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The Effects of Dexketoprofen on Endogenous Leptin and Lipid Peroxidation During Liver Ischemia Reperfusion Injury

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Hepatic ischemia reperfusion (IR) injury has complex mechanisms. We investigated the effect of dexketoprofen on endogenous leptin and malondialdehyde (MDA) levels. Wistar albino rats were divided into 4 equal groups and were subjected to 1-hour ischemia and different subsequent reperfusion intervals. Dexketoprofen was administered in a dose of 25 mg/kg 15 minutes before ischemia induction and 1-hour reperfusion to the Dexketoprofen one-hour reperfusion group, n = 6 (DIR1) group and 6-hour reperfusion to the Dexketoprofen six-hour reperfusion group, n = 6 (DIR6) group. In the control groups, 0.9% physiologic serum (SF) was administered 15 minutes before ischemia induction and 1-hour reperfusion to the one-hour reperfusion group, n = 6 (IR1) group and 6-hour reperfusion to the six-hour reperfusion group, n = 6 (IR6) group. Although serum leptin ($P = 0.044$) and hepatic tissue MDA levels ($P = 0.004$) were significantly higher in the IR6 group than in the IR1 group, there were no significant differences in dexketoprofen pretreatment between the DIR1 and DIR6 groups. There were no differences in serum MDA levels among the 4 groups, and serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were

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significantly higher in the IR1 ($P = 0.026$ and $P = 0.018$, respectively) and IR6 ($P = 0.000$ and $P = 0.002$, respectively) groups than in the DIR1 and DIR6 groups. Dexketoprofen pretreatment can protect the liver from IR injury by decreasing inflammation and lipid peroxidation. Our study shows that dexketoprofen has no effects on endogenous leptin during IR injury.

Key words: Ischemia-reperfusion injury – Liver – Ketoprofen – Malondialdehyde – Leptin

Hepatic ischemia reperfusion (IR) injury is a complication of several surgical conditions, such as liver resection and transplantation, and prolonged states of shock that lead to local injury or remote dysfunction of multiple organs.[1,2] Incipient tissue hypoxia; production of reactive oxygen species (ROS); activation of the inflammatory cascade, resulting in inflammatory responses[3,4] and microcirculatory problems[5] further aggravate injury. Although ischemic stress eventually causes cell death, cell injury often does not manifest itself until after the ischemic liver is reperfused.[6] ROS are highly reactive ions that include hydrogen peroxide (H_2O_2), lipid peroxides, hypochlorous acid (HOCl), and free oxygen radicals.[7] Malondialdehyde (MDA) is the end product of lipid peroxidation; increased MDA levels reflect excessive production of free oxygen radicals and indicate organ damage.[8,9]

The role of polymorphonuclear leukocytes (neutrophils) in the acute inflammatory response during IR injury has been investigated in several studies.[10,11] Vane and Botting described inflammatory response and the role of chemical mediators, such as prostaglandins, platelet-activating factor, interleukin-1, histamine, and bradykinin.[12] That study was followed by studies showing the ROS scavenging effects of nonsteroidal anti-inflammatory drugs (NSAIDs).[13] Dexketoprofen trometamol, the active enantiomer of racemic ketoprofen, possesses cyclooxygenase inhibitory effects, as do other members of the NSAID family. Properties of this formulation are more rapidly absorbed and have a faster onset of action than does ketoprofen.[14]

Leptin, an adipose tissue–derived hormone, decreases body weight by both suppressing appetite and promoting energy expenditure.[15] It also regulates inflammatory response, primarily by exerting pro-inflammatory actions.[16] The structure of leptin and its receptor suggest that leptin should be classified as a cytokine. The helical structure of leptin is similar to the structures of the long-chain helical cytokine family, which includes interleukin (IL)-6, IL-11, IL-12, leukemia inhibitory factor (LIF), and granulocyte colony-stimulating factor (G-CSF). Cytokines play an important role in the host response to infectious and inflammatory stimuli. Previous studies have shown the importance of leptin in the activation of the immune system and as a mediator of inflammation.[17–19] Faggioni and colleagues (1998) demonstrated that leptin production does not increase during inflammation in IL-1b-deficient mice.[20] Thus, the increase in leptin during infection and inflammation indicates that leptin is part of the immune response and host defense mechanism.[21] Leptin-deficient (*ob/ob*) and leptin-receptor–deficient (*db/db*) mice are not only obese but they also show immune/endocrine abnormalities.[22] While dexketoprofen inhibits inflammation, its effects on the level of leptin, which plays an important role in immune response, are unknown.

The aim of this study was to evaluate the role of dexketoprofen on endogenous leptin levels and lipid peroxidation at different reperfusion intervals during IR injury.

Material and Methods

Animals

A total of 24 male Wistar albino rats (weighing 200–250 g, 12 weeks of age) were supplied by the Experimental Research Center of Ondokuz Mayis University. All of the animals were housed under controlled environmental conditions (21°C ± 1°C, 40%–70% humidity, 12/12 dark–light cycle, standard rat diet and water). The experimental protocol was approved by the Ethics Committee for Experimental Animal Studies of Ondokuz Mayis University.

Experimental groups

All surgical procedures were conducted under anesthesia after intraperitoneal injection of ketamine (50 mg/kg) and xylazine (10 mg/kg). Dexketoprofen was supplied as pure powder by Menarini Ricerchere S.P.A. Sede e Laboratory, Pomezia, Rome.

Powder dexketoprofen trometamol was prepared in 25 mg/mL aliquots with physiologic serum (SF). Before surgery, the ventral abdomen was shaved and cleaned with 10% povidone iodine. The 24 rats were divided into 4 groups to undergo 1-hour ischemia and different subsequent reperfusion intervals, as described below.

Dexketoprofen was administered (25 mg/kg, intraperitoneally) 15 minutes before induction of ischemia. One-hour reperfusion (group DIR1, n = 6) and 6-hour reperfusion (group DIR6, n = 6) were produced by removing the vascular clamps after 1 hour of partial hepatic ischemia.

Physiologic serum (1 mL/kg) was administered to the control groups 15 minutes before induction of ischemia. One-hour reperfusion (group IR1, n = 6) and 6-hour reperfusion (group IR6, n = 6) were produced by removing the vascular clamps after 1 hour of partial hepatic ischemia.

After SF or dexketoprofen administration, a midline laparotomy was performed, and a rat model of 70% hepatic ischemia was achieved by occluding the portal circulation with a traumatic vascular clamp to the median and left lateral lobs of the liver, as described by Lin *et al*.[23] Reperfusion was produced by removing the vascular clamps after 1 hour of partial hepatic ischemia. The rats were humanely killed after the reperfusion period.

Measurement of hepatic tissue and serum MDA

The hepatic tissue homogenate was prepared as described by Celik and Suzek,[24] with minor modifications. The tissues were homogenized for 5 minutes in 50 mM ice-cold KH_2PO_4 solution (1:5 wt/vol) using a Dounce homogenizer (Sigma-Aldrich Co, LLC, St Louis, Missouri) and then centrifuged at 2142*g* for 20 minutes at 4°C.

A 0.5-mL aliquot of supernatant was added to 2.5 mL 20% trichloroacetic acid and 1 mL 67% thiobarbituric acid in a 10-mL centrifuge tube. The mixture was then incubated at 90°C for 30 minutes in a water bath, after which the tubes were rapidly cooled to stop the reaction. Following the addition of 4 mL n-butanol, the mixture was vortexed and centrifuged. The supernatants (20 μL) were injected into a high-performance liquid chromatography - fluorescence detection (HPLC-FLD) (Ex: 515 nm; Em: 553 nm) system. Serum MDA levels were measured according to the method described by Yoshioka *et al*[25] and Agarwal and Chase.[26]

Measurement of serum aspartate aminotransferase and alanine aminotransferase

The blood samples were centrifuged at 3000*g* at 4°C for 10 minutes. The serum of the centrifuged blood samples was transferred into microcentrifuge tubes and stored at –80°C until enzyme analyses were performed. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) measurements were performed by the endpoint method using an autoanalyzer (Autolab, AMS Srl, Selective Access, Saba, The Netherlands) and commercial kits (Audit Diagnostics, Carrigtwohill, Ireland) according to the manufacturers' instructions, as previously described.[27]

Measurement of serum leptin

The rat serum leptin levels were detected using a commercial enzyme-linked immunosorbent assay kit.[28]

Histopathologic examination

Fresh portions of the left and median lobes of the liver of each rat were excised rapidly, fixed in 10% buffered formalin, and embedded in paraffin. Sections were cut to 5-μm thickness and stained with hematoxylin and eosin for routine microscopic examination. The stained sections were examined for the presence of hydropic degeneration, necrosis, and polymorphonuclear cell infiltration. The histopathologic changes in the liver specimens were analyzed at 10 different high-power fields on a scale of 0 to 3 (none = 0, mild = 1, moderate = 2, severe = 3), as described by Demirel *et al*.[29]

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) for Windows (Version 15.0, SPSS Inc, Chicago, Illinois). All results were expressed as mean ± SD. Differences between 2 groups (*e.g.*, IR1 versus IR6, DIR1 versus DIR6) were analyzed using an independent sample *t* test and a Mann-Whitney *U* test. A value of $P \leq 0.05$ was considered statistically significant.

Results

Serum leptin levels after IR injury

Our results demonstrated significant alterations in serum leptin levels between the 1-hour and 6-hour

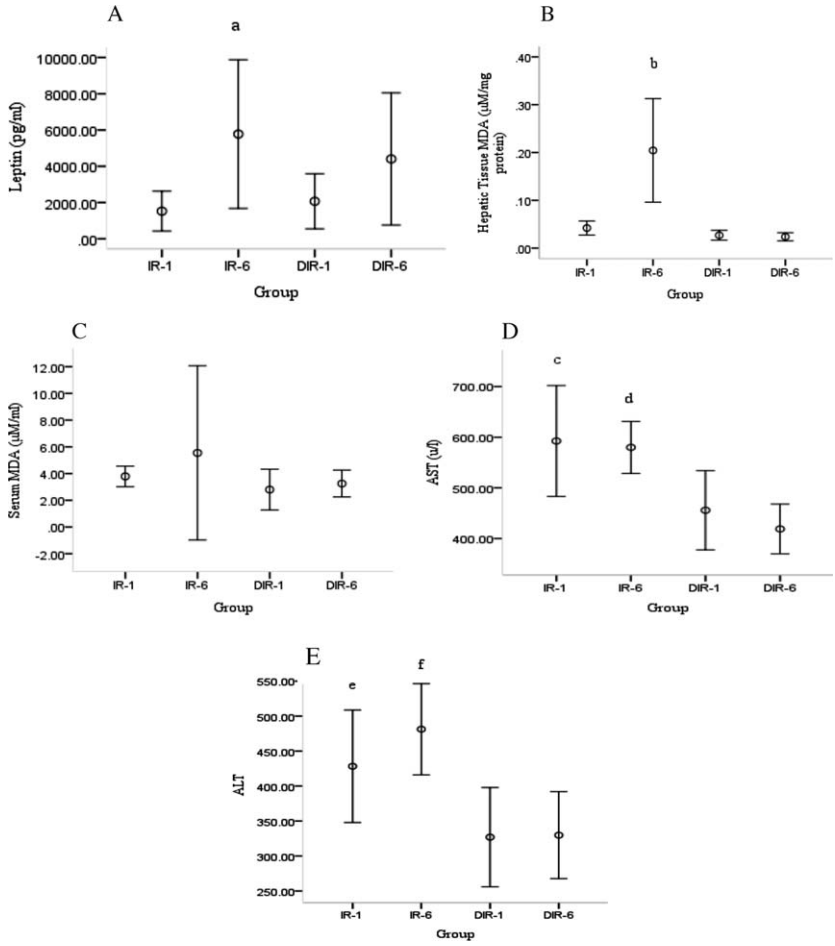

Fig. 1 (a) Serum leptin levels; (b) MDA concentration in liver tissue; (c) serum MDA levels; (d) serum AST activities; and (e) serum ALT activities. [a]$P = 0.044$ versus IR1; [b]$P = 0.004$ versus IR1; [c]$P = 0.026$ versus DIR1; [d]$P = 0.000$ versus DIR6; [e]$P = 0.018$ versus DIR1; [f]$P = 0.002$ versus DIR6.

reperfusion groups after 1-hour partial hepatic ischemia. Serum leptin levels were significantly higher in the IR6 group than in the IR1 group ($P = 0.044$) without medical intervention (Fig. 1A). There were no significant differences in serum leptin levels between the 1-hour (DIR1) and 6-hour (DIR6) reperfusion groups when the rats were pretreated with dexketoprofen. There were no significant differences in serum leptin levels between the dexketoprofen-treated and the nontreated groups (IR1 versus DIR1, IR6 versus DIR6).

Serum MDA levels and liver tissue concentration

Tissue MDA levels were significantly higher ($P = 0.004$) in the IR6 group than in the IR1 group; however, there was no significant difference in tissue MDA concentration between the dexketoprofen-treated groups (DIR1 versus DIR6) (Fig. 1B). In addition, there were no significant differences in serum MDA levels among the 4 reperfusion groups (IR1 versus DIR1, $P = 0.873$; IR6 versus DIR6, $P = 0.539$) (Fig. 1C).

Serum activities of AST and ALT after injury

Our results demonstrated that serum AST and ALT activities in the IR6 group were not significantly higher than in the IR1 group, and there was no significant difference between the treated groups (DIR1, DIR6). In addition, serum AST and ALT activities were significantly higher in the IR1and IR6 groups than in the DIR1and DIR6 groups (Fig. 1D and 1E).

Histopathologic observations

Liver histopathology was evaluated on the basis of neutrophil infiltration, hydropic degeneration, and necrosis.

In the IR1 group, there was no appreciable neutrophil infiltration into the liver tissue; however, we observed mild or moderate necrosis and

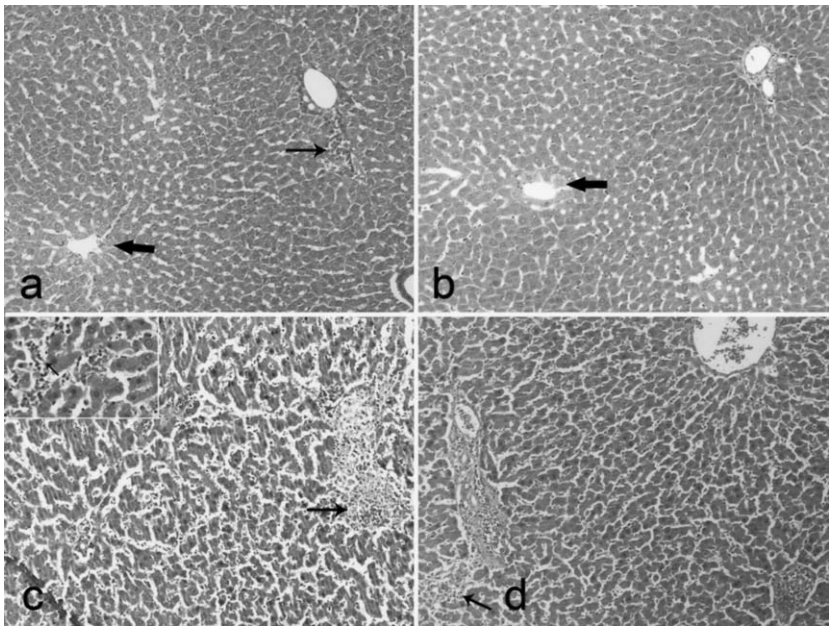

Fig. 2 (a) Mild hydropic degeneration in the centrilobular region (thick arrow) and mild necrosis (thin arrow) in IR1; Hematoxylin and Eosin (HE) HE ×200. (b) Mild hydropic degeneration in the centrilobular region in DIR1 (thick arrow); HE ×200. (c) Moderate necrosis (thin arrow) in IR6; HE ×200 (inset: higher magnification of neutrophil infiltration in sinusoid, HE ×400). (d) Moderate necrosis (thin arrow) in DIR6; HE ×200.

moderate hydropic degeneration in this group. In the dexketoprofen-treated (DIR1) group, we observed only mild hydropic degeneration, and there was no appreciable necrosis or neutrophil infiltration in the liver tissue.

After 6 hours of reperfusion (IR6), neutrophil infiltration, moderate necrosis, and moderate–severe hydropic degeneration were observed in the liver tissues. Similarly, mild or moderate neutrophil infiltration, hydropic degeneration, and necrosis were observed in the dexketoprofen-treated (DIR6) group (Fig. 2).

Discussion

IR injury is a multifactorial process that includes ischemic organ damage and inflammation-related reperfusion injury. Kupffer cells, lymphocytes, polymorphonuclear neutrophils, endothelial cells, and ROS play significant roles in the pathogenesis of liver IR injury.[30]

Neutrophil-induced parenchymal cell injury, as described by Jaeschke, includes 3 steps: sequestration of neutrophils, transendothelial migration, and adherence-dependent cytotoxicity.[31] Previous studies have investigated the association between hepatic IR injury and NSAIDs, such as ibuprofen[32] and diclofenac.[33]

In our study, hepatic parenchymal damage was assessed by measuring serum AST and ALT activities and by histopathologic evaluation. Although serum AST and ALT activities were similar in the IR1 and IR6 groups, the values obtained were higher than those of sham groups in previous studies.[34–37] Serum AST and ALT activities were decreased by the administration of dexketoprofen, indicating that it might reduce liver damage in IR injury.

The present study shows that leptin levels were not altered by the administration of dexketoprofen. To our knowledge, no previous study has reported the effect of NSAIDs on endogenous leptin levels during IR injury. Leptin, a circulating hormone produced by adipose tissue, plays a significant role in energy homeostasis, angiogenesis, inflammation,[38] blood pressure homeostasis,[39] and innate and adaptive immunity.[16] Leptin is a 167 aa peptide that is structurally similar to cytokines such as IL-6, IL-11, and IL-12. It acts via the Ob-R receptor, which is structurally similar to other class I cytokine receptors, and it is expressed by immune cells: neutrophils, monocytes, and macrophages.[40] Recent studies have shown that leptin attenuates IR injury in various tissues, such as the brain,[41,42] liver,[43] and intestine.[44] Increased leptin levels during infection and inflammation might indicate that leptin plays a protective role in host response to inflammation.[45] Lin *et al* established a rat model of 70% liver IR injury[23] and found that serum leptin levels were significantly higher in a 6-hour reperfusion group than in a 4-hour reperfusion group. Similarly, our results suggest that endogenous leptin levels were elevated after 6 hours of reperfusion, but there were no significant differences in serum leptin levels between the dexketoprofen-treated and nontreated groups.

IR injury is a multifactorial process that includes complex molecular mechanisms. Activation of the inflammatory response, calcium overload, and the production of free oxygen radicals play important roles in lipid peroxidation and ultimately result in liver damage.[46] MDA is the most frequently used indicator of lipid peroxidation and indicates oxidative tissue damage.[47] In the present study, tissue MDA concentration was significantly higher during IR in the IR6 group than in the IR1 group, as demonstrated in previous studies.[41,34,48] In addition, our results showed that tissue MDA concentration was suppressed by the administration of dexketoprofen. This effect was also related to dexketoprofen-reduced neutrophil infiltration, as well as lipid peroxidation.

After dexketoprofen pretreatment, no necrosis was observed in the 1-hour reperfusion group, and intraperitoneal dexketoprofen treatment resulted in reduced hydropic degeneration, neutrophil recruitment, and necrosis in the 6-hour reperfusion group. Our results demonstrate that dexketoprofen pretreatment can reduce the histopathologic changes associated with IR injury.

In conclusion, this study focused on the effect of dexketoprofen on endogenous leptin levels and lipid peroxidation during different time points of liver IR injury. Our results suggest that dexketoprofen administration can protect the liver from IR injury by decreasing inflammation and lipid peroxidation, but there is no association between serum leptin levels and dexketoprofen. As there are insufficient studies on this subject, we suggest that further studies are required to determine leptin fluctuation during IR injury.

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