The Protective Effect of L-carnitine on Ionizing Radiation-induced Free Oxygen Radicals

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Summary

Ionizing radiation is known to generate reactive oxygen species (ROS) that can be removed by antioxidants. L-carnitine, a natural component of mammalian tissue, is a necessary factor in the utilization of long-chain fatty acids to produce energy. Furthermore it has been shown that L-carnitine is an antioxidant which has a scavenger effect on ROS and a stabilizing effect on damaged cell membranes. The aim of the study was to evaluate the potential protective effect of L-carnitine on radiation-induced free radicals in hamsters. L-carnitine was given by gavage at a dose of 50 mg/kg for 15 consecutive days before irradiation with a single dose of 8 Gy. 24 h after radiation exposure, the hamsters were sacrificed and samples were taken from blood and tissues, and the biochemical and histopatological determinations were carried out. In the irradiated group, there were significant increases in plasma and liver malondialdehyde (MDA) with marked reduction in glutathione (GSH) levels in the liver, compared with controls. In red blood cells, superoxide dismutase (SOD) and catalase activities were also reduced. All these effects were reversed by L-carnitine. In conclusion, L-carnitine with its antioxidant and free radical scavenging properties could play a modulatory role against the cellular damage produced by free radicals induced by ionizing radiation.

Introduction

Radiation therapy plays an important role in the curative and palliative treatment of malignant diseases. Exposure of the body to ionizing radiation produces reactive oxygen species (ROS) that damages proteins, lipids and nucleic acids. Because of the lipid component in the membrane, lipid peroxidation is reported to be particularly susceptible to radiation damage (*Riley, 1994; Chevion et al., 1999*). In addition, cell lipid peroxidation is related to radiation-induced cell death, changes in membrane fluidity (*Berroud, et al. 1996*) and in the activities of some membrane enzymes (*Yukawa et al., 1983*). Furthermore, it has been shown that irradiation causes a marked change in the plasma total antioxidant capacity and total body irradiation (TBI) is known to cause a pronounced decrease in antioxidant capacity and large increase in oxidant stress (*Chevion et al., 1999*). Mammals are endowed with antioxidant defense systems that scavenge and minimize the formation of ROS. However, these systems are not always fully operative. Therefore, diet-derived antioxidants become particularly important in diminishing cumulative oxidative damage (*Duthie et al., 1996*) and a number of dietary antioxidants have been reported to decrease free radical attack on biomolecules (*Saada & Azab, 2001*).

L-carnitine is a vitamin-like substance that is structurally similar to amino acids. Most carnitine is obtained from diet. It can also be synthesized

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Published in the Scandinavian Journal of Laboratory Animal Science - an international journal of laboratory animal science

endogenously by skeletal muscle, heart, liver, kidney and brain from the essential amino acids lysine and methionine. It is essential for the normal oxidation of fatty acids by the mitochondria and is involved in the trans-esterification and excretion of acyl-CoA esters, the oxidation of branched chain a-ketoacids, and removal of potentially toxic acylcarnitine esters from within mitochondria (*Rebouche & Seim, 1998*). It is known that L-carnitine and its derivatives prevent the formation of ROS, scavenge free radicals and protect cells from peroxidative stress (*Packeret al., 1991; Luo et al., 1999; Arockia Rani & Panneerselvam, 2001; Izgut-Uysal et al., 2001; Sener et al., 2004; Dokmeci, 2005; Dokmeci et al., 2005*). It is therefore possible that L-carnitine could scavenge free radicals and produce beneficial effects against radiation damage. Hence, we hypothesized that L-carnitine, because of its antiperoxidative and scavenger effects, may be useful to prevent ionizing radiation-induced lipid peroxidation.

Materials and Methods

Animals

Twenty-four adult male Syrian golden hamsters, weighing 80-100 g, were used in this study. They were obtained from the Istanbul University Experimental Research Center and were housed in stainless cages with 3 hamsters per cage. The animals were maintained under standard laboratory conditions (temperature 22 ± 1 °C, 12-h light/dark cycles, relative humidity 55 %) and had free access to standard pellet diet (Gebze Food Factory, Kocaeli, Turkey) and tap water. The protocol for the study was approved by the Ethical Committee of Trakya University Medical Faculty, Animal Breeding and Research. Animal weights were measured at the beginning and the end of the experiment.

Experimental Design

The animals were divided into the following four groups (n=6): group 1: control; group 2: L-carnitine administered; group 3: irradiated-control group; and group 4: irradiated and L-carnitine administered group.

L-carnitine (Sigma-Aldrich Chemical Co., St Louis, Mo, USA) was dissolved in isotonic NaCl and given by gavage to the animals in groups 2 and 4 in a concentration of 50 mg/kg body weight for 15 consecutive days. Vehicle (saline, 1 ml/100 g) was administered to the hamsters by the same route to groups 1 (control) and 3 (irradiated-control) for 15 consecutive days. The hamsters of groups 3 and 4 were exposed to total body gamma irradiation (Table 1).

Table 1. Experimental design

Day	
1	Randomization Administration of saline to the hamsters of groups 1 and 3 Administration of L-carnitine to the hamsters of groups 2 and 4
15	Irradiation of the hamsters in group 3 and 4
16	Sacrification of all hamsters Measurement of lipid peroxidation and GSH and, enzymes SOD and catalase

Group 1: control; group 2: L-carnitine administered; group 3: irradiated-control group; group 4: irradiated and L-carnitine administered group.

Total Body Irradiation

A cobalt 60 teletherapy instrument (Cirus, Cis-Bio Int-France) was used to deliver a single peak whole body dose of 8 Gy (1 Gy/min) to a depth of 3 cm as described (*Feurgard et al., 1999; Sener et al., 2003; Dokmeci et al., 2004*). A single anterior field was used for irradiation and three animals were treated at a time. The cobalt 60 unit was calibrated with a Exradin Farmer type ionization chamber (Keithley 35040 radiation dosimeter, Cleveland, Ohio, USA). A ±3 % uncertainty in absorbed dose was estimated. 24 h after radiation exposure, the hamsters were

sacrificed and samples were taken from blood and tissues, and the biochemical and histopatological determinations were carried out.

Biochemical Analysis

At the end of the study, the animals were anaesthetized by intraperitoneal injection of ketamine, 200 mg/kg (Ketalar, Pfizer, Istanbul, Turkey) and xylazine, 10 mg/kg (Rompun, Bayer, Istanbul, Turkey). Blood samples were collected into heparin-treated (50 IU/ml) tubes by cardiac puncture. Plasma samples were obtained by centrifugation at 3000 rpm for 10 min at 4°C to determine the plasma malondialdehyde (MDA) levels. The leukocyte layer was taken off from the erythrocyte phase and the separated red blood cells were used for evaluation of superoxide dismutase (SOD) and catalase activities. Tissue samples were obtained from the liver to determine the liver MDA and glutathione (GSH).

The malondialdehyde in plasma and liver, a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid reacting substances (TBARS) (*Ohkawa et al., 1979*). GSH was determined by the spectrophotometric method which was based on the use of Ellman's reagent (*Ellman, 1959*). Superoxide dismutase activity was measured according to Sun et al. (*Sun et al, 1988*) by reduction of nitrobluetetrazolium by the xanthine-xanthine oxidase system, which is a superoxide generator. Enzyme activity leading to 50% inhibition was accepted as one unit. Results were expressed as unit per gram heamoglobin. Catalase assay was performed by the method of Aebi (*Aebi, 1984*) by measuring the decomposition of H_2O_2 at 240 nm.

Bone Marrow Determination

The femur was removed and fixed overnight in 10% formalin and decalcified with hydrochloric acid. The transverse sections were processed routinely for histological examination. They were embedded in paraffin wax and stained with hematoxylin and eosin (H&E), in 4 µm sections. The severity of histological lesions was evaluated in each group.

Statistical Analysis

The results are presented as the mean ± S.E.M. Enzyme parameters and lipid peroxidation levels of matched experimental and control pairs were analyzed with the Kruskal Wallis test. When there was a significant difference among groups, the Mann-Whitney U-test was used. P-values of less than 0.05 were considered statistically significant.

Results

Twenty-four hamsters entered the study and were randomized into four groups according to irradiation and drug administration. The animals were weighed at the beginning and end of the experiment. No weight difference was found in the groups of the irradiated and non-irradiated hamsters (data not shown).

The mean level of MDA, which is a major degradation product of lipid peroxidation, in plasma and liver, was increased in the irradiation group compared to controls ($p<0.001$ and $p<0.001$, respectively). L-carnitine alone had no effect on MDA levels. However, L-carnitine treatment in the irradiation group caused a marked decrease in mean MDA values of liver tissue compared with the untreated groups ($p<0.001$) (Fig. 1).

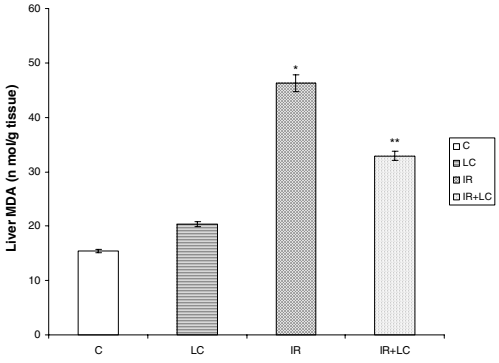

Figure 1. Malondialdehyde levels in liver of control, untreated and L-carnitine treated irradiation groups, and the effect of L-carnitine on control hamsters alone. Each group consisted of 6 hamsters. *$p<0.001$; compared to control group; **$p<0.01$; compared to non-treated irradiated group.

Similar to its effect at tissue level, L-carnitine treatment also abolished the increase in the plasma MDA level of the untreated irradiation group ($p<0.001$) (Fig. 2).

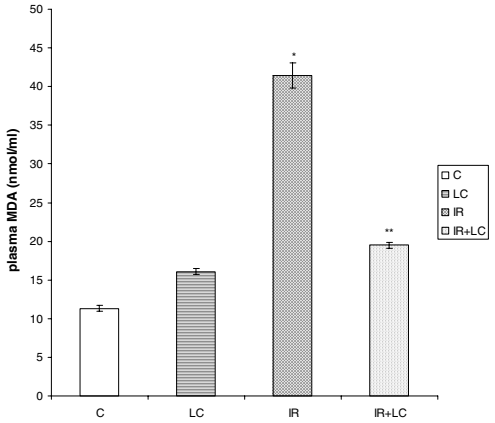

Figure 2. Malondialdehyde levels in plasma of control, untreated and L-carnitine treated irradiation groups, and the effect of L-carnitine on control hamsters alone. Each group consisted of 6 hamsters. *$p<0.01$; compared to control group; **$p<0.01$; compared to non-treated irradiated group.

The mean GSH level of the liver was dramatically decreased in the irradiation group compared to controls ($p<0.001$). L-carnitine treatment of the irradiation group increased the mean GSH level compared with the untreated group ($p<0.001$), whereas L-carnitine treatment alone had no significant effect (Fig. 3).

The activity of blood SOD and catalase significantly decreased after irradiation exposure ($p<0.05$ and $p<0.05$, respectively) (Fig. 4). L-carnitine treatment of the irradiation group reversed these enzymatic changes significantly ($p<0.05$ and $p<0.001$, respectively) (Figs. 4, 5).

After the TBI of the hamsters, the animal's bone marrow was evaluated (H&E X100, scale bar 50 μm). In the non-irradiated control and L-carnitine groups, bone marrow structures were observed to

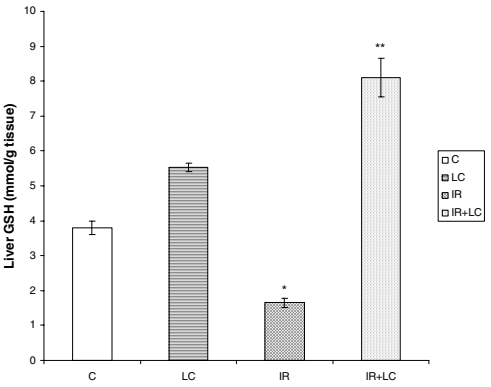

Figure 3. Glutathione levels in liver of control, untreated and L-carnitine treated irradiation groups, and the effect of L-carnitine on control hamsters alone. Each group consisted of 6 hamsters. *$p<0.01$; compared to control group; **$p<0.01$; compared to non-treated irradiated group.

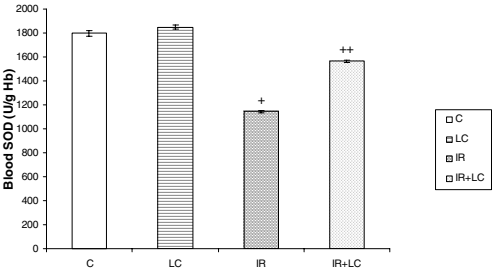

Figure 4. Blood superoxide dismutase activity. Each group consisted of 6 hamsters. +$p<0.05$; compared to control group; ++$p<0.05$; compared to non-treated irradiated group.

have normal morphology and ultrastructure (data not shown), while in the irradiated control group, enlarged sinusoids and bleeding were seen; L-carnitine significantly reduced the damage in the L-carnitine pre-irradiation group (Fig. 6, 7).

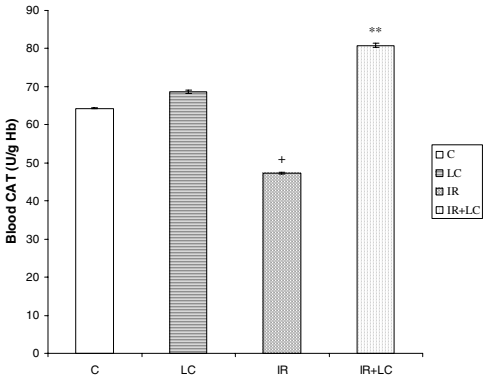

Figure 5. Blood catalase activity. Each group consisted of 6 hamsters. +p<0.05; compared to control group; **p<0.001; compared to non-treated irradiated group.

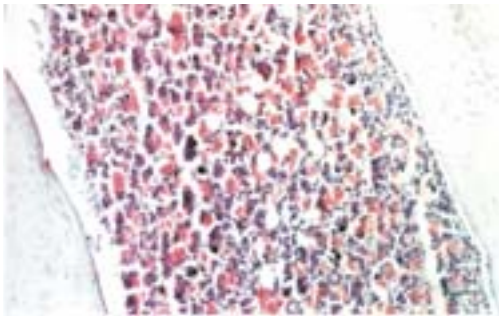

Figure 6. Group III: Irradiated control group (H&E X100, scale bar 50 μm)

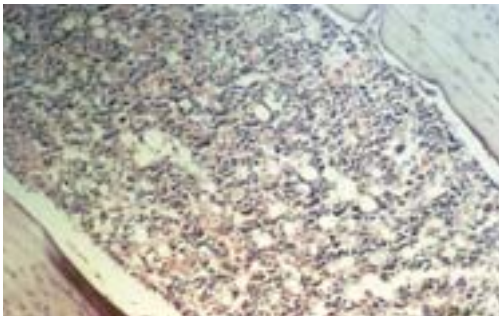

Figure 7. Group IV: L-carnitine pre-irradiation group (H&E X100, scale bar 50 μm)

Discussion

Global tissue damage due to oxygen-derived free radicals has been implicated in several pathological processes including exposure to ionizing radiation, ischemia-postischemic reperfusion and the inflammatory response. The damaging effect of ionizing radiation on living cells is predominantly due to ROS, and there are two available antioxidant strategies in oxidative stress. In the first, ROS are removed using specific enzymes such as SOD and glutathione peroxidase, either by administration of these enzymes or by increasing their in vitro activities. In the second, radical generation is prevented. Since biological antioxidants inactivate free radicals and their products, the enzymes involved in the metabolism of ROS are expected to play an important role in the sensitivity of cells to radiation. Hence, chemical repair may be provided by protective compounds present during exposure to ionizing radiation (*Riklis et al., 1996; Saada & Azab, 2001; Sener et al., 2003; Dokmeci et al, 2004*). A number of dietary antioxidants have been reported to decrease free radicals (*Duthie et al., 1996; Saada & Azab, 2001*).

L-carnitine, a naturally occurring enzymatic antioxidant, is a necessary factor in the utilization of long-chain fatty acids to produce energy. Furthermore, carnitine improves the turnover of fatty acids peroxidated by the free oxygen radicals produced during normal metabolism (*Rebouche & Seim, 1998*). Ronca et al. (1992) have shown that L-carnitine supressed hydroxyl radical production in the Fenton reaction, probably by chelating the iron required for the generation of hydroxyl radicals. Furthermore, the preventive effect of L-carnitine on the formation of ROS due to the xanthine/XO system has been demonstrated by Di Giacomo et al. (1993). Although our data do not reveal the mechanism of the action of L-carnitine, prevention of lipid peroxidation, scavenging of ROS and/or reducing antioxidant depletion might be the putative mechanisms. To our knowledge, this is the first report of the effect of L-carnitine on the formation of ROS by ionizing radiation.

Many studies showed previously that Total Body Irradiation (8Gy) produces increased lipid peroxidation in several tissues and body fluids, and changes antioxidant enzymes activities (*Kergonou et al., 1981; Feurgard et al., 1999; Sener et al., 2003; Dokmeci et al., 2004*). In our study, the antioxidant effect of L-carnitine having been proven in many previous studies, its radiation–protective action was investigated. Several pathological conditions that cause elevation of MDA due to lipid peroxidation are prevented by carnitine and its derivatives such as ischemia/reperfusion injury (*Packer et al., 1991*), adriamycin-induced (*Kawasaki et al., 1996*) and doxorubicin-induced (*Luo et al., 1999*) cardiomyopathy and myocardial infarction (*Pauly & Pepine, 2003*). Previous studies have shown that L-carnitine intake decreases the production of superoxide anion by exudate cells in a rat model of carrageenan-induced inflammation (*Izgut-Uysal et al., 2003*) and has neuroprotective action with free radical scavenging effects (*Binienda, 2003*).

Recently, it has also been suggested that L-carnitine derivatives may reduce the age-dependent accumulation of lipofucsines (*Arockia Rani & Panneerselvam, 2001*), which are considered to be by-products of lipid peroxidation. Besides, L-carnitine increases the synthesis of phospholipids required for membrane formation and integrity and plays a role in membrane repair by reacylation of phospholipids (*Kashiwagi et al, 2001*). Moreover, carnitines protect erythrocytes and low density lipoproteins against peroxidation induced by ROS (*Bertelli et al., 1994*). Recently we have reported for the first time that L-carnitine attenuated ethanol-induced gastric mucosal injury. This gastroprotective effect may be mediated by its well-known antioxidant potential (*Dokmeci et al., 2005*). In the present study, the increase in the level of MDA in plasma and liver after irradiation indicates an increase in lipid peroxidation. The significant protective role of L-carnitine against lipid peroxidation has been demonstrated by improvement in the levels of MDA in the plasma and tissue of hamsters pretreated with L-carnitine before irradiation. Even though the antioxidant and/or free radical scavenging mechanism of action of L-carnitine await elucidation, the results obtained by us indicate that L-carnitine modifies irradiation-induced elevation of oxidative reactions. Our findings suggest for the first time that L-carnitine has radiation-protective activity.

Radiation induces changes in antioxidant activities expressed as a decrease in blood SOD and catalase activities, and radiation resistance of various mammalian cell lines was shown to correlate with their SOD activity (*Yamaguchi et al., 1994*). Our results also demonstrated that irradiation decreased blood SOD and catalase activities in irradiated-hamsters. Superoxide radical is one of the major products of radiolysis of cellular constituents in the presence of oxygen and might continue to be formed in the post-irradiation period (*Yukawa et al, 1983; Riley, 1994; Berroud et al., 1996; Chevion et al., 1999*). Since intracellular SOD is a natural antioxidant and acts as a selective scavenger of superoxide radical, the enhanced specific activity of SOD (up to 4 Gy) is likely to catalytically metabolize superoxide radical leading to radiation protection of tumors. In the case of radiation doses greater than 6 Gy, the activity of SOD might have been inhibited due to adverse effects of radiolytically generated free radicals on the enzyme itself (*Sharma & Kale, 1993*). In the present study, the modulatory role of L-carnitine on SOD and catalase activities induced by exposure to ionizing radiation becomes evident as L-carnitine offered significant protection expressed as increased activity of the two enzymes.

Navarro et al. (1997) reported that tissue GSH levels are significantly reduced by oxidative stress and proposed that impairment of antioxidant defense mechanisms could permit enhanced free-radical-induced tissue damage. Furthermore, GSH, the most prevalent nonprotein thiol in mammalian cells, protects against radiation-induced cell damage. Radiation resistance of many cells is associated with high intracellular levels of GSH (*Mitchell & Russo, 1987*). In the present study, the decrease in tissue GSH levels after irradiation may be due to its

consumption during the oxidative stress induced by ionizing radiation. Taken together with these previous reports, the results of the present study suggest that L-carnitine is a very potent agent in replenishing the tissue GSH, which has a major role in the antioxidant defense mechanisms against irradiation injury.

It is known that carnitines have beneficial effects on sickle-cell (*Ronca et al., 1994*), thalassaemic (*Palmieri et al., 1994*) and chronic renal failure anemia (*Golper et al., 2003*). In addition, oral or intravenous L-carnitine therapy results in an increase in haematocrit, mean reticulocyte count, haemoglobin levels, erythrocyte count and survival time, and a significant decrease in erythropoietin requirement in hemodialysis patients. L-carnitine stabilizes cellular membranes, prolongs their lives and raises red blood cell osmotic resistance (*Nikolaos et al., 2000; Matsumoto et al. 2001*). In the present study, L-carnitine protected the bone marrow from radiation injury.

The antioxidative and/or free radical scavenging effects of L-carnitine have been proven in many previous studies, but our study investigated for the first time its protective action on ionizing radiation-induced lipid peroxidation. Our findings strongly support that L-carnitine modulates the radiation-induced changes in the antioxidant activities of SOD and catalase and consequently reduces lipid peroxidation and counteracts the imbalance produced by the release of excessive quantities of ROS. Based on these observations, this radioprotective effect might be induced, at least partly, through antioxidant mechanisms and free radical scavenging capacity. However, further studies need to be done to determine the mechanism involved.

Abbreviations: GSH – glutathione, H&E – hematoxylin and eosin, MDA – malondialdehyde; ROS – reactive oxygen species, SOD – superoxide dismutase, TBARS – thiobarbituric acid reactive substances, TBI – total body irradiation

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