The Protective Effects of L-Carnitine and Vitamin E in Rat Lenses in Irradiation-Induced Oxidative Injury

Abstract

Objectives. The aim of this study was to evaluate the antioxidant role of L-carnitine (LC) and vitamin E against radiation-induced cataracts in rat lenses after total cranial irradiation with a single 5 Gray (Gy) dose of gamma irradiation.

Material and Methods. Thirty two Sprague-Dawley rats were used for the experiment. The control group did not receive LC and vitamin E or irradiation but received both 0.1 ml physiological saline intraperitoneally and sham irradiation. The irradiation (IR) group received 5 Gy gamma irradiation to the total cranium as a single dose plus 0.1 ml physiological saline intraperitoneally. The IR plus vitamin E group received irradiation to total cranium plus 10 mg/kg/day vitamin E intraperitoneally. The IR plus LC group received irradiation to total cranium plus 100 mg/kg/day LC intraperitoneally. Biochemical parameters measured in murine lenses were carried out using spectrophotometric techniques.

Results. Total superoxide scavenger activity (TSSA), non-enzymatic superoxide scavenger activity (NSSA), glutathione-S-transferase (GST) and glutathione reductase (GRD) activities, significantly increased in the control, IR plus vitamin E and LC plus IR groups when compared with the IR only group. Lens TSSA and NSSA activities in the control group were significantly increased compared to that of the IR only group, but decreased compared to those of the IR plus vitamin E and IR plus LC groups. Lens xanthine oxidase (XO) activity in the IR group significantly increased compared to those of other groups.


Key words: carnitine, vitamin e, antioxidant enzymes, irradiation, oxidative stress, lens.
Radiation therapy is a common and important tool for cancer treatment [1]. Eighty percent of cancer patients need radiotherapy at some time or other, either for curative or palliative purposes. The radiosensitivity of normal tissue adjacent to the tumor limits therapeutic gain. The responses of normal tissues to therapeutic radiation exposure range from those that cause mild discomfort to others that are life threatening. The speed at which a response develops varies widely from one tissue to another and often depends on the dose of radiation that the tissue receives [2–4]. Ionizing radiation is known to generate reactive oxygen species (ROS) in irradiated tissue. Because human tissues contain 80% water, the major radiation damage is due to aqueous free radicals, generated by the action of radiation on water. Advanced glycation end products (AGEs) are known to be generated in foods and biological systems. Recently, these AGEs were reported to be considered to generate reactive oxygen species (ROS) in irradiated tissue. Because human tissues contain 80% water, the major radiation damage is due to aqueous free radicals, generated by the action of radiation on water. Advanced glycation end products (AGEs) are known to be generated in foods and biological systems. They were also considered to generate reactive oxygen species (ROS) under the presence of transition metals in vitro and in vivo [5]. These free radicals react with cellular macromolecules, such as deoxyribonucleic acid (DNA), ribonucleic acid (RNA), proteins, membranes, etc., and cause cell dysfunction and mortality. These reactions take place in tumors as well as normal cells when exposed to radiation [6].

A cataract is opacity of the eye lens that interferes with vision. Cataracts are formed in response to a variety of different agents and environmental stresses, and this damage seems in almost all cases to have an oxidative damage component. Although cataract of the eye lens is a known late effect of ionizing radiation exposure, most of the experimental work to date has concentrated on single, acute, high doses or multiple, fractionated, and chronic exposures [7, 8]. Radiation cataracts are expressed after latency. The duration of the latency depends inversely on dose: the higher the dose, the more rapidly the cataract develops. For a single treatment, the lowest cataractogenic dose was reported to be 2 Gy [9, 10]. Vitamin E not only acts as an effective lipophilic antioxidant and radical scavenger but also stabilizes cellular membranes [11]. The protective role of vitamin E against radiation-induced oxidative damage was demonstrated in vitro [12]. Using an injectable form of vitamin E (α-tocopherol), there was a clear improvement in post-irradiation survival compared with the results for dietary administration of vitamin E [7, 12].

L-carnitine (LC) is a natural substance that acts as a carrier for fatty acids across the inner mitochondrial membrane for subsequent beta-oxidation, and for the removal of potentially toxic metabolites from the inner aspect of mitochondrion as acyl-CoA and acylcarnitines. LC and its short chain esters, propionyl-LC and acetyl-L-carnitine, are endogenously synthesized in man and also found in diet. Carnitines are essential factors of several enzymes necessary for the transformation of long-chain fatty acids, and act also as scavengers of oxygen free radicals in mammalian tissues [6]. LC prevents oxidative stress and regulates nitric oxide, cellular respiration and the activity of enzymes involved in defense against oxidative damage [13]. It has been shown that acetyl-L-carnitine has an antioxidant activity towards oxidative stress and that the improvement in cognitive ability seen with acetyl-L-carnitine may occur through an amelioration of cellular dysfunction via an inhibition of the increase in lipid hydroperoxidation observed in the brain tissue of untreated senescence-acceleration-prone mice [14].

Cells have developed different antioxidant systems and various antioxidant enzymes to defend themselves against free radical attacks. Superoxide dismutase (SOD) catalyses the dismutation of the O₂⁻ into hydrogen peroxide (H₂O₂). The glutathione-dependent antioxidant system consisting of reduced glutathione and an array of functionally related enzymes plays a fundamental role in cellular defense against reactive free radicals and other oxidant species. Of these enzymes, glutathione peroxidase (GSH-Px) is a selenoprotein that
Carnitine and Vitamin E in Irradiation

Material and Methods

Rats and Experiments

Thirty-two Sprague-Dawley rats, 10–14 weeks old, weighing 195 ± 18 g at the time of radiation, were used for the experiment. All procedures involving the Sprague-Dawley rats adhered to the ARVO Resolution on the Use of Animals in Research. The rats were quarantined for at least 3 days before gamma irradiation and fed standard laboratory chow and water ad libitum. The laboratory was windowless with automatic temperature (22 ± 1°C) and lighting controls (14 h light/10 h dark). We divided the rats into four equal groups of eight animals each and housed them in different cages. The control group did not receive LC, vitamin E or animals each and housed them in different cages.

Biochemical Analysis

Ten days after irradiation, all animals were killed by decapitation, their eyes were enucleated, and the lenses were dissected immediately. Lenses were homogenized in physiological saline solution (Omni Accessory Pack International Homogenizer, Warrenton, VA, USA). The homogenate was centrifuged at 10,000 g for 1 hr to remove debris. The clear upper supernatant was collected and all assays were carried out on this fraction. All the procedures were performed at +4°C.

TSSA and NSSA assays, as indicators of tissue antioxidant capacity, were performed in the samples before and after the addition of trichloroacetic acid (TCA, 20%), as described by Durak et al [18]. First, TSSA is measured by the method where xanthine-xanthine oxidase complex produces superoxide radicals that react with nitroblue tetrazolium (NBT) to form a formazan compound. TSSA activity is measured at 560 nm by detecting inhibition of this reaction. By using a blank reaction in which all reagents are present except the supernatant sample and by determining the absorbance of the sample and blank, TSSA activity is calculated. Second, NSSA activity is measured in TCA-treated fractions, which are prepared by treating part of

reduces hydroperoxides as well as H2O2 while oxidizing glutathione. A number of potentially toxic electrophilic xenobiotics are conjugated to nucleophilic glutathione by glutathione-S-transferases (GSTs) present in high amounts in cell cytosol. GST can also catalyze reactions reducing peroxides like GSH-Px. Reduction of oxidized glutathione (GSSG) to GSH is mediated by the widely distributed enzyme glutathione reductase (GRD) that uses NADPH as the reductant [15, 16]. Xanthine oxidase (XO) functions in purine and free radical metabolism. It also catalyses the conversion of xanthine and hypoxanthine to uric acid and the production of superoxide radicals (O2•−), which are potentially toxic to cellular structures [17].

To the best of our knowledge, there are no studies on the simultaneous effects of both LC and vitamin E on total (enzymatic plus non-enzymatic) superoxide scavenger activity (TSSA), non-enzymatic superoxide scavenger activity (NSSA, GRD, GST, XO) activities in the lenses of rats with ionizing-induced cataracts. Therefore, in the present study, we aimed to investigate the effects of these antioxidant substances on antioxidant (TSSA, NSSA, GRD, GST) and oxidant parameters (XO) in the lenses of rats with or without exposure to total cranium irradiation with a single dose of 5 Gy of gamma rays.

Second, NSSA activity is measured in TCA-treated fractions, which are prepared by treating part of
the sample with 20% (w/v) TCA solution (to removed all enzymes and proteins), and centrifuging at 5000 × g for 30 min. After the elimination of proteins by this procedure, NSSA activity assay is performed in the supernatant fraction.

GRD activity was determined by coupled spectrophotometric registration at 340 nm, using GSSG as substrate and NADPH at 37 °C [19]. Glutathione S-transferase (GST) activity of the cell supernatant was measured by using 1-chloro-2,4-dinitrobenzene (CDNB) and GSH as described [20]. XO activity was measured spectrophotometrically by the formation of uric acid from xanthine [21]. The protein content was determined by using the Bradford method [22]. Results were expressed in U/mg protein for TSSA, NSSA; mU/mg protein for GRD, GST and XO activities. One unit of TSSA, NSSA was defined as the amount of enzyme protein causing 50% inhibition in nitrobluetetrazolium reduction rate. Biochemical measurements were carried out using a spectrophotometer (CECIL CE 3041, Cambridge, UK).

### Statistical Analyses

Statistical and correlation analyses were undertaken using a one-way variance analysis and Spearman’s rank correlation test, respectively. Least significant difference (LSD) multiple range tests were used to compare the mean values. Acceptable significance was recorded when P values were < 0.05. Statistical analysis was performed with Statistical Package for the Social Sciences for Windows (SPSS, version 10.0, Chicago, IL, USA).

### Results

#### Antioxidant Parameters

All parameters are shown in Table 1. Lens TSSA, NSSA, GST and GRD activities significantly increased in the control, IR plus vitamin E and LC plus IR groups when compared with the IR group. Lens TSSA and NSSA activities in the IR plus vitamin E and IR plus LC groups were significantly increased compared to those of the control group.

#### Oxidative Stress Parameters

Lens XO activity in the IR group significantly increased compared to those of all other groups. Correlation analyses were shown in Table 2. As seen in Table 2, correlation analysis revealed a significant negative correlation between XO and NSSA ($r = -0.893$, $p < 0.001$), XO and GST ($r = -0.83$, $p < 0.01$), XO and GRD ($r = -0.79$, $p < 0.05$) in the IR group (Fig. 1). There were significant positive correlations between GST and NSSA ($r = 0.78$, $p < 0.05$), and GST and GRD ($r = 0.71$, $p < 0.05$) in the IR plus vitamin E group (Fig. 2) and IR plus L-carnitine group (Fig. 3), respectively. However, no correlation could be found among the parameters in other groups.

### Discussion

As the world’s population ages, cataract-induced visual dysfunction and blindness are on the

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### Table 1. Antioxidant (TSSA, NSSA, GRD, GST) and oxidative stress (XO) parameters in rat lenses

<table>
<thead>
<tr>
<th></th>
<th>Control group (Grupa kontrolna)</th>
<th>IR group (Grupa IR)</th>
<th>IR + Vitamin E group (Grupa IR plus witamina E)</th>
<th>IR + L-carnitine group (Grupa IR plus LC)</th>
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</thead>
<tbody>
<tr>
<td>TSSA – U/mg protein</td>
<td>50.6 ± 11.6a</td>
<td>35.2 ± 7.8d</td>
<td>64.3 ± 16.6cd</td>
<td>63.5 ± 11.7cd</td>
</tr>
<tr>
<td>(U/mg białka)</td>
<td></td>
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<tr>
<td>NSSA – U/mg protein</td>
<td>9.87 ± 1.76a</td>
<td>7.30 ± 1.53d</td>
<td>14.38 ± 3.12ce</td>
<td>12.81 ± 3.14ce</td>
</tr>
<tr>
<td>(U/mg białka)</td>
<td></td>
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<tr>
<td>GRD – mU/mg protein</td>
<td>33.9 ± 6.1b</td>
<td>24.5 ± 4.3</td>
<td>33.7 ± 5.2b</td>
<td>34.7 ± 8.3b</td>
</tr>
<tr>
<td>(mU/mg białka)</td>
<td></td>
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<tr>
<td>GST – mU/mg protein</td>
<td>37.9 ± 8.5a</td>
<td>23.6 ± 8.4</td>
<td>49.3 ± 18.6b</td>
<td>46.7 ± 16.5c</td>
</tr>
<tr>
<td>(mU/mg białka)</td>
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<tr>
<td>XO – mU/mg protein</td>
<td>1.81 ± 0.20a</td>
<td>2.36 ± 0.58</td>
<td>1.90 ± 049a</td>
<td>1.88 ± 0.25a</td>
</tr>
<tr>
<td>(mU/mg białka)</td>
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a – $p < 0.05$, b – $p < 0.01$, c – $p < 0.001$, vs. irradiation group.

d – $p < 0.05$, e – $p < 0.001$, vs. control group.

a – $p < 0.05$; b – $p < 0.01$; c: $p < 0.001$, vs grupa napromieniana.

d – $p < 0.05$; e – $p < 0.001$, vs grupa kontrolna.
increase. Cataracts are a major cause of blindness and of severe visual impairment leading to bilateral blindness in an estimated 20 million people worldwide. In developing countries, 50–90% of all blindness is caused by cataracts. Pharmacological treatment to prevent human cataracts have so far not been achieved. Therefore, surgery to remove the opacified lens is the only effective treatment for the cataract. The challenges are to prevent or delay cataract formation and also to treat cataracts if they occur. The exact mechanism of cataract formation is still not very clear [23].

The aim of radiation treatment is to deliver carefully determined doses of ionizing radiation to a defined tumor volume to eliminate tumor cells, to cause minimal injurious effects to surrounding healthy tissue given by eliminating tumor cells, giving a high quality of life and to prolong survival, all at a reasonable cost to cancer patients [3, 24]. But, cataract is an unavoidable complication if radiotherapy includes the orbit of eye in the treated volume, even with very low doses of radiation. Ionizing radiation, such as X and gamma rays and ultraviolet light, is known to be a cataractogenic factor for rat lenses [7, 25]. Multiple processes may lead to endothelial damage under irradiation but the generation of oxygen free radicals and the following lipid peroxidation may be one of the key components in this cascade of events. Radiation generates ROS that interact with cellular molecules, including DNA, lipids, and proteins [6].

In the present study, irradiation caused a significant decrease in the activities of antioxidant enzymes and also increases in oxidant enzyme activities in rat lenses. These results are in agreement with the previous findings of Karslioglu et al. [7, 8], Kocer et al. [10] and Yagci et al. [23]. They reported a significant depletion in the antioxidant system accompanied by enhancement of lipid peroxidation after irradiation. Under normal conditions the inherent defense system protects against oxidative damage.

In this study, we showed a significant reduction in GRD and GST activities in the IR group and also a significant increase in GRD and GST activities in the LC plus IR and IR plus vitamin E groups in rat lenses. This reduction in the IR group could be due to an enhanced utilization of glutathione reduct cycle as an attempt to detoxify the free radicals generated by irradiation. Supplementation of LC and vitamin E protects the endogenous GRD, GST depletion resulting from irradiation. The increase in GRD, GST, TSSA and NSSA activities suggests that protection of LC and vitamin E may be mediated through the modulation of lens antioxidant system. These results suggest that these substances have a free radical scavenging activity. Some studies have reported that LC and vitamin E pretreat-

<p>| Table 2. Spearman’s rank correlation coefficients in IR, IR plus Vitamin E and IR plus L-carnitine groups |
|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|</p>
<table>
<thead>
<tr>
<th>IR group (Grupa IR)</th>
<th>IR + Vitamin E group (Grupa IR plus witamina E)</th>
<th>IR + L-carnitine group (Grupa IR plus LC)</th>
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<tr>
<td>r =</td>
<td>p &lt;</td>
<td>r =</td>
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<tr>
<td>XO-NSSA</td>
<td>-0.93</td>
<td>-</td>
</tr>
<tr>
<td>XO-GST</td>
<td>-0.83</td>
<td>-</td>
</tr>
<tr>
<td>XO-GRD</td>
<td>-0.79</td>
<td>-</td>
</tr>
<tr>
<td>GST-NSSA</td>
<td>-</td>
<td>0.78</td>
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<tr>
<td>GST-GRD</td>
<td>-</td>
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Fig. 1. A negative correlation between lens tissue XO and NSSA (r = -0.893, p < 0.001), XO and GST (r = -0.83, p < 0.01), XO and GRD (r = -0.79, p < 0.05) in IR group

Ryc. 1. Korelacja negatywna między XO i NSSA (r = –0,893; p < 0,001), XO i GST (r = –0,83; p < 0,01), XO i GRD (r = –0,79; p < 0,05) w soczewkach w grupie IR
ment significantly lowered the radiation-induced lipid peroxidation in terms of malondialdehyde and increased antioxidant enzyme activities in rat lenses [8, 10]. The inhibition of lipid peroxidation in biomembranes can be caused by antioxidants. Significant increases in the levels of free radicals has been reported to be present in both the lens and the aqueous humor of cataract patients when compared with age-matched controls, emphasizing the role of oxidative damage in the pathogenesis of cataracts. A decrease in the antioxidant system could be responsible for increased lens oxidation and cataract development [10, 26–28].

A significant negative correlation was present between XO and such parameters as NSSA, GST, and GRD in the IR group. This result can be seen in Table 2. However, a positive correlation was seen between GST and NSSA in the IR plus vitamin E group, and GST and GRD in the IR plus L-carnitine group. These positive correlations might be indicators of the compensatory mechanism in these groups.

In conclusion, LC and vitamin E have clear antioxidant properties and are likely to be valuable drugs for protection against gamma-irradiation and/or to be used as antioxidants against oxidative stress. By increasing antioxidant enzyme activities and decreasing oxidant enzyme activities, LC and vitamin E prevented oxidative stress by scavenging free radicals generated by ionizing radiation in rat lenses. These results also show the need for further studies on this subject.

References


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