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L-carnitine mitigates UVA-induced skin tissue injury in rats through downregulation of oxidative stress, p38/c-Fos signaling, and the proinflammatory cytokines

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ABSTRACT

UVA comprises more than 90% of the solar UV radiation reaching the Earth. Artificial lightening lamps have also been reported to emit significant amounts of UVA. Exposure to UVA has been associated with dermatological disorders including skin cancer. At the molecular level, UVA damages different cellular biomolecules and triggers inflammatory responses. The current study was devoted to investigate the potential protective effect of L-carnitine against UVA-induced skin tissue injury using rats as a mammalian model. Rats were distributed into normal control group (NC), L-carnitine control group (LC), UVA-Exposed group (UVA), and UVA-Exposed and L-carnitine-treated group (UVA-LC). L-carnitine significantly attenuated UVA-induced elevation of the DNA damage markers 8-oxo-2′-deoxyguanosine (8-oxo-dG) and cyclobutane pyrimidine dimers (CPDs) as well as decreased DNA fragmentation and the activity of the apoptotic marker caspase-3. In addition, L-carnitine substantially reduced the levels of lipid peroxidation marker (TBARS) and protein oxidation marker (PCC) and significantly elevated the levels of the total antioxidant capacity (TAC) and the antioxidant reduced glutathione (GSH) in the skin tissues. Interestingly, L-carnitine upregulated the level of the DNA repair protein proliferating cell nuclear antigen (PCNA). Besides it mitigated the UVA-induced activation of the oxidative stress-sensitive signaling protein p38 and its downstream target c-Fos. Moreover, L-carnitine significantly downregulated the levels of the early response proinflammatory cytokines TNF-α, IL-6, and IL-1β. Collectively, our results highlight, for the first time, the potential attenuating effects of L-carnitine on UVA-induced skin tissue injury in rats that is potentially mediated through suppression of UVA-induced oxidative stress and inflammatory responses.

1. Introduction

Solar light is the major source of UVA reaching the Earth [1]. In addition, several artificial lightening sources have been reported to emit variable amounts of UVA [2,3]. Although UVA has relatively low energy as compared to other UV components, it penetrates deeper into the skin and is considered as a major risk factor in the skin carcinogenesis [4–7]. UVA induces damage to a variety of critical biomolecules [1,8–10]. Its damaging effects are largely mediated through generation of reactive oxygen species, ROS [10–12]. It has been reported that UVA generates a variety of ROS including superoxide anion radical, hydroxyl radical, hydrogen peroxide, and singlet oxygen [10,13–16]. ROS interact with DNA, causing DNA lesions particularly 8-oxo-2′-deoxyguanosine, 8-oxo-dG [9,12,17]. Energy transfer to DNA induces cyclobutane pyrimidines dimers, CPDs [17,18]. Accumulation of DNA lesion such as 8-oxo-dG and CPDs has been linked to mutagenesis and carcinogenesis [19,20]. In the same context, ROS interact with other cellular biomolecules, causing lipid peroxidation and protein oxidative modifications as well as initiation of inflammatory responses [9,21,22]. Importantly, modification of proteins that are involved in DNA repair may boost mutagenicity of UVA [10,19].

L-carnitine is a naturally occurring quaternary amine that plays

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important role in transport of fatty acids across the mitochondrial membrane for subsequent oxidation and energy production [23]. In addition to it critical role in energy metabolism, L-carnitine exhibits antioxidant and antiapoptotic properties [24–27]. It has been shown that L-carnitine scavenges superoxide anion radical and the strong oxidant hydrogen peroxide [24]. In addition, it scavenges hydroxyl radical and prevents its generation through Fenton reaction [28,29]. L-carnitine also increases the activity and expression of the antioxidant enzymes superoxide dismutase and catalase in human hepatocytes [30].

Based on its antioxidant properties, L-carnitine may protect against UVA-induced, ROS- mediated skin tissue injury. The aim of the current study, thus, was to evaluate the potential protective effect of L-carnitine against UVA-induced skin tissue injury using rats as an experimental mammalian model.

2. Material and methods

2.1. Animals

Male Wistar rats weighing 200–230 g (50 days old) were housed in groups of four per polypropylene cage. Rats were acclimatized to Taif University animal facility for ten days before starting the experimental work. Constant conditions of temperature (23 ± 2 °C), humidity (60 ± 10%) and light/dark cycle (12 h/12 h) were maintained all over the experimental period. Standard commercial rat chow and water were allowed *ad libitum*. All procedures related to animal care, treatment, and sampling were conducted in compliance with the guidelines of National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). Ethical committee approval number (2017/TU/Pharmacy/02).

2.2. Chemicals and kits

Thiobarbituric acid (TBA), trichloroacetic acid (TCA), 2, 4-dinitrophenyl hydrazine (DNPH), 5, 5′- dithiobis [2-nitrobenzoic acid] (DTNB), and L-carnitine were purchased from Sigma-Aldrich (St Louis, MO, USA). All other chemicals were of high purity. Total antioxidant capacity (TAC) kit was purchased from Cayman Chemical Company (Ann Arbor, MI, USA). TNF-α, IL-6 and IL-1β kits were purchased from Ray Biotech (Norcross, GA, USA). Caspase-3 colorimetric assay kit and DNA fragmentation assay kit (TiterTACS In Situ Detection Kit) were purchased from R & D systems (Minneapolis, MN, USA). 8-oxo-2′-deoxyguanosine assay kit was purchased from Trevigen (HT 8-oxo-dG, Trevigen, Inc., Gaithersburg, MD, USA). Cyclobutane pyrimidine dimer assay kit was purchased from Cell Biolab (OxiSelect™, Cell Biolab, Inc, San Diego, CA, USA). DNA extraction kit was purchased from Qiagen (DNeasy Blood & Tissue Kit, Qiagen, Hilden, Germany). Rat skin dissociation kit (Rat Skin PrimaCell™ I) were purchased from Chi Scientific (Chi Scientific, Inc, USA). Antibodies for PCNA and actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies for total and phosphorylated forms of p38 and c-Fos were purchased from cell signaling (Cell Signaling Technology, Inc, USA).

2.3. Experimental design and treatment protocol

Thirty-two male Wistar rats were randomly distributed into four groups of eight-animal each. The dorsal area of all rats was shaved (4 cm^2) using a shaving machine. The shaved skin in different rat groups was clean and free of any injury. The treatment protocol was as the following: Group-1 (Normal control, NC): animals in this group were not exposed to any UVA or any treatment all over the experimental period. Group-2 (L-carnitine control, LC): animals in this group were treated with 300 mg/kg body weight of L-carnitine orally by gastric gavage once daily for one week. Group-3 (UVA-Exposed, UVA): rats in this group were directly illuminated with a single dose of UVA (20 J cm^{-2}) using the commercially available high intensity UVA lamp (BlakRay-B100-A, 230 W, 8900 μW/cm^2, main peak emission 365 nm at 25 cm distance, UVP, Cambridge, UK, exposure period was 37.5 min). During illumination, the animals were in their polypropylene cages and the distance between the UV light source and the animals was *ca.* 25 cm. Group-4 (UVA-Exposed and L-carnitine-treated group, UVA-LC): rats in this group were handled the same way as UVA group except that the animals were treated with 300 mg/kg body weight of L-carnitine orally by gastric gavage once daily for one week before exposure to UVA. UVA exposure was done 2 h after the last dose of L-carnitine. Doses of UVA and L-carnitine were consistence with previously published work [1,31].

2.4. Sample preparation

Twelve hours after the exposure to UVA, rats in all groups were euthanized by decapitation under pentobarbital sodium anesthesia (65 mg/kg, ip) [32] to collect skin tissue samples. The skin tissues were quickly removed, rinsed in ice cold saline, and divided into three parts for DNA extraction, tissue homogenization and skin tissue dissociation. DNA was extracted using the commercially available Qiagen kit (DNeasy Blood & Tissue Kit, Qiagen, Hilden, Germany) according to the manufacturer's instructions. Skin tissue samples designated for homogenization were weighed and homogenized (10% w/v) in phosphate-buffered saline (PBS) using T25 digital ultra-turrax homogenizer (IKA-Werke GmbH & Co. KG, Germany). Homogenates were then centrifuged for 15 min at 10000 × g and 4 °C. The supernatant was used for determination of the proposed biochemical parameters. Skin tissues designated for tissue dissociation was used to make a single cell suspension of epidermal keratenocytes using the commercially available Rat Skin PrimaCell™ I (Chi Scientific, Inc, USA). The resulted single cell suspension was used for determination of the level of DNA fragmentation in different experimental groups.

2.5. Measured parameters

2.5.1. Measurement of 8-oxo-2′–deoxyguanosine

Levels of the oxidative DNA damage biomarker 8-oxo-2′–deoxyguanosine (8-oxo-dG) in skin tissues of UVA-Exposed and control rats were evaluated using the commercially available HT 8-oxo-dG ELISA II kit (Trevigen, Inc., Gaithersburg, MD, USA). The assay employs standard 8-oxo-dG immobilized to 96-well plate. Specific antibody (anti 8-oxo-dG monoclonal antibody) is employed to bind competitively to the immobilized 8-oxo-dG and to that in test samples. Upon washing, antibodies that bind to 8-oxo-dG in the test samples are washed away while antibodies bind to the immobilized 8-oxo-dG are retained. Detection was performed with HRP-conjugated secondary antibody and a colorimetric substrate. Optical density is inversely proportional to the level of 8-oxo-dG present in the test samples [33]. The optical density was measured using SPECTRAmax PLUS^{384} microplate spectrophotometer (Molecular devices, Sunnyvale, California, USA).

2.5.2. Measurement of cyclobutane pyrimidine dimer

CPDs level was determined using the commercially available immunoassay colorimetric ELISA kit (OxiSelect, Cell Biolab, INC.) according to the manufacturer's instructions. CPD-DNA standards or test DNA samples were first heat-denatured before adsorption onto a 96-well DNA high-binding plate. The CPDs present in the standard or the test sample were probed with anti-CPD antibody followed by HRP-conjugated secondary antibody. The level of CPD in the test sample was determined using a standard curve that is prepared from a predetermined CPD-DNA standard [34].

2.5.3. Determination of caspase-3 activity

The commercially available colorimetric assay kit (R &D systems) was used for determination of the activity of the apoptotic marker caspase-3 according to the manufacturer's instructions as described