Co-administration of Melatonin and Dexamethasone Attenuates Lung Tissue Injury after Liver Ischemia/Reperfusion

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Abstract: Ischemia-reperfusion injury (IRI) is a common and important clinical problem in many different organ systems. Once the blood flow and oxygen supply are reestablished, reperfusion enhances the injury caused by the ischemic period .This phenomenon, known as ischemia/reperfusion (IR) injury, impacts directly on liver viability, especially during transplantation and liver surgery. The purpose of this study was to evaluate combination pretreatment of melatonin (MEL) and dexamethasone (DEX) on liver I/R model of lung injury. Male Wistar rats (n=60) were assigned to 5 groups of 12 animals each: 1: Sham; Laparatomy without I/R 2: I/R; underwent hepatic I/R. 3: I/R+MEL; hepatic I/R+ injected intraperitoneally melatonin (20 mg/kg). 4: I/R+DEX; hepatic I/R+ injected intravenously dexamethasone (10 mg/kg). 5: I/R+MEL+DEX; hepatic I/R+ injected intraperitoneally melatonin + injected intravenously dexamethasone. Liver subjected to ischemia by clamping portal triad for 30 minutes and was reperfused for 6 hours after ischemia by removing the clamps. Levels of glutathione peroxidase (GPx) and superoxide dismutase (SOD) decreased after hepatic I/R in all groups; levels of GPx and SOD were higher in I/R+MEL+DEX group comparing to I/R, I/R+MEL and I/R+DEX groups significantly($P<0.05$), and they were higher in I/R+MEL group comparing to I/R and I/R+DEX groups but it was not significant. Lung tissue malondialdehyde (MDA) ,lung injury index, and apoptotic index increased after hepatic I/R. Tissue MDA, tissue injury index and apoptotic index were lower in I/R+MEL+DEX group comparing to I/R, I/R+MEL and I/R+DEX groups significantly($P<0.05$), and in I/R+MEL was lower than I/R+DEX group but it was not significant. Co-administration of melatonin and dexamethasone had better results in decreasing the lung injury after hepatic I/R , comparing to administering each of them alone.

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1. Introduction

Ischemia-reperfusion injury (IRI) is a common and important clinical problem in many different organ systems. Once the blood flow and oxygen supply are reestablished, reperfusion enhances the injury caused by the ischemic period, aggravating the damage caused at the cellular level (Romanque et al., 2005). This phenomenon, known as ischemia/reperfusion (IR) injury, impacts directly on liver viability, especially during transplantation and liver surgery (Henderson et al., 1999). It involves the transient deprivation of blood flow and oxygen, and the return of blood flow during reperfusion with concomitant release of oxygen-free radicals (OFR), cytokines/chemokines, and up-regulation of adhesion molecules with consequent cellular and organ dysfunction. Ischemia/reperfusion injury is a multi-factorial antigen independent inflammatory condition that has both immediate and long-term effects on the allograft. According to the classic understanding, acute ischemia leads to the activation of the endothelium with an increase in permeability and expression of many adhesion molecules. These molecules are crucial for the recruitment and infiltration of effector cells into the post-ischemic tissue. The adherent leukocytes release reactive oxygen species (ROS) and a variety of cytokines, enhancing the inflammatory reaction. Subsequently, the leukocytes transmigrate and enter the subendothelial space (Boros et al., 2006). Oxygen free radicals (OFR) are one of the earliest and most important components of tissue injury after reperfusion of ischemic organs. The major OFR include the superoxide radical, hydroxyl, and hydrogen peroxide. The prime sources of OFR production in ischemic tissue include cytosolic xanthine oxidase (XO), Kupffer cells, and adherent PMN (Jaeschke et al., 1991). OFR-induced injury targets proteins, enzymes, nucleic acids, cytoskeleton, cell membranes, and lipid peroxides,

resulting in decreased mitochondrial function, and lipid peroxidation (McCord et al., 1985). The damage caused by OFR leads to the loss of microvascular integrity and decreased blood flow. Endogenous antioxidant compounds, such as superoxide dismutase, catalase, glutathione, and beta-carotene, may limit the effects of OFR but these systems can become overwhelmed by large quantities of OFR (Atalla et al., 1985). Melatonin, a known neuroendocrine mediator has pleiotropic bioactivities such as hormonal, neurotransmitter, immunomodulator, and biological modifier actions, which are mediated through interactions with high-affinity membrane-bound or nuclear receptors (Lerner et al., 1958). Moreover, melatonin itself can function as a free-radical scavenger and broad-spectrum antioxidant or as activator of pathways protective against oxidative stress or metabolic modulator (Reiter et al., 2003; Reiter et al., 2002). Melatonin is also an anti-inflammatory agent known to reduce several hallmarks of inflammation. Melatonin down-regulates a variety of pro-inflammatory cytokines such as interleukin (IL)-1b, IL-6 and tumour necrosis factor-a by preventing the translocation of nuclear factorkappa B to the nucleus and its binding to DNA (Reiter et al., 2000; Rodriguez et al., 2007). The glucocorticoid dexamethasone is currently used as an anti-inflammatory and immunosuppressive drug. Steroid therapy suppresses tissue injury by a variety of mechanisms that include the suppression of oxygen free radicals, the formation of arachidonic acid derivatives, and the production of lysosomal proteases (cathepsins) and cytokines (Wang et al., 2008). A possible explanation includes the protection of the cell membrane by stabilization of the lysosomal membrane or the inhibition of circulating toxic substances liberated from the liver during ischemia. Corticosteroids have been used for the suppression of inflammatory mechanisms and are used in warm and cold hepatic I-R injury (Saidi et al., 2007). The aim of this study was to evaluate protective effect of melatonin and dexamethasone on lung tissue after liver ischemia reperfusion.

2. Materials and methods

2.1. Animals

Male Wistar rats were obtained from laboratory animals care center of Tabriz University of Medical Sciences (Tabriz, Iran). Rats were weighing 240-285 g and they were approximately 6-7 weeks old. Fluorescent lights were on for 12 h/day. The experiments were performed according to the guidelines of our Ethics Committee. After an adaptation period of 3 days, rats (n=60) were assigned to 5 groups of 12 animals each: Group 1: Sham; injected with (0.9% saline). Group 2: I/R; injected with (0.9% saline) and underwent hepatic I/R. Group 3: I/R+MEL; hepatic I/R+ injected intraperitoneally (i.p.) melatonin (20 mg/kg). Group 4: I/R+DEX; hepatic I/R+ injected intravenously (i.v) dexamethasone (10 mg/kg). Group 5: I/R+MEL+DEX; hepatic I/R+ injected intraperitoneally (i.p) melatonin (20 mg/kg) + injected intravenously (i.v) dexamethasone (10 mg/kg).

2.2. Melatonin treatment

Melatonin (MEL) was purchased from Merck Chemical Co (Darnstadt, Germany). MEL was freshly dissolved in absolute ethanol and further dilutions were made in saline. The final concentration of ethanol was 1%. An equal volume of ethanol, as used in the MEL solution, was added to the saline solution. MEL was administered intraperitoneally (i.p.) at 20 mg/kg, 1 h prior to the trial (Zencirci et al., 2010).

2.3. Dexamethasone treatment

Dexamethasone was administered as a single injection of 10 mg/kg. Drug was injected intravenously (i.v.), 20 min before the trial (Chen et al., 2009).

2.4. Surgery protocol

Rats were anesthetized with ketamin (50mg/kg) and xylazine (10mg/kg). They were placed in the supine position. After the midline laparotomy, the liver was made ischemic by clamping both the portal vein and the hepatic artery, After 30 min of ischemia, the clamp was released to allow reperfusion of the liver for 6 hours. The abdominal cavity was closed, and the animals were allowed to recover with free access to food and water. After 6 h rats were anesthetized and lung tissue samples were excised from the same region of the liver for determination of biomarkers of oxidative stress and histopathologic examination (Dibazar et al., 2008).

2.5. Assay of antioxidant enzymes

The left lung was frozen in liquid nitrogen and stored at -80°C until further preparation. In order to measure anti-oxidant enzyme activity, the lung samples were homogenized in 1.15% KCl solution. Superoxide dismutase (SOD) activity in lung tissue was determined by using xanthine and xanthine oxidase to generate superoxide radicals which then react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye. The SOD activity was then measured by the degree of inhibition of this reaction (Ransod, Randox

Laboratories Ltd., Antrim, United Kingdom). Results obtained as SOD Unit/mg protein (Paoletti et al., 1986). Glutathione peroxidase (GPx) activity in lung tissue was measured using the method described by Paglia and Valentine. GPx catalyses the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidised glutathione is immediately is converted to reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm is measured (Ransod, Randox Laboratories Ltd., Antrim, UK). Results obtained as GPx Unit/mg protein (Paglia et al., 1967).

2.6. Tissue MDA level

Tissue malondialdehyde was determined by the method of Uchiyama and Mihara (Mihara et al., 1983) 3-mL aliquot of 1% phosphoric acid and 1 mL of 0.6% thiobarbituric acid solution were added to 0.5 mL of 10% tissue homogenate. The mixture was heated in boiling water for 45 minutes. After cooling, the color was extracted into 4 mL of n-butanol. The absorbance was measured in a spectrophotometer (Amersham Pharmacia Biotech UK Ltd., Little Chalfont, Buckinghamshire, UK) at 532 nm ($\acute{\varepsilon} = 0.56 \times 10^5$ mol/L^{-1} cm^{-1}). The amounts of lipid peroxides calculated as thiobarbituric acid reactive substances (TBARS) of lipid peroxidation were expressed as nMol/ml.

2.7. Histopathology

Lung tissue samples were saved and fixed in 10% formalin. Paraffin-embedded liver tissue was cut into 5-µm thick sections, deparaffinized in xylene, and rehydrated through a series of decreasing concentrations of ethanol. The sections were stained with hematoxylin and eosin and analyzed under a light microscope. The lung samples were then graded histologically according to the severity of injury by using a predetermined scoring system for lung tissue. The assessment was expressed as sum of the individual score grades from 0(no findings), 1(mild), 2(moderate), to 3(severe) for each of the following 6 parameters from lung sections: interstitial edema, alveolar edema, alveolar hemorrhage, atelectasis, inflammatory cellular infiltration, and pulmonary congestion (Kirimlioglu et al., 2008).

2.8. TUNEL assay

Terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL) assay was used to assess the DNA fragmentation in paraffin-embedded tissue sections. The assay was conducted according to the manufacturer's instructions (Roche, Mannheim, Germany). Total cell population and TUNEL positive cells were counted. TUNEL positive cells were expressed as percentage of total cells (Zegdi et al., 2003).

2.9. Statistical analysis

Data were expressed as means ± SD. Differences among various groups were tested for statistical significance using the one-way ANOVA test and Tukeys posttest. A P value of less than 0.05 denoted the presence of a statistically significant difference.

3. Results

3.1. SOD and GPx level

Level of SOD and GPx antioxidant enzymes were decreased in all of the groups subjected to hepatic I/R, but it was less severe in melatonin and dexamethasone pretreated groups. SOD and GPx levels in I/R+MEL+DEX group were higher than I/R+ MEL and I/R+DEX groups significantly ($P<0.05$,Table 1). SOD and GPx levels were higher in I/R+ MEL group comparing to I/R+DEX group but it was not significant.

3.2. MDA level

MDA level, as an index of lipid peroxidation, increased significantly in all of the groups subjected to hepatic I/R. MDA level was lower in I/R+MEL+DEX group comparing to I/R+ MEL group and I/R+DEX group significantly ($p<0.05$, Table 1). MDA level was lower in I/R+ MEL group comparing to I/R+DEX group but it was not significant.

3.3. Histological changes

At 6 hours post I/R, lung tissue injury index in I/R group was significantly higher than I/R+ MEL, I/R+DEX and I/R+MEL+DEX groups. Tissue injury index in I/R+ MEL group was lower than I/R+DEX group but it was not significant, and in I/R+MEL+DEX group was lower than I/R+ MEL and I/R+DEX groups significantly ($P<0.05$,Table 2).

3.4. Apoptosis

A significant ($P<0.05$) increase in the number of TUNEL positive cells was observed in rats undergoing I/R. The MEL and DEX treatment led to a significant decrease in the number of TUNEL positive cells compared with the I/R group. Number of TUNEL positive cells was higher in I/R+DEX group comparing to MEL+I/R group but it was not significant. However, fewer number of TUNEL positive cells was observed in the group receiving both MEL and DEX ($P<0.05$, Table 2).

Table 1: Glutathione peroxidase (GPx), Superoxide dismutase (SOD) and Malondialdehyde (MDA) levels in lung tissue of rats after hepatic I/R.

	Sham	I/R	I/R+MEL	I/R+DEX	I/R+MEL+DEX
GPx(U/mg protein)	3.47±0.44	1.34±0.17	1.85 ±0.23	2.12±0.40	2.67±0.21
SOD(U/mg protein)	3.80±0.51	1.41±0.21	2.04±0.40	2.27±0.34	2.14±0.33
MDA MDA(nMol/ml)	0.67±0.18	4.24±0.72	3.45±0.59	2.77±0.34	2.14±0.33

Levels of SOD and GPx antioxidant enzymes were decreased in lung tissue in the groups subjected to hepatic I/R, and elevated the MDA levels , but it was less severe in I/R+MEL+DEX group comparing to other groups significantly ($P<0.05$).

Table 2: Lung injury index and apoptosis index in lung tissue after hepatic I/R

	Sham	I/R	I/R+MEL	I/R+DEX	I/R+MEL+DEX
Pathology	3.00±0.81	14.85±1.34	12.42±0.97	12.42±1.13	10.00±1.29
TUNEL positive cells	2.00±0.81	21.00±2.94	16.71±1.49	17.00±1.63	13.00±2.00

Lung injury index and apoptosis index were elevated in lung tissue in the groups subjected to hepatic I/R, but it was less severe in I/R+MEL+DEX group comparing to other groups significantly ($P<0.05$).

4. Discussion

The ischemic damage is resulted from a decrease in the blood flow to an organ. When restoring blood flow a more pronounced damage, so called reperfusion injury, occurs. In the development of I/R injury, the enhanced generation of oxygen radicals has been suggested (Schoenberg et al., 1993). Oxidative pathway has been previously demonstrated to play a significant role in the etiology of remote lung injury in the rabbit model of hepatoenteric ischemia-reperfusion, and in other animal models as well (Okutan et al., 2004). Reperfusion of an ischemic organ causes the activation and adhesion of polymorphonuclea neutrophils (PMN), with the release of proinflamatory substances and the formation of free radicals, which are nitrogen-derived reactive nitrogen species (RNS) or oxygen-derived reactive oxygen species (ROS), such as superoxide (O_2), peroxide (H_2O_2), and hydroxyl radicals (OH) (Beckman et al., 1996; Erdogan et al., 2006). In this study we tried to examine the effect of melatonin and dexamethasone in lung injury induced by liver I/R. Degradation of polyunsaturated fatty acids in cell membranes by ROS results in the destruction of membranes and formation of MDA, which is an indicator of ROS generation (Dabrowski et al., 1988). In the present study, the level of MDA, an end product of lipid peroxidation, significantly increased in lung at the end of reperfusion in I/R group. However, MDA level was reduced in lung by melatonin and dexamethasone after I/R. Our results demonstrated that melatonin significantly inhibits MDA elevations, but the extent of reduction in MDA level did not reach to the level in control groups, indicating that melatonin could provide a protection for lung injury. Dexamethasone also attenuated lung injury induced by liver I/R, but coadministration of both melatonin and dexmethosone had better result, although there was no significant difference between groups receiving dexamethasoe or melatonin, MDA levels were higher in dexamethasone group than melatonin group. During reperfusion, however, an excessive concentration of free radicals occurs, eliciting a process called oxidative stress, with deleterious effects. Highly reactive ROS directly attacks lipids, proteins in the biological membranes and cause their dysfunction (Chang et al., 2013). Free radicals are normally removed by antioxidant enzymes such as superoxide dismutase (SOD), which rapidly and specifically reduce O2 to H2O2. The other endogenous antioxidant enzyme, glutathione peroxidase (GPx), degrades H2O2 to water (Biewenga et al., 1997; Klotz et al., 2003; Sun et al., 1988). Hepatic I/R decreased lung cellular GPx and SOD, melatonin and dexamethasone administration attenuated destruction of cellular GPx and SOD levels, but co administration of melatonin and dexamethasone had better effects significantly.

There are two distinct phases of liver injury after warm I/R (Lentsch et al., 2000). The initial phase (<2 h after reperfusion) is characterized by oxidant stress, where production and release of reactive oxygen species (ROS) appears to directly result in hepatocellular injury. Although data on the activation of Kupffer cells by hypoxia–reoxygenation in vitro are conflicting, blockade of Kupffer cell activity with gadolinium chloride reduces hepatocyte damage in animal models (Shiratori et al., 1998; Rizzardini et al., 1998) Complement activation is also critical in the initial phase of IR injury as depletion of complement reduces Kupffer cell-induced oxidant

stress (Jaeschke et al., 1991; Jaeschke et al., 1993). The late phase of liver injury from 6 to 48 h after hepatic reperfusion is an inflammatory disorder mediated by recruited neutrophils; these damage hepatocytes, at least partly through release of ROS. The primary neutrophil ROS-generating pathway involves reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Thus, knockout mice deficient in the gp91 phox component of NADPH are protected against hepatic IR injury (Ozaki et al., 2000). Activated neutrophils also release elastase, cathepsin G, heparanase, collagenase and hydrolytic enzymes that are likely to be directly cytotoxic to hepatocytes. The mechanisms that link the first 'biochemical' phase of hepatic I/R injury to the later inflammatory phase are elaborated below.

PMNs are well known for their destructive capabilities. Not only can the PMN produce such toxic enzymes as elastase, serine protease, and metalloproteinases, but it can also produce such reactive oxygen species as the superoxide anion, hydrogen peroxide, and hydroxide radical. All toxic products of PMN activation can act alone or in concert to produce cellular destruction. The PMN has been implicated in hepatic I/R injury. Its level of involvement may be quite extensive, ranging from mechanical plugging of the microvasculature (the no-reflow phenomenon previously discussed) to the release of toxic mediators and recruitment of other cellular mediators of injury (Jaeschke et al., 1990).
Massive infiltration of ischemic hepatic tissue by PMNs has been noted after reperfusion by many investigators. In general, the timing of PMN infiltration coincides with the progression of tissue I/R injury noted on histopathologic examination in that the initial injury is exacerbated after PMN infiltration. Some investigators have correlated the severity of tissue tissue injury with the degree of PMN infiltration however; others have not noted this correlation (Kim et al., 1994). In an effort to confirm this role, several methods of PMN depletion or functional inactivation have been applied to I/R injury. Chavez- Chavez--Cartaya et al., (1995) showed that induction of neutropenia with the administration of vinblastine resulted in improved hepatic blood flow after I/R injury.

Corticosteroids are important anti-inflammatory agents for different inflammatory processes including asthma, trauma, surgical procedures and many other inflammatory and immune diseases. The predominant effect of corticosteroids is to switch off multiple inflammatory genes (encoding cytokines, chemokines, adhesion molecules, inflammatory enzymes, receptors and proteins) that have been activated during the inflammatory process. They have additional effects on the synthesis of anti inflammatory proteins, and also post-genomic effects (Odermatt et al., 2008). It is reported that dexamethasone can suppress inflammatory processes and ROS production (Simons et al., 2008). Dexamethasone is known to decrease I/R injury in different organs (Morariu et al., 2005). In our study, dexamethasone administration decreased MDA production after I/R and also maintained cellular anti-oxidant enzymes levels but It was less effective than melatonin.

In conclusion, hepatic and lung tissue injury attenuated through melatonin and dexamethasone pretreatment ,but combined treatment with melatonin and dexamethasone had better protection comparing to administration of melatonin and dexamethasone alone in rat model of hepatic I/R. Cellular anti-oxidant enzymes were improved by the administration of melatonin and dexamethasone; however, the combination therapy had better effects. Regarding that dexamethasone is usually used for suppressing inflammation and melatonin is an endogenous anti-oxidant and has not so much side effects, combination therapy of these drugs may be a safe and an effective protocol for a protection against the hepatic I/R injury .

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References:

1. Atalla SL, Toledo-Pereyra LH, MacKenzie GH, et al. Influence of oxygen-derived free radical scavengers on ischemic livers. Transplantation 1985;40:584.
2. Beckman JS, Koppenol WH. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. Am J Physiol 1996;271:1424–1437.
3. Biewenga GP, Haenen GR, Bast A. The pharmacology of the antioxidant lipoic acid. Gen Pharmacol 1997;29:315–31.
4. Boros P, Bromberg JS.New cellular and molecular immune pathways in ischemia/reperfusion injury. Am J Transplant 2006;6:652-658.
5. Chang KC, Hsu CC, Liu SH, Su CC, Yen CC, Lee MJ, Chen KL, Ho TJ, Hung DZ, Wu CC, Lu TH, Su YC, Chen YW, Huang CF. Cadmium Induces Apoptosis in Pancreatic β-Cells through a Mitochondria-Dependent Pathway: The Role of Oxidative Stress-Mediated c-Jun N-Terminal Kinase Activation. PLoS One 2013;8:e54374.
6. Chavez-Cartaya RE, DeSola GP, Wright L, et al. Regulation of the complement cascade by soluble complement receptor type I. Protective effect in

experimental liver ischemia and reperfusion. Transplantation 1995;59:1047-1052.
7. Chen X, Zhang KL, Yang SY, Dong JF, Zhang JN. Glucocorticoids aggravate retrograde memory deficiency associated with traumatic brain injury in rats. J Neurotrauma 2009;26:253-60.
8. Dabrowski A, Gabryelewicz A, Wereszczynska-Siemiatkowska U, Chyczewski L. Oxygen-derived free radicals in cerulein-induced acute pancreatitis. Scand J Gastroenterol 1988;23:1245-1249.
9. Dibazar F, Hajipour B, Hosseinian MM, Hemmati MR, Ghandiha A. Simvastatin decreases hepatic ischaemia/reperfusion-induced liver and lung injury in rats. Folia Morphol 2008;67:231-235
10. Erdogan H, Fadillioglu E, Yagmurca M, Ucar M, Irmak MK. Protein oxidation and lipid peroxidation after renal ischemiareperfusion injury: protective effects of erdosteine and Nacetylcysteine. Urol Res 2006;34:41–46.
11. Henderson JM. Liver transplantation and rejection: an overview. Hepatogastroenterology 1999;46:1482–4.
12. Jaeschke H, Farhood A, Bautista AP. Complement activates Kupffer cells and neutrophils during reperfusion after hepatic ischemia. Am J Physiol 1993;264:801–809.
13. Jaeschke H, Farhood A, Smith CW. Neutrophils contribute to ischemia/reperfusion injury in rat liver in viva. FASEB J I990;4:3355-3360.
14. Jaeschke H, Farhood A. Neutrophil and Kupffer cellinduced oxidant stress and ischemia-reperfusion injury in rat liver. Am J Physiol 1991;260:355–362.
15. Jaeschke H. Reactive oxygen and ischemia/reperfusion injury of the liver. Chem Biol Interact 1991;79:115.
16. Kim YI, Akizuki S, Kawano K, et al. Intrahepatic neutrophil accumulation in ischemia/reperfusion injury of pig liver. Transplant Proc 1994;26:2392-2399.
17. Kirimlioglu H, Ecevit A, Yilmaz S, Kirimlioglu V, Karabulut AB. Effect of resveratrol and melatonin on oxidative stress enzymes, regeneration, and hepatocyte ultrastructure in rats subjected to 70% partial hepatectomy. Transplant Proc 2008;40:285-289.
18. Klotz LO, Sies H. Defenses against peroxynitrite: selenocompounds and flavonoids. Toxicol Lett 2003;140–141:125–32.
19. Lentsch AB, Atsushi K, Yoshidome H, McMasters KM, Edwards MJ. Inflammatory mechanisms and therapeutic strategies for warm hepatic ischemia/reperfusion injury. Hepatology 2000;32:169–73.
20. Lerner AB, Case J, Takahashi Y. Isolation of melatonin, the pineal gland factor that lightens melanocytes J Am Chem Soc 1958;80:2587.
21. McCord JM. Oxygen-derived free radicals in postischemic tissue injury. N Engl J Med 1985;312:159.
22. Mihara M, Uchiyama M. Effects of antioxidants on the TBA reaction of various rat liver homogenates. Biochem Med 1983;30:131.
23. Morariu AM, Loef BG, Aarts LP, Rietman GW, Rakhorst G, van Oeveren W, Epema AH. Dexamethasone: benefit and prejudice for patients undergoing on-pump coronary artery bypass grafting: a study on myocardial, pulmonary, renal, intestinal, and hepatic injury. Chest 2005;128:2677-2687.
24. Odermatt A, Gumy C. Glucocorticoid and mineralocorticoid action: why should we consider influences by environmental chemicals? Biochem Pharmacol 2008;76:1184-1193.
25. Okutan H, Savas C, Delibas N. The antioxidant effect of melatonin in lung injury after aortic occlusion-reperfusion. Interact Cardiovasc Thorac Surg 2004;3:519-22.
26. Ozaki M, Deshpande SS, Angkeow P, et al. Inhibition of the Rac1 GTPase protects against non-lethal ischemic/reperfusion-induced necrosis and apoptosis in vivo. FASEB J 2000;14:418–429.
27. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967;70:158-69.
28. Paoletti F, Aldinucci D, Mocali A, Caparrini A. A sensitive spectrophotometric method for the determination of superoxide dismutase activity in tissue extracts. Anal Biochem 1986;154:536-41.
29. Reiter RJ, Tan DX, Manchester LC, El Sawi MR. Antioxidative capacity of melatonin. In: Cadenas E, Ann NY (Eds.). Acad Sci 2002;959:238–250.
30. Reiter RJ, Tan DX, Osuna C, Gitto E. Actions of melatonin in the reduction of oxidative stress: a review. J Biomed Sci 2000;7:444–458.
31. Reiter RJ. Melatonin: clinical relevance. Best Pract Res Clin Endocrinol Metab 2003;17:273–285.
32. Rizzardini M, Zappone M, Villa P, et al. Kupffer cell depletion partially prevents hepatic heme oxygenase 1 messenger RNA accumulation in systemic inflammation in mice: role of interleukin 1b. Hepatology 1998;27:703– 10.
33. Rodrı´guez MI, Escames G, Lo´pez LC et al. Chronic melatonin treatment reduces the age-dependent inflammatory process in senescence-accelerated mice. J Pineal Res 2007;42:272–279.

34. Romanque UP, Uribe MM, Videla LA. Molecular mechani9sms in liver ischemic-reperfusion injury and ischemic preconditioning. Rev Med Chil 2005;133:469–76.
35. Saidi RF, Chang J, Verb S, et al. The effect of methylprednisolone on warm ischemia-reperfusion injury in the liver. Am J Surg 2007;193:345–348.
36. Schoenberg MH, Beger HG. Reperfusion injury after intestinal ischemia. Crit Care Med 1993;21:1376–86.
37. Shiratori Y, Ohmura K, Hikiba Y. Hepatocyte nitricoxide production is induced by Kupffer cells. Dig Dis Sci 1998;43:1737–1745.
38. Simons SS. What goes on behind closed doors: physiological versus pharmacological steroid hormone actions. Bioessays 2008;30:744-756.
39. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem 1988;34:497–500.
40. Wang M, Shen F, Shi LH et al. Protective effect of prednisolone on ischemia-induced liver injury in rats. World J Gastroenterol 2008;14:4332–4337.
41. Zegdi R, Fabre O, Cambillau M, et al. Exhaled nitric oxide and acute lung injury in a rat model of extracorporeal circulation. Shock 2003;20:569-74.
42. Zencirci SG, Bilgin MD, Yaraneri H. Electrophysiological and theoretical analysis of melatonin in peripheral nerve crushes injury. J Neurosci Methods 2010;191:277-82.

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