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Carnitine as a preventive agent in experimental renal ischemia-reperfusion injury

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Abstract Reactive oxygen species generated during the reperfusion of ischemic kidney, as well as any other tissue, cause lipid peroxidation damaging the cell membrane. The aim of this study was to investigate the effect of carnitine in reperfusion injury of the kidney. Male albino rabbits were subjected to unilateral renal 1-h warm ischemia followed by 15 min of reperfusion. Group I ($n = 9$): *control group* received 3 cc of isotonic saline solution and group II ($n = 9$): *carnitine group* received 100 mg/kg of carnitine. Blood samples were collected at the 15th min of reperfusion from the left renal vein selectively. Preischemic and post-reperfusion serum and renal tissue MDA levels were measured by thiobarbituric acid reactive substances (TBARS) spectrophotometric analysis. The preischemic serum and tissue MDA values (sham values) for groups I and II were statistically comparable ($P > 0.01$). Serum and tissue MDA levels were markedly elevated after 15 min of reperfusion in group I ($P < 0.01$), while the values remained in the baseline levels following reperfusion in group II ($P > 0.01$). In group I, the major histological differences observed in the reperfused kidneys were marked edema and congestion whereas glomerular and tubular cellular integrity were well preserved in group II. Pre-treatment with carnitine in solid organ transplantations, preschock states, surgical procedures that require temporary vascular clamping etc. may be helpful to minimize the reperfusion injury in the involved tissue, reducing morbidity and mortality.

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Introduction

Ischemic injury occurs when the blood supply to an area of tissue is cut off. The incidence of ischemic injury is high; myocardial infarction and cerebrovascular incidents and other thrombotic events affect millions of people in varying degrees each year. Ischemic injury also occurs during surgery when the blood vessels are cross-clamped, especially in transplantation surgery.

Each tissue has a variable limit of time to preserve vitality during oxygen deprivation. This period depends on the metabolic activity of the specified organ/tissue system in the organism, and the result is tissue necrosis. Although restoration of blood supply was expected to minimize the damage, the observations (e.g., thrombolytic therapy and angioplasty) have shown that the extent of tissue injury increases with reperfusion.

Now it is well known that production of oxygen free radicals are responsible for the reperfusion injury. Free radicals such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2) are normally generated during the aerobic metabolism and are used for biosynthesis, intracellular signaling and even in destruction of microorganisms, and mammalian cells have mechanisms to eliminate these free radicals under normal physiologic conditions [2, 12]. When the cellular antioxidant system is overwhelmed by reperfusion, the reactive oxygen species, particularly the hydroxyl radical ($OH^{\cdot}$), react with membrane phospholipids and cause lipid peroxidation. Damage to the cell membrane induces the synthesis of inflammatory cytokines and displays a chemotactic role for inflammatory cells to condense in the reperfused tissue. If the involved tissue is big enough, the cytokines act systemically, causing remote injury to lungs and other organs including brain, heart, liver and kidneys.

Many pharmacological agents have been investigated to reduce the reperfusion injury. Although some are clinically applicable, many continue to be experimental. Carnitine is a widely used agent both experimentally and clinically in myocardial and muscle ischemia [3–6, 13], but its effects in renal ischemia-reperfusion injury have not been studied. This study was designed to investigate the possible preventive effect of carnitine in reperfusion injury of the kidney.

Materials and methods

Eighteen 12-week-old male albino rabbits, weights ranging between 1497.6 ± 286.8 g, were used in this study. All animals were maintained following the guidelines of the Surgical Research Committee of Ege University, and the experimental studies were performed with the approval of the Ethical Committee of Surgical and Medical Research. Rabbits were brought to the laboratory 4 days prior to the experiment in order to adapt to the environment and were fed with laboratory animal chow regularly. All animals survived throughout the study and none were excluded from the study for any reason.

Animals were randomly assigned to two groups: control group (group I, $n = 9$) and carnitine group (group II, $n = 9$). Rabbits were anesthetized by intramuscular (i.m.) injection of 50 mg/kg of ketamine sulfate. Group I received 3 ml of isotonic saline solution from the left ear vein, and group II 100 mg/kg of carnitine 20 min prior to ischemia.

The abdomen was opened by a mid-line incision and 2 ml of blood samples were collected from the inferior vena cava via right renal vein for both groups and right nephrectomy was performed. These blood and kidney samples prior to left renal arterial clamping served as sham values of the study. Left renal vasculature was prepared, and the renal artery was clamped by an atraumatic vascular clamp upon the 20th min of carnitine (or saline) administration. The rabbits were maintained in an ambient temperature of 24 °C and the peritoneal cavity was periodically treated with warm saline to keep a constant body temperature and hydration. Following 60 min of warm ischemia, the clamp was opened and the left kidney was allowed to reperfuse for 15 min. At the end of 15 min of reperfusion blood samples were collected from the left renal vein selectively, while the vena caval end was controlled to prevent backflow of systemic blood. The procedure was completed by left nephrectomy. Animals were sacrificed by high dose intravenous ketamine injections.

Hematocrit values were controlled before and at the end of the procedure to rule out the possible effects of possible blood loss or dehydration during the study.

Each kidney specimen was divided into two pieces. One piece of each kidney was fixed in 10% formaldehyde solution for pathological examination and preserved at 4 °C. The other piece was placed in liquid nitrogen for tissue malonyldialdehyde (MDA) contents. All blood centrifugates were also preserved in liquid nitrogen.

Kidney samples reserved for pathological analysis were embedded into paraffin blocks and were sliced into 3–5 μ cross-section slides. They were examined under light microscopy after periodic acid Schiff (PAS) and hematoxylin-eosin (H&E) stains in a blinded fashion for the presence of tissue injury characteristics such as interstitial edema and congestion, cellular vacuolization, sloughing, tubular necrosis and luminal obstruction.

Serum and renal tissue MDA levels were measured quantitatively by thiobarbituric acid reactive substances (TBARS) spectrophotometric analysis. Serum MDA values were calculated in micromole/l and renal tissue contents of MDA in nmol/g wet weight (ww) (means ± SD). Mann-Whitney *U*-test and Wilcoxon rank sum test were used for the statistical analysis of the data using SPSS for Windows 6.0.

Determination of malondialdehyde

Since it is impossible to measure free radicals directly in vivo, it is necessary to rely on the quantitation of their reaction products. Malondialdehyde (MDA) is a byproduct of the lipid peroxidation process. It is the most widely used biomarker, providing information about overall lipid peroxidation level. It is formed during iron ion catalysed decomposition of hydroxy endoperoxides, and can be easily assayed by the thiobarbituric acid (TBA) reaction. One molecule of MDA binds two molecules of TBA, forming a pink-colored compound. Then the peak value of this chromogen is measured by spectrophotometric analysis at a wavelength of 532–535 nm, and compared with its standard curve.

Results

Mean body weights of groups I and II were 1332.6 ± 214.7 g and 1628.6 ± 335.1 g, respectively ($P > 0.05$). Preischemic and postreperfusion serum and tissue MDA values for both groups are given in Figs. 1 and 2. The preischemic serum and tissue MDA values (sham values) for groups I and II were statistically comparable ($P > 0.05$). Serum and tissue MDA contents were markedly elevated after 15 min of reperfusion in group I (serum MDA: $P < 0.01$; $P = 0.0039$, and tissue MDA: $P < 0.01$; $P = 0.002$). These values remained at the baseline levels following reperfusion in group II (serum MDA: $P > 0.05$; $P = 0.674$, and tissue MDA: $P > 0.05$; $P = 0.391$).

There were no significant differences in hematocrit values before and at the end of the study for either groups ($P > 0.05$; Table 1).

Histological findings

In group I, the major histological differences observed in the reperfused kidneys compared to normal kidneys were marked edema and congestion. Cellular vacuoli-

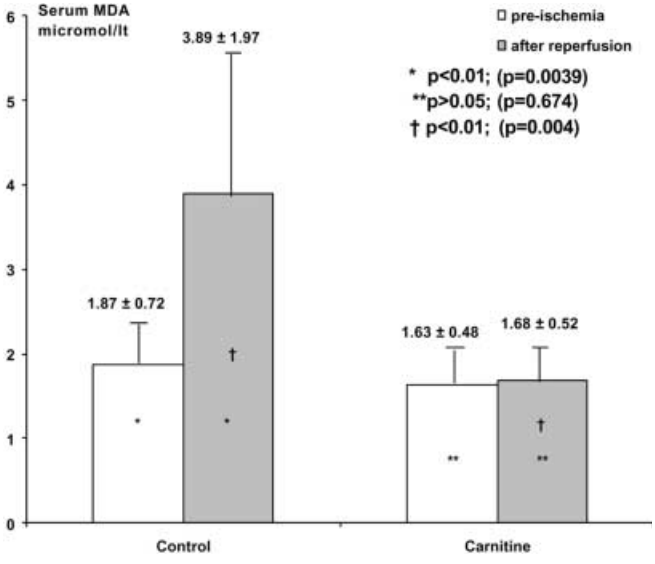

Fig. 1 Serum MDA values of the two groups before ischemia and after 15 min of reperfusion

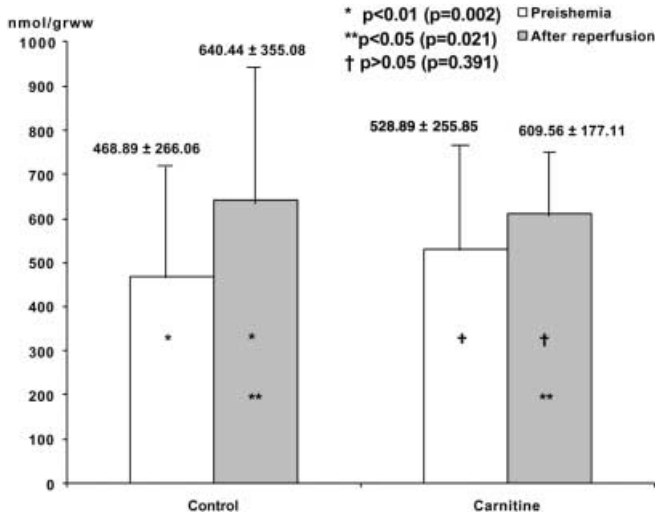

Fig. 2 Renal tissue MDA levels of the two groups before ischemia and after reperfusion

Table 1 Hematocrit values of the two groups

Hematocrit	Control	Carnitine
Pre-ischemia	34.30 ± 3.87	35.7 ± 2.87
Reperfusion (15 min)	32.56 ± 4.88	33.0 ± 3.67
P	>0.05	>0.05

zation, sloughing and granular debris in the tubular lumen were other findings observed in the control group (Fig. 3), but these findings were not as broad as edema and congestion.

Glomerular and tubular cellular integrity were well preserved in group II (Fig. 4). Cellular swelling and vacuolization and granular debris in the tubular lumen were not observed. Edema was seen in some areas but was not a constant finding for the carnitine group.

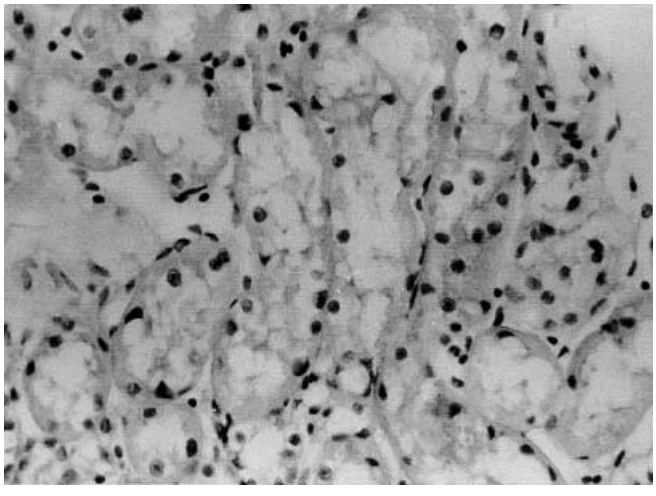

Fig. 3 Cellular vacuolization, edema, and sloughing and granular debris in the tubular lumen in the control group. H&E, ×200

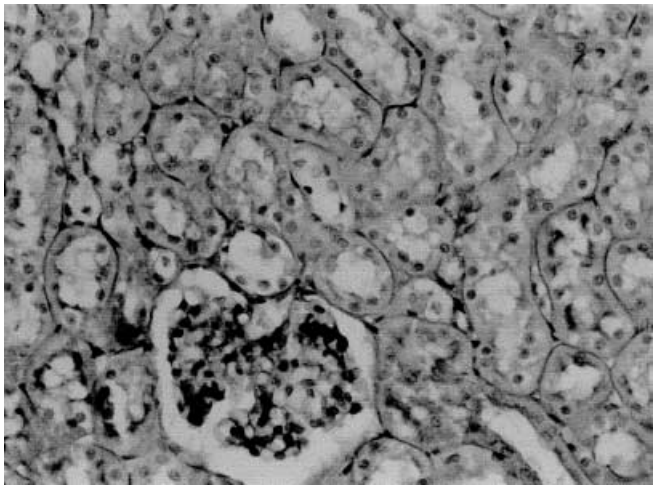

Fig. 4 Glomerular and tubular cellular integrity well preserved in group II after carnitine pretreatment. PAS, ×200

Discussion

Kidneys, like other organs in the organism, may be exposed to ischemic conditions in many clinical situations. Renal ischemia injures the renal tubular cell by disrupting the vital cellular metabolic machinery [7]. Further damage is caused by restoration of blood flow by which the oxygen free radicals are produced. Shock and renal transplantation are the most frequent clinical conditions that expose the kidneys to ischemia and subsequent reperfusion. Renal transplantation is the treatment of choice at the end stage renal failure. Blood flow to the transplanted kidney is interrupted starting from harvesting from the donor until the anastomoses at the recipient are completed. The incidence of ischemic allograft damage during the first postoperative week is around 30–40% [1]. Such damage plays an important role in the late term graft dysfunction as well [1]. There are many other clinical conditions besides renal transplantation that may cause ischemic renal failure due to deficient renal blood flow, and mortality rates may be as high as 50% [17].

Postischemic reperfusion injury to kidney is often attributed to generation of oxygen free radicals subsequently promoting lipid peroxidation to cause cellular injury by tubular cell swelling, luminal obstruction and apoptosis [8, 11, 15, 17]. The protective effects of a variety of antioxidants and free radical scavengers (e.g. α-tocopherol, allopurinol, glutathione, superoxide dismutase, desferroxamine etc.) have been well documented [11, 15]. Carnitine is another agent whose anti-ischemic effects on myocardial and skeletal muscles have been demonstrated in a series of clinical and experimental studies [4–6, 13]. However, looking at the current literature, we realized that its effect in renal ischemia-reperfusion injury is underinvestigated.

In this study, serum and tissue MDA levels were significantly elevated after 60 min of left renal arterial

clamping followed by 15 min of reperfusion in group I. This finding is consistent with the data in the literature [7, 9, 14, 18]. The 60-min ischemia is a realistic time period for which transplanted kidney is exposed starting from renal arterial clamping until the anastomoses are completed in transplant surgery from living related donors. The reperfusion injury to kidneys from cadavers is another issue that might require additional factors that we did not address in this particular study. As can be seen in Figs. 1 and 2, serum and tissue MDA values are preserved at baseline levels after 15 min of reperfusion following carnitine administration. This finding supports that carnitine prevents lipid peroxidation after reperfusion. Histological findings also sustain this assumption, that is in the control group, marked edema and congestion were observed in the reperfused kidneys whereas cellular vacuolization, sloughing and granular debris in the tubular lumen were other findings, but were not as broad as edema and congestion. On the other hand, glomerular and tubular cellular integrity were well preserved in group II. Cellular swelling and vacuolization and granular debris in the tubular lumen were not observed. Edema was seen in some areas but was not a consistent finding for the carnitine group.

Free L-carnitine is found in many foods, mainly those from animal sources. L-carnitine is not an essential nutrient but endogenous synthesis may fail to ensure adequate L-carnitine levels in neonates, especially in premature infants. All tissues in the body can produce deoxy-carnitine but, in humans, the enzyme that enables hydroxylation of deoxy-carnitine to carnitine is found only in liver, brain and kidneys. L-carnitine ensures transfer of fatty acids to mitochondria where they undergo oxidation, and regeneration of coenzyme-A, and are thus involved in energy metabolism. L-carnitine and its esters are eliminated mainly through kidneys [10].

Although produced endogenously, carnitine is stored mainly in myocardium and skeletal muscles that are responsible for regulating energy consumption according to the organism's metabolic demand. Renal carnitine content is low, and pre-treatment with carnitine prior to ischemia provides increased carnitine delivery to the kidneys. Ischemia reduces mitochondrial metabolism that slows down β-oxidation. This results in reduction of energy production that hinders the mechanisms that preserve cellular integrity. Carnitine is not an antioxidant agent nor a free radical scavanger. Its preventive effect in renal reperfusion injury is probably more diverse than the myocardial or skeletal muscle reperfusion injuries. By stimulating mitochondrial fatty acid oxidation, it may act as a pro-oxidant agent providing renal protection in general. Protection of renovascular endothelial and epithelial integrity providing preservation of renal blood flow may play a role in reduction of reperfusion injury.

In conclusion, pre-treatment with carnitine significantly reduces reperfusion injury in ischemic kidneys experimentally. This may be beneficial in terms of reducing morbidity and mortality under clinical conditions including solid organ transplantations, preshock states, and surgical procedures that require temporary vascular clamping.

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