

Evaluation of the Protective Effect of L-Carnitine on Radiation Induced Free Oxygen Radicals and Genotoxicity in Male Mice

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Abstract: The aim of the study was to evaluate the potential protective effect of L-carnitine on radiation induced free radicals in mice .To achieve the ultimate goal of this study ,122 adult Swiss male mice were randomly divided into 4 groups. Group 1: Did not receive L-carnitine or irradiated and served as control group. Group 2: Daily injected subcutaneous with L-carnitine (200 mg/Kg) for 3 successive weeks. Group 3: Irradiated with fractionated dose of 6 Gy where the dose was 2 Gy once per week for 3 successive weeks. Group 4: Received a daily subcutaneous injection of L-carnitine (200 mg/Kg) for 7 consecutive days before the first dose of irradiation and continuous together through the experimental period. The animals were sacrificed at 1, 7 and 14 days after last dose of irradiation. The samples were taken from the blood and some organs, heart, spleen, testes and bone marrow for the biochemical and cytogenetics analysis. In the irradiated group, there were a significant decrease in RBCs ,WBCs, Hb, Hct, plasma levels of testosterone and ferritin. Also, significant increase in TBARS in both cardiac and spleen homogenates tissues which accompanied with significant decrease in reduced GSH content in both cardiac and spleen tissues as compared to control group. We analysed micronuclei in bone marrow cells and chromosome aberrations in spermatocyte cells, which represent an appropriate cytogenetic model to study compounds that enhance cell protection against externally induced DNA damage. Our results showed that L-carnitine has the ability to reduce the haematological, some biochemical changes and chromosomal damage induced by γ -rays. In conclusion, L-carnitine has a potential effect for scavenging ROS induced by radiation. [Researcher, 2009; 1(6):7-15]. (ISSN: 1553-9865).

Key words: L-carnitine, γ -irradiation, Haematopiotic, GSH, Testosterone, Genotoxicity, Oxidative stress, Mice

1. Introduction

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 endogenously by skeletal muscle, heart, liver, kidney and brain from the essential amino acids lysine and methionine (Rebouche and Seim, 1998). It is known that L-carnitine and its derivatives prevent the formation of reactive oxygen species (ROS) and protect cells from per oxidative stress (Sener *et al*, 2004; Dokmeci *et al*, 2006). The dependence on carnitine uptake is evident from patients suffering from primary systemic carnitine deficiency (CDSP), an autosomal recessive disorder of fatty acid oxidation, caused by mutations in the *OCTN2* gene encoding an organic cation/carnitine transporter (Wang *et al*, 1999). It has been reported that carnitine protects the myocardium against ischemia (Reznick *et al*, 1992), myocardial infarction (Singh *et al*, 1996) and skeletal muscle myopathy in heart failure (Vescovo *et al*, 2002).

The interaction of radiation with the component of living organs results in the generation of ROS

which is responsible for many injurious in living cells, including gene mutations, cellular transformation and cell death. These effects have been attributed to radiation induced DNA damage. However, the cytotoxicity may be delayed for up to six generations for cell replication (Wright *et al*, 1998), and chromosomal aberrations due to destabilization of the genome (Wang *et al*, 2000). Ionizing radiation damage to cells is oxidant dependent and can be inhibited with exogenous antioxidant supplementations (Reeves, 2003).

So, this study was designed to detect the capability of L-carnitine to protect mammalian cells from oxidative stress induced haematological, biochemical and DNA damage as a consequence of gamma irradiation in male mice.

2. Material and Methods

2.1 Experimental animals

Male Swiss mice (28 \pm 2 gm) obtained from the animal house of National Research Center, Dokki, Giza, Egypt. Animals were kept under standard conditions through the experimental period. The

mice were fed on pellet concentrated diet containing all the necessary nutritive elements. Liberal water intakes were available.

2.2 Radiation Facility

Whole body gamma irradiation was performed using gamma cell Co-60 unit installed at the Middle Eastern Regional Radioisotopes center for the Arab Countries (MERRCAC), Dokki, Giza, Egypt. Mice were exposed to fractionated gamma irradiation delivered as 2 Gy once per week up to cumulative dose of 6Gy through 3 successive weeks.

2.3 Chemical

L-carnitine was purchased from Arab Company For Pharmaceuticals and Medicinal Plants, Egypt. The product is provided as 350 mg capsules. A capsule content was dissolved in distilled water. All other chemicals used of analytical grade.

2.4 Experimental design

Animals were divided into 4 groups. Group 1: Mice were neither treated with L-carnitine nor irradiated and were considered as control. Group 2: Mice received daily subcutaneous injection of 200 mg /Kg body weight of L-carnitine for 21 consecutive days. Group 3: Mice were exposed to fractionated gamma irradiation 2 Gy once per week up to a cumulative dose of 6Gy. Group 4 : Mice were received a daily subcutaneous injection of L-carnitine (200 mg/Kg) for 7 consecutive days before the first dose of irradiation and continuous simultaneously through the experimental period. Mice from each group were slightly anaesthetized with ether and sacrificed at every experimental time intervals 1, 7 and 14 days after last irradiation dose.

2.4 Biochemical analysis

Heart and spleen were removed from six mice per each group to run biochemical analysis. Blood samples were obtained on EDTA for RBCs, WBCs, Hb and Hct. RBCs and WBCs determination using improved Neubauer chamber according to **Dacie and Lewis (1991)**. Blood haemoglobin was determined calorimetrically as cyanmethaemoglobin in grams per deciliter using spectrum Diagnostic kit according to **Teitz (1990)**. The ratio of erythrocytes to plasma in percent (Hct) was measured as the volume of erythrocytes per 100 ml blood after **Seivered (1964)**. GSH content was determined according to **Beutler et al (1963)** in both cardiac and spleen homogenates. Lipid peroxide content was determined by quantifying the thiobarbituric acid reactive substances (TBARS) in cardiac and spleen tissue homogenates after the method described by **Yoshioka et al (1979)**. Plasma

levels of testosterone and ferritin were assayed by radioimmunoassay (RIA) techniques using commercial kits depending on solid phase RIA (Coat-A-Count) Diagnostic product corporation (DPC), Los Angeles, USA.

2.5 Slide preparation and scoring for cytogenetic analysis.

2.5.1 Micronucleus test.

The micronucleus assay from mouse bone - marrow cells was performed following the standard procedure described by **Schmid (1973)**. The significance of the experimental groups from control data was calculated using differences between 2 proportions (**Daniel, 1974**), in the case of PE's and the tables of **Kastenbaum and Bowman (1970)** for PE's with micronuclei. Five animals per group were examined for assessment of micronucleus test.

2.5.2 Chromosomal aberrations.

Chromosomal preparations from testes were made according to the technique developed by **Evans et al (1964)** and 100 well-spread diakinesis metaphase-I cells were analyzed per animal to assess abnormalities in five mice per group. Metaphases with translocations were recorded.

2.6 Statistical analysis

The significance of the difference between different treated groups versus control and between L-carnitin plus radiation against radiation group alone was calculated using the *t*-test according to **Snedecor and Cochran (1982)**. The differences were considered significant at $P < 0.05$.

3. Results

Table (1) showed that mean circulating RBCs, WBCs, Hb and Hct % exhibited significant decrease with irradiation compared to control group at ($P < 0.05$) through the experimental period. Also, significant decrease in plasma testosterone and ferritin levels in irradiated group as compared to control was recorded. The treatment with L-carnitine revealed significant improvement in all the tested parameters.

Table (2) showed that fractionated gamma irradiation of 6Gy significantly depressed GSH content in both cardiac and spleen homogenates tissues. L-carnitine together with irradiation ameliorated that value through time intervals. Significant increase in TBARS levels ($P < 0.05$) above the control level is detected post gamma irradiation, treatment with L-carnitine alone and L-carnitine together with irradiation ameliorated that value through time intervals.

Table (1): Effect of L-carnitine on RBCs, WBCs, Hb and Hct% in mice exposed to fractionated dose of 6 Gy.

Parameters	Time post irradiation (days)	control	L-carnitine	Irrad.	L-carn. +irrad.
RBCs $10^6/cc^3$	1	6.49±0.32	6.82±0.24	4.09±0.25 ^{ab}	4.67±0.27 ^{ab}
	7	6.16±0.24	6.79±0.23	4.03±0.06 ^{ab}	4.95±0.06 ^{abc}
	14	6.44±0.30	6.96±0.09	4.92±0.24 ^{ab}	5.92±0.31 ^{bc}
WBCs $10^3/cc^3$	1	11.22±0.52	11.25±0.53	5.48±0.76 ^{ab}	9.07±0.49 ^{abc}
	7	11.88±0.87	12.02±0.40	5.13±0.18 ^{ab}	8.83±0.44 ^{abc}
	14	11.12±0.41	11.88±0.56	7.39±0.64 ^{ab}	10.65±0.44 ^c
Hb g/dl	1	14.11±0.31	14.19±0.16	12.83±0.28 ^{ab}	13.43±0.28 ^b
	7	14.21±0.19	14.55±0.27	11.81±0.17 ^{ab}	13.63±0.36 ^c
	14	14.16±0.16	14.15±0.19	11.56±0.29 ^{ab}	13.81±0.30 ^c
Hct %	1	52.67±0.85	54.50±0.52	41.00±0.48 ^{ab}	43.00±0.48 ^{abc}
	7	54.83±0.77	55.83±0.71	43.86±0.58 ^{ab}	49.25±0.67 ^{abc}
	14	55.75±0.67	56.08±0.62	50.83±0.74 ^{ab}	55.33±0.61 ^c
Testosterone ng/ml	1	1.62±0.11	1.50±0.05	0.66±0.14 ^{ab}	1.27±0.20 ^c
	7	1.58±0.12	1.47±0.17	0.98±0.06 ^{ab}	1.45±0.04 ^c
	14	1.88±0.15	1.85±0.08	1.27±0.12 ^{ab}	1.75±0.07 ^c
Ferritin ng/ml	1	0.58±0.01	0.57±0.03	0.40±0.02 ^{ab}	0.45±0.01 ^{ab c}
	7	0.59±0.01	0.58±0.02	0.45±0.01 ^{ab}	0.49±0.001 ^{ab c}
	14	0.57±0.01	0.59±0.03	0.41±0.02 ^{ab}	0.53±0.05 ^c

Number of animals/group 6±S.E. and are considered significant at P<0.05 a:significant different from control group.
b:significant different from L-carnitine group. c:significant different from irradiated group

Significant increase in TBARS levels (P<0.05) above the control level is detected post gamma irradiation, treatment with L-carnitine alone

and L-carnitine with irradiation decreased the level of TBARS to near the control level.

Table (2): Effect of L-carnitin on reduced glutathione content(GSH) and lipid peroxidation(TBARS)in cardiac and spleen tissue in male mice exposed to fractionated dose of 6 Gy

Parameters	Time post irradiation (days)	Control	L-Carnitine	Irradiated	L-Carnitine + Irradiation
Cardiac GSH µg/ g wet tissue	1	13.38±0.31	13.22± 0.37	11.44± 0.17 ^{ab}	13.13± 0.34 ^c
	7	14.71±0.79	15.07± 0.74	12.29± 0.07 ^{ab}	13.41± 0.44 ^c
	14	13.26±0.32	13.41± 0.59	9.61± 0.36 ^{ab}	12.32± 0.43 ^c
Cardiac TBARS µmol/ g wet tissue	1	15.87±0.61	15.21± 0.55	26.92± 0.48 ^{ab}	20.18± 0.61 ^{abc}
	7	18.71±0.49	18.77± 0.66	24.43± 0.63 ^{ab}	21.79± 0.39 ^{abc}
	14	18.41±0.37	18.25± 0.57	27.91± 0.61 ^{ab}	19.64± 0.76 ^c
Spleen GSH µg/ g wet tissue	1	23.16±0.82	23.23±0.88	15.03± 0.76 ^{ab}	17.59± 0.79 ^{abc}
	7	23.08±0.87	25.05±0.66	16.56±0.48 ^{ab}	18.34± 0.57 ^{abc}
	14	23.25±0.77	24.75±0.35	16.51± 0.77 ^{ab}	21.39± 0.68 ^{bc}
Spleen TBARS µmol/ g wet tissue	1	20.29±0.42	20.01±0.36	43.63± 0.59 ^{ab}	38.46± 0.46 ^{abc}
	7	22.66±0.31	22.39±0.32	35.65± 0.55 ^{ab}	30.46± 0.48 ^{abc}
	14	21.02±0.57	21.01±0.27	30.59± 0.62 ^b	23.02± 0.84 ^{bc}

Legends as in Table 1

From the results presented in Table (3) we found that the percentage of micronuclei in polychromatic erythrocytes (MPCE) statistically significant after all treatment compared to control. The highest level reached after 1 day then declined at 7 and 14 days post irradiation. The protective effect of L-carnitine administered as successive dose for 3 weeks with γ -rays exposure reduced MPCE after 1, 7 and 14 days compared to irradiated group alone.

Fractionated dose of 6 Gy induced a significant elevation in the percentage of chromosomal translocations compared to the control. The maximum percentage observed after 1 day post-irradiation in mouse spermatocytes. It reached of 10.4 ± 0.52 then the percentage of observations decreased with longer duration after exposure. This maximum percentage decreased to 9.0 ± 0.54 in the treated group received L-carnitine with irradiation (Table 4).

Table (3): Percentage of MPCE in mouse bone – marrow cells exposure to γ -rays and L-carnitine with γ -rays for 3 weeks.

Treatment	Time post irradiation (days)	No of PE with micronuclei	No. of PCE's	% MN (PCE's ± S.E.)
Control	—	14	2630	0.53 ± 0.35
L-carnitine (200 mg kg ⁻¹ b.wt.)	1	12	2421	0.49 ± 0.41
	7	12	2401	0.50 ± 0.53
	14	10	2385	0.42 ± 0.50
	γ -rays 2 Gy once/week/3weeks	1	412	3011
	7	328	2978	11.01± 0.81 ^{ab}
	14	301	3112	9.67 ± 0.73 ^{ab}
L-carnitine + γ -rays	1	327	2851	11.46 ± 0.55 ^{abc}
	7	307	3272	9.38 ± 0.75 ^{ab}
	14	218	2601	8.38 ± 0.70 ^{ab}

Legends as in table 1

Table 4: Detailed results of translocations induced in mouse spermatocytes exposure to γ -rays and L-carnitine with γ -rays for 3 weeks.

Treatment	Time post irradiation (days)	No of abnormal cells	Chromosome aberrations				Translocations % \pm S.E.
			Chain IV	Ring IV	Chain VI	Ring VI	
Control	—	—	—	—	—	—	—
L-carnitine (200 mg kg ⁻¹ b.wt.)	1	—	—	—	—	—	—
	7	—	—	—	—	—	—
	14	—	—	—	—	—	—
γ -rays 2Gy once/week/3weeks	1	52	23	15	8	6	10.40 \pm 0.52 ^{ab}
	7	48	20	14	9	5	9.60 \pm 0.63 ^{ab}
	14	40	18	14	6	2	8.0 \pm 0.57 ^{ab}
L-carnitine + γ -rays	1	45	23	13	6	3	9.0 \pm 0.54 ^{ab}
	7	40		23	10	4	8.0 \pm 0.48 ^{ab}
	14	30				3	6.0 \pm 0.64 ^{ab}
						4	
					17	8	1

No. of examined metaphases = 500

Legends as in table 1

4. Discussion

Radiation can have tremendous therapeutic benefits for humans. It is also associated with the risk of serious adverse effects (Borek, 2004; Jagetia *et al*, 2006). It produces reactive oxygen species (ROS) that damage proteins, lipids and nucleic acid (Nair *et al*, 2001). Haematopoietic system mainly bone marrow is known to be one of the most radiosensitive and its damage may be critical for the survival due to haematopoietic syndrome (Tukov *et al*, 2002).

The present study demonstrated that fractionated whole body gamma irradiation dose of 6Gy significantly affected red blood cells number, haemoglobin content and haematocrit, which is a sign of anemia. This may be attributed to lysis of circulating RBCs due to a decreased production of erythropoietin (Alfrey *et al*, 1997) and haemorrhages caused by structural changes in membrane proteins, modification in internal peptides as well as internal viscosity of RBCs (Gwozdinski 1991). Moreover, radiation induced haemodilution shown by decreased haematocrite value. The circulating WBCs were affected by fractionated whole body gamma irradiation dose of 6 Gy. It is known that lymphocytes are replication components that normally survive in the blood for 2-4 days and are highly radiosensitive (Sado *et al*,

1998). Radiation induced depletion in lymphocytes is primarily due to apoptosis, although necrotic death occurs (Kajioka *et al*, 2000).

The obtained results observed that whole body gamma irradiation caused dramatic decrease in plasma testosterone levels. This finding is in accordance with Jégou *et al* (1991) and Dygalo *et al* (1997). They reported that radiation has particularly severe adverse effect on gonads and therefore on fertility in both animals and man. Also, irradiated animals showed significant decrease in plasma ferritin level. Oxidative stress induced by irradiation causes damage in the liver of rats resulting in ferritin degradation, increase in the intracellular free iron levels (Atkinson *et al*, 2005). However, the increase in iron level may be resulted from releasing iron from haemoglobin in irradiated animals and releasing iron from its macromolecular complexes ferritin and transferring (Comporti *et al*, 2002).

Exposure of animals to fractionated dose of 6 Gy affected seriously the biological cell membrane. Gatsko *et al* (1990), attributed the intensity of lipid peroxidation to the inhibition occurring in the antioxidant system caused by irradiation. Also, Zheng *et al* (1996) demonstrated that the increase in lipid peroxidation was related to the decrease in the biooxidase activities after irradiation. The anion

radicals formed by ionizing radiation react with polyunsaturated fatty acids in biological membrane forming lipid peroxides which result in severe damage to cellular membrane, organelles and their associated enzymes (Poli *et al*, 1985). The observed decrease in GSH content after irradiation may be due to the diminished activity of glutathione reductase and to the deficiency of NADPH formed by glucose -6-phosphate -dehydrogenase which is necessary to change the oxidized glutathione to its reduced form (Pulpanova *et al*, 1982).

In the present study animals injected with L-carnitine before irradiation had significant improvement in RBCs, WBCs, Hb and Hct as compared to irradiated group. This may be due to that L-carnitine had beneficial effects on stabilized cellular membranes prolongs their lives and raises red blood cell osmotic resistance (Nikolaos *et al*, 2000 ; Matsumoto *et al*, 2001). Also, L-carnitine induced elevation in GSH content in both cardiac and spleen homogenates which is accompanied with significant decrease in TBARS in the same tissue homogenates. However, GSH has a major role in the antioxidant defense mechanisms against irradiation injury (Harun *et al*, 2006 ; Ibrahim *et al*, 2007). Carnitine improves the turnover of fatty acids peroxidated by the free oxygen radicals produced during normal metabolism (Rebouch and Seim, 1998). Ronca *et al* (1992) showed that L-carnitine suppressed 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).

In the absence of direct data on the genetic radio sensitivity of human germ cells, the assumption is made that, unless there is evidence to the contrary, the response of human germ cells is similar to those of the mouse (Paul and Van Buul ,1973). In recent years it has become clear that the biology of spermatogenesis plays a major role in determining yields of genetic damage from mammalian spermatogonial stem cells following γ -rays. This has been best demonstrated in the mouse using reciprocal translocations as the genetic endpoint (Cattanach and Crocker, 1980). The micronucleus test is found to be a sensitive marker for clastogenic and mutagenic effects to detect chromosomal damage caused by different types of mutagens (Mavrounic *et al*, 1990).

Our results showed that the frequency of micronuclei in polychromatic erythrocyte (MPCE) and chromosome translocation in spermatocytes increased significantly after 1 day post exposure then declined up to 14 days. The same results observed by Cole *et al*, (1981) and Jagetia and

Ganapathi (1994) they found that the frequency of MPCE increased significantly at 12h post – exposure, reached the highest percentage after 24h, thereafter it declined. Zahran *et al* (2004) reported that the percentage of MPCE from rat bone marrow cells reduced from 1 to 10 days after exposure to 6 Gy gamma rays.

Winegar *et al* (1994) found that γ -rays at doses ranging from 0.1 to 14 Gy induced point mutations, deletions and micronuclei in lacI transgenic mice. The induction of MPCE of bone-marrow of Swiss mice exposure to γ -rays was dose dependent (Frash *et al*, 1999). The induction of translocations in the spermatocytes of the mouse by radiation has been extensively studied because of its relevance to the induction of hereditary defects in man (Leenhouts and Chadwick, 1981).

The decreased in the frequency of MPCE and chromosome translocation with increasing the time after irradiation may be attributed to decline the stem cell killing levels gradually and for rapid stem cell repopulation (Leenhouts and Chadwick, 1981). Cytological evidence for rapid stem cell repopulation has indeed been found several days after radiation exposure in both rat and mice (Huckins and Oakberg, 1978; Zahran *et al*, 2004).

Our study observed that injection of L-carnitine with irradiation reduced DNA damage in mouse somatic and germ cells. The modulation of DNA repair by L-carnitine, as an alternative mechanism to counteract radiation-mediated clastogenicity, is supported by many studies suggest that L-carnitine could help the cells to repair single-strand breaks (SSBs) induced in DNA (Boerrigter *et al*, 1993) and to protect it from oxygen free radicals (Vanella *et al*, 2000). Other studies indicated that L-carnitine-dependent reduction of DNA single-strand breaks after *in vitro* treatment with an oxygen radical-generating system, in isolated human lymphocytes (Garcia *et al*, 2006). Santoro *et al* (2005) reported that L-carnitine have the ability to inhibition chromosomal damage induced by H₂O₂ as an oxidative stress in CHO cells. Pre-treatment of mice with L-carnitine 1h before exposure to magnetic field caused a significant recovery of mice testes damage induced by high magnetic field (Ramadan *et al*, 2002). Berni *et al* (2008) demonstrated that L-carnitine pre-treatment on oxidative DNA damage (tert-butyl-hydroperoxide) produced an enhancement of the rate and extent of DNA repair in *Ataxia telangiectasia* (A-T) patient's cell lines at early recovery time. Furthermore, a reduction of all types of chromosomal aberrations was observed, both in A-T and in wild-type cell lines.

It could be concluded that, the data obtained *in vivo* represent a possible strategy to reduce oxidative stress and protect mammalian cells from the damage

caused by reactive oxygen species using a natural compound like L-carnitine.

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