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Short communication

EVALUATION OF PROTECTIVE EFFECTS OF VERAPAMIL AGAINST LENS EPITHELIAL TISSUE INJURY INDUCED BY IRRADIATION IN RATS

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Abstract

In this study, we investigated the possible protectant effects of verapamil against lens epithelial tissue injury induced by irradiation in rats. Forty female Wistar Albino rats were divided into four groups (n=10). Group 1 received 10 mg/kg verapamil and underwent 20 Gy irradiation. Group 2 received no verapamil and underwent 20 Gy irradiation. Group 3 received 10 mg/kg verapamil and underwent sham irradiation. Group 4 received no verapamil and underwent sham irradiation. The lens tissues were dissected and measured malondialdehyde levels in all groups. The mean malondialdehyde levels (nmol/mg prt) were determined as 5,59 for Group 1, 7,04 for Group 2, 3,95 for Group 3 and 3,25 for Group 4. In conclusion, we can suggest that verapamil as a protectant agent against radiation induced lens epithelial tissue injury.

Key words: *Radiation, Lens injury, Lipid peroxidation, Verapamil*

Ratlarda Radyasyonla İndüklenen Lens Epitel Doku Hasarına Karşı Verapamil'in Koruyucu Etkilerinin Değerlendirilmesi

Bu çalışmada, ratlarda radyasyonla indüklenen lens epitel hücre hasarına karşı verapamilin olası koruyucu etkisini inceledik. Kırk adet dişi Wistar Albino türü rat dört guruba ayrıldı (n=10). Gurup 1'e 20 Gy dozda radyasyon ve 10 mg/kg verapamil uygulandı. Gurup 2'ye 20 Gy radyasyon uygulandı ve verapamil verilmedi. Gurup 3'e 10 mg/kg dozda verapamil verildi, yalancı radyasyon uygulandı. Gurup 4'e verapamil verilmedi ve yalancı radyasyon uygulandı. Tüm guruplarda lens dokuları çıkarıldı ve malondialdehit (MDA) ölçümleri yapıldı. Ortalama MDA düzeyleri (nmol/mg prt); Gurup 1 için 5.59, Gurup 2 için 7.04, Gurup 3 için 3.95 ve Gurup 4 için 3.25 olarak belirlendi. Sonuç olarak, radyasyonla indüklenen lens epitel hücre hasarına karşı verapamili koruyucu bir ajan olarak önerebiliriz.

Anahtar kelimeler: *Radyasyon, Lens hasarı, Lipid peroksidasyonu,Verapamil*

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INTRODUCTION

The oxidative action of ionizing radiation is predominantly due to reactive oxygen species (ROS) such as hydroxyl radical ($OH^{\cdot}$), superoxide radical ($O^{\cdot}_2^{-}$) and hydrogen peroxide (H_2O_2) generated by the decomposition of water (1,2). ROS are associated with various degenerative diseases including cancer, cataract, macular degeneration, aging and arteriosclerosis (3,4). Since the lens epithelial cells are remarkably vulnerable to injury induced by irradiation through lipid peroxidation as a result of the reactive oxygen species due to the abundance of the unsaturated fatty acids in their membranes (5), the scavengers of the unstable reactive oxygen species need to be activated for protection of the lens epithelial cells against the damaging effects of irradiation (6). Verapamil, an L-type calcium channel blocker that acts as a scavenger of the unstable reactive oxygen species, might function as a protectant for the lens epithelial cells against the damaging effects of irradiation, presumably through the its radical scavenger effects and activation of the antioxidant defense system (7). This study presents the evaluation of verapamil as a protectant against lens epithelial tissue injury induced by irradiation in a rat model.

MATERIALS AND METHODS

Experimental design

The study was undertaken at the Laboratory for Experimental Studies in accordance with the guidelines for the care and use of the laboratory animals established by the Animal Ethics Committee following the approval of the design by the Animal Ethics Committee. Forty female Wistar Albino rats (ages between 10 and 12 months and weights between 250 and 300 grams) were divided into four groups (n=10). The rats in each group were kept in separate cages in rooms with controlled light and temperature and were fed with standard chow and water ad libitum. The rats in Group 1 received 10 mg/kg verapamil (in 0,9 % sodium chloride; Isoptin, Abbott Lab. Co. Istanbul, Turkey) and underwent 20 Gy irradiation. The rats in Group 2 received no verapamil and underwent 20 Gy irradiation. The rats in Group 3 received 10 mg/kg verapamil and underwent sham irradiation. The rats in Group 4 received no verapamil and underwent sham irradiation.

Irradiation

The rats were immobilized in the prone position in a custom-designed acrylic restrainer. For the rats in Group 1 and Group 2, irradiation was performed on a Cobalt-60 unit using a single fraction of 20 Gy defined at a depth of 2,5 cm through an anterior portal (with a 0,5 cm bolus) covering the brain in its entirety. For the rats in Group 3 and Group 4, sham irradiation was performed on a Cobalt-60 unit over the same fraction duration.

Euthanasia

The rats underwent euthanasia at 1 hour following irradiation or sham irradiation. Euthanasia was performed by way of cervical dislocation. The both eyes were enucleated and the lens tissues were dissected for immediate homogenization.

Biochemical evaluation

The both lens tissues were homogenized in 1 mL of 1.15 % potassium chloride solution using a homogenizer (T 25 Ultra-Turrax, IKA Werke GmbH, Staufen, Germany). The temperature was kept at + 4 °C throughout the preparation of the homogenates. The homogenates were immediately used for the measurement of the malondialdehyde (MDA) levels, in accordance with the method of Mihara and Uchiyama (8), with 1,1,3,3-tetramethoxypropane as the standard. The MDA level was expressed in nanomoles per

milligram of protein (nmol/mg). The protein concentration in the homogenates was measured in accordance with the method of Lowry et al.(9), with bovine serum albumin as the standard. Shimadzu UV-1601 Spectrophotometer (Shimadzu Corporation, Kyoto, Japan) was used for all spectrophotometric measurements.

Statistical analysis

The mean MDA levels were compared using the one-way analysis of variance (ANOVA). Statistical analysis was performed using the 10.0 version of the SPSS for Windows software package. Results were presented as mean±SD. Statistical significance was defined as the P value being less than or equal to 0.05.

RESULTS

The mean MDA levels (nmol/mg protein) were 5.59 ± 0.42 (ranging from 3.31 to 7.83) for the rats in Group 1, 7.04 ± 0.47 (ranging from 5.54 to 9.66) for the rats in Group 2, 3.95 ± 0.25 (ranging from 3.17 to 5.29) for the rats in Group 3 and 3.25 ± 0.30 (ranging from 2.04 to 4.72) for the rats in Group 4. All results were summarized in Table 1 and Figure 1.

Table 1. Malondialdehyde levels of all groups. Results are presented as mean ± SD.

	Group I (n=10)	Group II (n=10)	Group III (n=10)	Group IV (n=10)
MDA (nmol/mg prt)	5.59 ± 0.42^{b}	$7.04 \pm 0.47^{a,c}$	3.95 ± 0.25^{b}	$3.25 \pm 0.30^{a,b}$

a: $p<0.05$, compared with Group I ; **b:** $p<0.05$, compared with Group II ; **c:** $p<0.05$, compared with Group III.

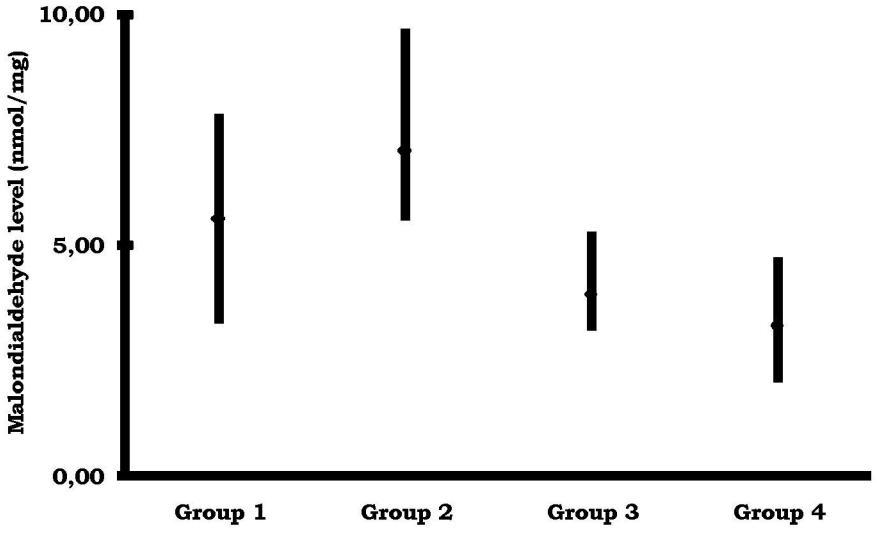

Figure 1. Mean malondialdehyde levels (nmol/mg prt) with the highest and the lowest values of groups.

DISCUSSION

The lens is an avascular transparent structure located in the visual axis of the eye between the anterior aqueous humour and the posterior vitreous humour. The anterior surface of the lens is lined by a single layer of epithelial cells that are mitotically active throughout life and, therefore, responsible for maintaining the lens homeostasis (10).

Lipid peroxidation of unsaturated fatty acids in the phospholipids of membranes changes the cellular permeability (11). Within the cellular membranes, the calcium pumps and the L-type calcium channels regulate the entry of calcium into the epithelial cells (12). As the consequence of the lipid peroxidation mediated insults that involve the cellular membranes, the intracellular overload of calcium in the lens epithelial cells trigger a series of events such as the activation of calpains that are calcium dependent cysteine proteases and the irreversible proteolysis of the cytoskeletal and junctional proteins (13) that eventually lead to cataractogenesis (14). Therefore, the limitation of the intracellular calcium through the regulatory activity of the calcium pumps and the L-type calcium channels and the inhibition of the calcium dependent enzymes might help in the prevention of cataractogenesis (15). Owing largely to the disproportionate distribution of the antioxidant defense system in the lens, the use of protectants that act against the lipid hydroperoxides through the stabilization of the integrity of the cellular structures or that attempt to strengthen the antioxidant defense system is prompted (16). The effectiveness of distinct pharmacological agents as protectants against lipid peroxidation mediated lens epithelial tissue injury induced by irradiation was evaluated in rat models. Ertekin et al.(1) compared the MDA levels, the SOD activities and the GPx activities of the lens tissues in the rats that underwent irradiation in a single fraction of 5 Gy and received Ginkgo biloba in comparison to the rats that underwent irradiation in a single fraction of 5 Gy but did not receive Ginkgo biloba. At ten days following irradiation, the mean MDA levels were significantly decreased whereas the mean SOD activities and the mean GPx activities were significantly increased for the rats that underwent irradiation and received Ginkgo biloba as compared to the rats that underwent irradiation but did not receive Ginkgo biloba. Karslioglu et al.(17) reported the similar protective effects of melatonin in the epithelial tissue injury induced by irradiation in a rat model. An increase in lenticular calcium concentration has been shown to be closely correlated with cataract formation following irradiation. Cengiz et al.(7) reported that at the end of 7.5 weeks following irradiation, the mean calcium levels were significantly lower for the rats that underwent irradiation and received verapamil as compared to the rats that underwent irradiation but did not receive verapamil.

In this study, the oxidant injury in the lens epithelial tissue was evaluated at 1 hour following irradiation in the rats that underwent irradiation in a single fraction of 20 Gy and received verapamil in comparison to the rats that underwent irradiation and received no verapamil. The oxidant injury induced by irradiation resulted in the increased production of the reactive oxygen species to initiate the oxidative degradation of the unsaturated fatty acids in the cellular membranes, as reflected in the substantial increase in the mean MDA levels. Given the abundance of the unsaturated fatty acids in the cellular membranes in contrast to the modest antioxidant defense system in the lens epithelial cells, the prompt increase in the MDA levels indicate that the depletion of the enzymes involved in the antioxidant defense system following a less than adequate response leads to an accentuated oxidant injury. The possible antiperoxidative mechanism of the calcium channel blocker verapamil might be similar to a chain-breaking reaction at the level of membrane phospholipids. Because the structure of verapamil is lipophilic, it appears likely that it would partition differantially into the lens epitelial membranes according to its lipophilicity.

In conclusion, this study suggests a probable protectant role for verapamil in radiation induced lens injury, presumably through the activation of the antioxidant defense system and its chain-breaking property. Nevertheless, the use of verapamil, as well as other calcium channel

blockers, on radiation induced lens injury warrants further evaluation regarding both effectiveness and safety.

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