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L-Carnitine protects against testicular dysfunction caused by gamma irradiation in mice

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ABSTRACT

This study was conducted on mice to evaluate the radioprotective role of L-carnitine against γ-ray irradiation-induced testicular damage. Adult male mice were exposed to whole body irradiation at a total dose of 1 Gy. Radiation exposure was continued 24 h a day (0.1 Gy/day) throughout the 10 days exposure period either in the absence and/or presence of L-carnitine at an i.p. dose of 10 mg/kg body weight/day. Results revealed that γ-rays irradiation suppressed the expression of ABP and $CYP450_{SCC}$ mRNA, whereas treatment with L-carnitine prior and throughout γ-rays irradiation exposure inhibited this suppression. Treatment with γ-ray irradiation or L-carnitine down-regulated expression of aromatase mRNA. With combined treatment, L-carnitine significantly normalized aromatase expression. γ-Ray irradiation up-regulated expression of FasL and Cyclin D2 mRNA, while L-carnitine inhibited these up-regulations. Results also showed that γ-ray-irradiation up-regulated TNF-α, IL1-β and IFN-γ mRNA expressions compared to either controls or the L-carnitine treated group. Moreover, γ-irradiation greatly reduced serum testosterone levels, while L-carnitine, either alone or in combination with irradiation, significantly increased serum testosterone levels compared to controls. In addition, γ-irradiation induced high levels of sperm abnormalities (43%) which were decreased to 12% in the presence of L-carnitine. In parallel with these findings, histological examination showed that γ-irradiation induced severe tubular degenerative changes, which were reduced by L-carnitine pre-treatment. These results clarified the immunostimulatory effects of L-carnitine and its radioprotective role against testicular injury.

Introduction

Nowadays, there is an increased use of irradiation in clinical therapy and industrial fields causing undesired side effects often with delayed action to persons exposed to the irradiation. Therefore, studying the biological damage induced by ionizing radiation is necessary for assessment of maximum absorbed dose during radiotherapy or diagnosis, and is essential for development of protective tools for protection or recovery of undesired tissue damage (Borek, 2004). Irradiation causes direct damage to cells by ionization of DNA and indirect damage by increasing free radicals and reactive oxygen species (ROS) (Epperly et al., 2001, 2003a; Borek, 2004). Radiotherapy causes destruction of tumor cells, but threatens the integrity and survival of surrounding normal cells (Konopacka and Rogoliński, 2010). The diversity of irradiation used in medicine, industry, agriculture, scientific research and the military increases the need to find protective measures (Epperly et al., 2003b). It is well known that irradiation causes activation of apoptosis and related genes such as caspase-3 by DNA fragmentation (Epperly et al., 2002) and mediation of some cytokines such as TNF-α, IL-1 and IL-8 (Epperly et al., 2003a). L-Carnitine is a naturally occurring compound synthesized in human body and is also

Abbreviations: ABP, androgen binding protein; $CYP450_{SCC}$, cholesterol side-chain cleavage enzyme; DEPC, diethylpyrocarbonate; FSH, follicle stimulating hormone; GLUT1, glucose transporter member 1; Gy, gray; IFN-γ, interferon gamma; IL1-β, interleukin 1 beta; IGFBP-4, insulin-like growth factor-binding protein 4; ROS, reactive oxygen species; TAE, Tris–acetate–EDTA; TNF-α, tumor necrosis factor alpha; Esr1, estrogen receptor 1; Esr2, estrogen receptor 2.

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Table 1
PCR primer names, annealing temperatures, nucleotide sequences and products size.

Gene	Annealing temperature	Primer sequence (5′–3′)	Product size
β-Actin	54 °C	Forward – TTCTTTGCAGCTCCTTCGTTGCCG Reverse – TGGATGGCTACGTACATGGCTGGG	457 bp
Cyclin D2	62 °C	Forward – GGAACCTGGCGGCAGTCACC Reverse – AATCATCGACGGCGGGTACATG	163 bp
$CYP450_{SCC}$	65 °C	Forward – AGAAGCTGGGCACTTTGGAGTCAG Reverse – TCACATCCCAGGCAGCTGCATGGT	536 bp
FasL	53 °C	Forward – GAGAATTGCTGAAGACATGACAATCC Reverse – GTAGTTTTCACTCCAGACATTGTCC	314 bp
ABP	65 °C	Forward – CCATTCCTCCTTTGAGTTTCGA Reverse – CAGCTCCACCCGGGTGC	195 bp
Aromatase	54 °C	Forward – GCCTGTCGTGGACTTGGT Reverse – GGTAAATTCATTGGGGTTGG	142 bp
TNF-α	54 °C	Forward – TTCTTTGCAGCTCCTTCGTTGCCG Reverse – CAGAGAGGAGGTTGACTTTC	429 bp
IL-1β	54 °C	Forward – 5′-GTGACGTTCCCATTAGACAA Reverse – 5′-TGTCCTGACCACTGTTGTTT	431 bp
INF-γ	54 °C	Forward – TGCATCTTGGCTTTGCAGCTCTTC Reverse – GGGTTGTTGACCTCAAACTTGGCA	350 bp

found in the diet (Izgut-Uysal et al., 2003). It is an essential cofactor of several enzymes necessary for energy metabolism and acts as a scavenger of free radicals in cells and also possesses anti-inflammatory effects (Izgut-Uysal et al., 2003). L-Carnitine has been shown to be immunomodulatory (Deufel, 1990), with both antioxidant and antiapoptotic effects (Monti et al., 1992). It decreases irradiation-induced increases of malondialdehyde levels and increases the activities of superoxide dismutase and catalase (Uçüncü et al., 2006). L-Carnitine has also been shown to play a role in the control of the male reproductive system and normal function of the testis (Ng et al., 2004).

Nutritional supplementation with L-carnitine improves sperm quality and/or quantity in the testis of mice exposed to physical insults, such as heat and X-ray irradiation, and in men with idiopathic oligoasthenospermia. The antiapoptotic effects of L-carnitine in the testis may also contribute to these benefits, but this remains speculative and requires further investigation (Bansal et al., 1990; Ng et al., 2004). Recently, it has been shown that L-carnitine and vitamin E have protective effects on the testis of atherosclerotic rats confirming its role in male fertility (Salama et al., 2013).

Androgen-binding protein (ABP) is a glycoprotein secreted by Sertoli cells in the seminiferous tubules of the testis. ABP is thought to regulate spermatogenesis by maintaining high androgen levels in testis and epididymis (Hammond, 1995). Aromatase, or estrogen synthase, is an enzyme responsible for a key step in the biosynthesis of estrogens. It is a member of the cytochrome P450 superfamily encoded by the CYP19 gene (Carreau et al., 2002). Aromatase expression is detected in Sertoli cells, Leydig cells, spermatogonia, spermatocytes, elongate spermatids and spermatozoa in adult mice and rats (Carreau et al., 2002). Estrogen plays an essential role in male fertility through regulating the ability of the efferent ductules to reabsorb the testicular fluid and to influence the seminal fluid composition (Lazari et al., 2009). The studies with knockout animals have shown that the spermatogenesis, steroidogenesis and fertility of Esr1−/−, Esr1−/−/Esr2−/− and animals with targeted disruption of the CYP19 gene (ArKO) are affected (Dupont et al., 2000).

In Leydig cells, at least four steroidogenic enzymes are involved in testosterone biosynthesis from cholesterol. The first steroidogenic enzyme is cholesterol side chain cleavage enzyme ($CYP450_{SCC}$) that is located in the inner membrane of the mitochondria and catalyzes three sequential reactions in androgen biosynthesis; from cholesterol to pregnenolone (Ge and Hardy, 2007). Testicular Leydig cells are the predominant source of circulating testosterone. Thus to study the effects of radiation on Leydig cell steroidogenesis and the mechanism behind such effects would be of great importance (Sivakumar et al., 2006).

The D-type cyclins consist of three family members: cyclins D1, D2, and D3. D2 cyclin is implicated in cell cycle regulation, differentiation, and oncogenic transformation (Meyyappan et al., 1998).

FasL is a trimetric type II membrane protein of approximately 37 kDa and belongs to the TNF superfamily. The Fas–FasL system is the best characterized member of the extrinsic pathway family. When FasL binds to Fas on target cells, caspases are activated and the cells die through programmed cell death (Gu et al., 2014). The present study aimed to evaluate the protective effects of L-carnitine against testicular damage induced by γ-ray irradiated mice.

Materials and methods

Experimental design and animal irradiation

Twenty four Swiss male mice of eight weeks old with body weight of 20–25 g each were purchased from the animal house of the College of Pharmacy, King Abdel Aziz University, Jeddah, Saudi Arabia. The mice were acclimatized for 7 days before the onset of the experiment. Animals were maintained at normal atmospheric temperature of 25 ± 5 °C, good ventilation and 12 h light period. The animal experimental protocols were approved by the Animal Care and Use Committee of Taif University. The mice were divided into four groups (6 mice per group) and housed in polycarbonate cages (3 mice per cage). The first group served as a negative control and was daily injected intra-peritoneally (i.p.) with 50 μl saline. The second group received L-carnitine (Sigma–Aldrich Co., St. Louis, MO, USA) at a dose 10 mg/kg BW in normal saline i.p. (Elshazly et al., 2012). The third group was exposed to whole body irradiation at a total dose of 1 Gy. Radiation exposure was continued 24 h a day (0.1 Gy/day) throughout the 10 days exposure period using a cesium-137 source irradiator (United Nuclear Scientific, East Lansing, MI, USA). The fourth group was pre-treated for one week with intra-peritoneal L-carnitine at a dose 10 mg/kg BW/day then, exposed to continuous 24 h a day whole body gamma ray irradiation at a total dose of 1 Gy (0.1 Gy/day) throughout additional 10 days along with L-carnitine treatment. The cage with irradiation source was designed and equipped by the irradiation source in a manner to provide an even distribution of radiation throughout the entire cage area and allow free movement of the animals inside the cage.