q. Ling, X.H. Zhou, Y. Wang, H.Y. Xie, J.R. Yu and S.S. Zheng, 2009. Urinary trypsin inhibitor attenuates hepatic ischemia-reperfusion injury by reducing nuclear factor-kappa B activation. *Hepatobiliary Pancreat. Dis. Int.*, 8: 53-58.
- Yazawa, H., I. Sasagawa, Y. Suzuki and T. Nakada, 2002. Glucocorticoid hormone can suppress apoptosis of rat testicular germ cells induced by testicular ischemia. *Fertil. Steril.*, 75: 980-985.