

Protective Effects of Ibuprofen and L-Carnitine Against Whole Body Gamma Irradiation-Induced Duodenal Mucosal Injury

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ABSTRACT

Objective: Ibuprofen and L-carnitine have been demonstrated to provide radioprotective activity to the hamster against whole body sublethal irradiation. The purpose of this study is to test those antioxidant drugs, each of which has the capacity of inhibiting mucosal injury, as topical radioprotectants for the intestine.

Material and Methods: The male hamsters were divided into the following four groups ($n=6$): group 1: control group, received saline, 1 ml/100 g by gavage, as placebo. Group 2: irradiated-control group, received whole body irradiation of 8 Gy as a single dose plus physiological saline. The animals in groups 3 and 4 were given a daily dose of 10 mg/kg of ibuprofen and 50 mg/kg of L-carnitine for 15 days respectively, before irradiation with a single dose of 8 Gy. Twenty-four hours after radiation exposure, the hamsters were sacrificed and samples were taken from the duodenum, and the histopathological determinations were carried out.

Results: Morphologically, examination of the gamma irradiated duodenum revealed the presence of shortening and thickening of villi and flattening of enterocytes, massive subepithelial lifting. Pretreatment of ibuprofen and L-carnitine with irradiation reduced these histopathological changes.

Conclusion: Ibuprofen and L-carnitine administrated by the oral route may be a good radioprotector against small intestinal damage in patients undergoing radiotherapy.

Key Words: Whole body irradiation, duodenum, ibuprofen, L-carnitine, hamster

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Introduction

Therapeutic irradiation regimens are designed to maximize tumoricidal effects while causing minimal damage to normal organs. The intestine is an important dose-limiting organ during radiation therapy of tumors in the pelvis or abdomen. It was previously believed that the severity of intestinal radiation toxicity depends solely on the extent of radiation-induced intestinal crypt cell death (1, 2). The crypts of Lieberkühn, which maintain the villus architecture, have continuously proliferating stem cells and are therefore highly radiosensitive (3). This damage to crypts by radiation leads to the gastrointestinal syndrome. After 5-8 days of irradiation with a dose of 8 Gy and higher, the inflammatory cascade, denudation of epithelium, and other morphological changes of the intestinal surface develop (4, 5). To evaluate the effect of modifiers of gastrointestinal injury, villus morphology and cellularity, crypt number and cellularity and pathological features like apoptotic cells, apoptotic bodies have been considered as reliable parameters (6). The damaged intestinal epithelium loses its function and septic shock becomes the most common cause of death.

These side effects may result in modification or cancellation of planned therapeutic regimens (7).

The effects of ionizing radiation are mediated mainly by oxygen free radicals that are generated by its action on water. These highly reactive oxidants remove hydrogen atoms from fatty acids, causing lipid peroxidation with resultant changes in membrane permeability and fluidity and ultimately in cell death. The oxyradicals also induce DNA strand breaks and protein oxidation (7). Ibuprofen is one of the most useful nonsteroidal anti-inflammatory agents available to humans. Originally intended as a therapy for arthritis, it is now available as a nonprescription antipyretic and analgesic. Ibuprofen has antiradical and antioxidant effects and scavenges reactive oxygen species. It protects the lipids of biological membranes from oxidation and consequently inhibits the accumulation of lipid peroxidation products such as malondialdehyde (8, 9). Several studies have shown that ibuprofen could scavenge hydroxyl (10) and superoxide radicals (11) and that it possessed radioprotective properties (12). L-carnitine, a naturally occurring antioxidant, is a necessary factor in the utilization of long chain fatty acids to produce energy. L-carnitine exhibits a wide range of biological activities including anti-inflammatory (13),

neuroprotective (14), gastroprotective (15) and radioprotective (16) properties. Furthermore, these effects are attributed to its antioxidative and free radical scavenging activity and it also acts on cellular DNA and membranes, protecting them against damage induced by free oxygen radicals. Currently, there are increasing data, from human and experimental studies suggesting that both ibuprofen and L-carnitine as antioxidant and anti-inflammatory and cytoprotective agents should be beneficial agents in the protection against cancer treatment-related normal tissue injury (17-19).

The aim of the present study is to determine the protective effects of ibuprofen and L-carnitine on whole body gamma irradiation-induced mucosal injury in the hamster duodenum.

Materials and Methods

Animals

The experiments were performed on twenty-four adult male Syrian golden hamsters weighing 80-100 g, which were kept at a constant temperature of $22\pm1^{\circ}\text{C}$. The animals were fed a standard pellet diet (Gebze Food Factory, Kocaeli, Turkey), had access to tap water ad libitum, and were synchronized by the maintenance of controlled environmental conditions (light, temperature, feeding time, etc.) for at least 2 weeks prior to, and throughout the experiments. The lighting regimen was 12 h of light alternating with 12 h of darkness. The protocol for the study was approved by the Ethical Committee of Animal Breeding and Research, Medical Faculty, Trakya University.

Experimental Design

The animals were divided into the following four groups ($n=6$): group 1: control; group 2: irradiated-control group; group 3: irradiated and ibuprofen administered group; and group 4: irradiated and L-carnitine administered group. Ibuprofen was given by gavage to the animals in groups 3 at a dose of 10 mg/kg body weight for 15 consecutive days before irradiation. L-carnitine was dissolved in isotonic NaCl and given by gavage to the animals in groups 4 at a dose of 50 mg/kg body weight for 15 consecutive days before irradiation. Vehicle (saline, 1 ml/100 g) was administered to the hamsters by the same route to groups 1 (control) and 2 (irradiated-control) for 15 consecutive days. The hamsters of groups 2, 3 and 4 were exposed to whole body gamma irradiation of 8 Gy in a single dose (Table 1).

Total Body Irradiation

A cobalt 60 teletherapy instrument (Cirus, Cis-Bio International, France) was used to deliver a single peak whole body sub-lethal irradiation dose of 8 Gy (1 Gy/min) to a depth of 3 cm as described (12, 20). A single anterior field was used for irradiation and three animals were treated at a time. The cobalt 60 unit was calibrated with an Exradin Farmer type ionization chamber (Keithley 35040 radiation dosimeter, Cleveland, Ohio, USA). A $\pm 3\%$ uncertainty in absorbed dose was estimated. Twenty-four hours after radiation exposure, the hamsters were killed and samples were taken from the duodenum, and the histopathological determinations were carried out.

Table 1. Experimental Design

Day	
1	Randomization Administration of saline to the hamsters of groups 1 and 2
	Administration of ibuprofen to the hamsters of group 3
	Administration of L-carnitine to the hamsters of group 4
15	Irradiation of the hamsters in group 2, 3 and 4
16	Sacrifice of all hamsters Group 1: control; group 2: irradiated-control group; 3: irradiated + ibuprofen group; group 4: irradiated + L-carnitine group

Light Microscopic Procedures and Histopathologic Analysis

Tissue samples were fixed overnight in 10% formalin and paraffin blocks were obtained and routinely processed for light microscopy. Slices of $4-5\text{ }\mu\text{m}$ were obtained from the prepared blocks and stained with hematoxylin-eosin (H-E), periodic acid-Schiff-hemalum (PAS-HL) and Masson's trichrome. The preparations obtained were visualized using a CX-31 microscope (Olympus Company, Japan).

Duodenum sections were stained with H-E, and mucosal injury, inflammation and hyperemia/hemorrhage were assessed and graded in a blinded manner by a histologist using the histological injury scale previously defined by Chiu et al. (21). Briefly, mucosal damage was graded from 0 to 5 according to the following criteria: grade 0, normal mucosal villi; grade 1, development of subepithelial Gruenhagen's space at the apex of the villus, often with capillary congestion; grade 2, extension of the subepithelial space with moderate lifting of the epithelial layer from the lamina propria; grade 3, massive epithelial lifting down the sides of villi, possibly with a few denuded tips; grade 4, denuded villi with lamina propria and dilated capillaries exposed, possibly with increased cellularity of lamina propria; and grade 5, digestion and disintegration of the lamina propria, hemorrhage, and ulceration. The sum of the scores for the individual alterations constitutes the radiation injury score.

Measurement of Morphometric Parameters

Under the microscope, the height of enterocyte on the top of villi, lengths of basal lamina of ten enterocytes (magnified 400-fold) were measured using a micrometric ocular. Twenty measurements were performed per animal, so we had 120 measurements in each group. All values were calculated in micrometers (μm) (22).

Statistical Analysis

The data were presented as the median (Min-Max), due to non-normal distribution. Normality distribution of the variables were tested using one sample Kolmogorov-Smirnov test. Differences in measured parameters among the groups were analyzed by the Kruskal Wallis test. When a significant difference was found, the Mann-Whitney U-test was used for multiple comparisons. P-values of less than 0.05 were considered statistically significant.

Results

Histopathologic Findings

By light microscopy, the duodenum from control hamsters showed normal histologic structure of the villi intestinalis and Lieberkühn cripts (Figs. 1a-c). Morphologically, as compared to the control duodenum, examination of the gamma irradiated

duodenum revealed the presence of shortening and thickening villi, massive lifting of epithelial layer from the lamina propria and capillary congestion in the villi. There was flattening of enterocytes due to depletion of epithelial cells (Figs. 1d-f). Pretreatment with ibuprofen (Figs. 1g-i) and L-carnitine (Figs. 1j-l) with irradiation reduced the histopathological changes. In the pretreatment groups, villi were generally normal although there was light subepithelial lifting in the apical region of some villi.

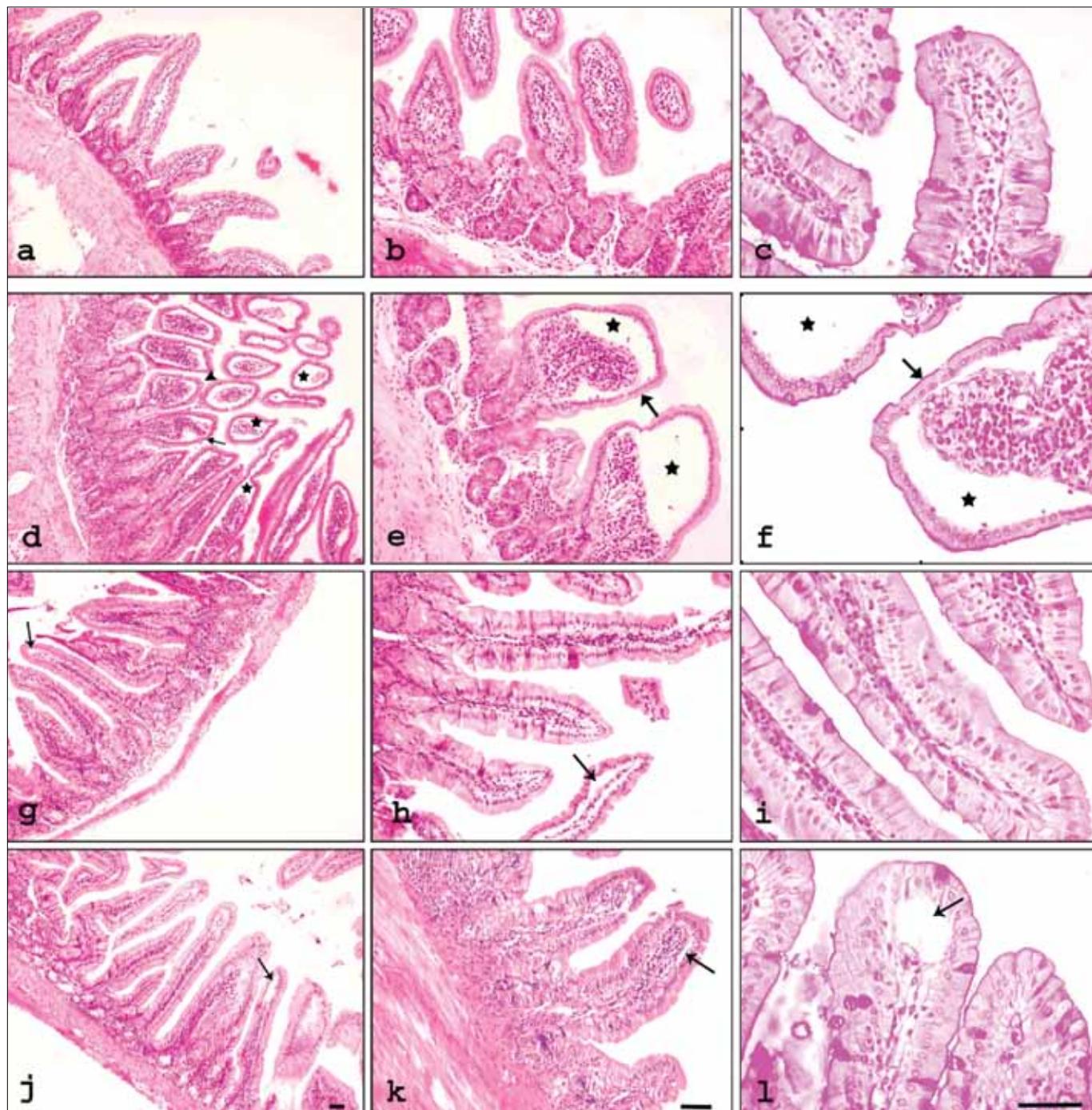


Figure 1. Photomicrographs of duodenal sections stained with H-E (a, b, d, e, g, h, j, k) and PAS-HI (c, f, i, l). (a-c) Control hamster duodenum; showing normal morphology. (d-f) Whole body irradiated-control hamster; showing shortening and thickening villi (arrowhead) and morphologic changes in the epithelial cells with flattening of enterocytes (arrow), massive subepithelial lifting (asterisks). (g-i) Radiation-ibuprofen treated and (j-l) radiation-L-carnitine treated hamsters; showing slight subepithelial lifting (thin arrow) in some villi. Scale bars: 50 µm

The radiation injury scores for each group are represented in Table 2. Whole body irradiation resulted in a marked increase in radiation injury score of duodenal mucosa compared to control duodenum ($p<0.01$). Pretreatment with ibuprofen and L-carnitine resulted in a significant decrease in mucosal damage in the irradiated duodenum ($p<0.01$), compared to irradiated-control group.

Morphometric Findings

The height of enterocytes on the top of the villus and the length of basal lamina of ten enterocytes were measured and the summary of those results is presented in Table 2. In comparison with the control group animals, the values of enterocyte height were significantly lower ($p<0.01$) and the values of basal lamina length were significantly higher ($p<0.01$) in hamsters examined 24 hours after irradiation with the dose of 8 Gy. In the ibuprofen pretreatment group and L-carnitine pretreatment group, the intensity of epithelial cell damages were less than in the irradiated-control group ($p<0.01$) (Figs. 2, 3).

Discussion

Intestinal radiation damage can be studied in different ways. Histologic changes in the intestine after irradiation have

been carefully studied and described. Production of new enterocytes in the crypts halts shortly after exposure to ionizing radiation. Crypts rapidly decrease in number. Surface cells continue to slough from the tips of the villi; the villi shorten and the luminal surface is denuded. Goblet cells largely disappear (23-25). Small intestinal epithelium are constantly renewed from undifferentiated progenitor cells located within the crypts of the intestine, and thus homeostasis is maintained (26). Upon exposure to irradiation, most progenitor cells in the intestinal crypts die, and the intestinal villi shrink within 48 hours, resulting in impaired absorption (27).

In this study, sublethal (8 Gy) irradiation caused severe degenerative changes in hamster duodenum, such as shortening and thickening of villi, massive subepithelial lifting and capillary congestion in the villus and flattening of enterocytes due to depletion of epithelial cells. These data corroborate previous studies reported by other investigators on radiation induced intestinal damage in animals (23, 25, 28, 29). Our results also indicate that oral administration of ibuprofen and L-carnitine proves to be protective by reducing the severity of radiotherapy-induced duodenal mucosal injury. In addition, histopathological evaluation also supports the idea of a reduction in the severity of radiotherapy-induced duodenal mucosal injury at 24 hours.

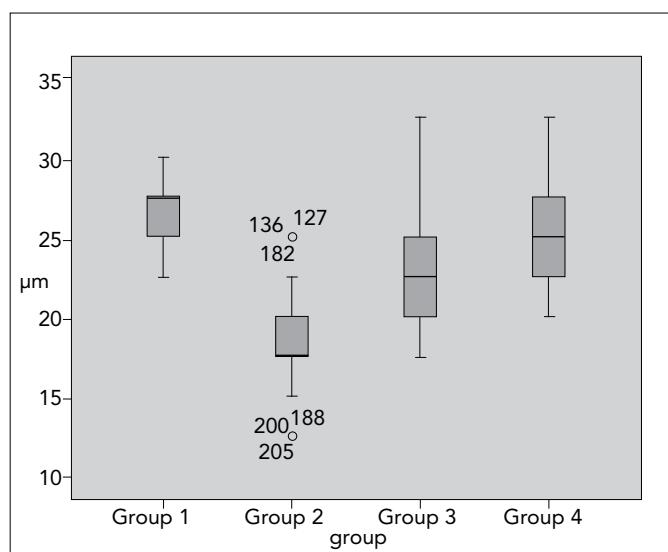


Figure 2. Height of enterocyte on the top of villus

Group 1: control; group 2: irradiated-control group; 3: irradiated + ibuprofen group; group 4: irradiated + L-carnitine group

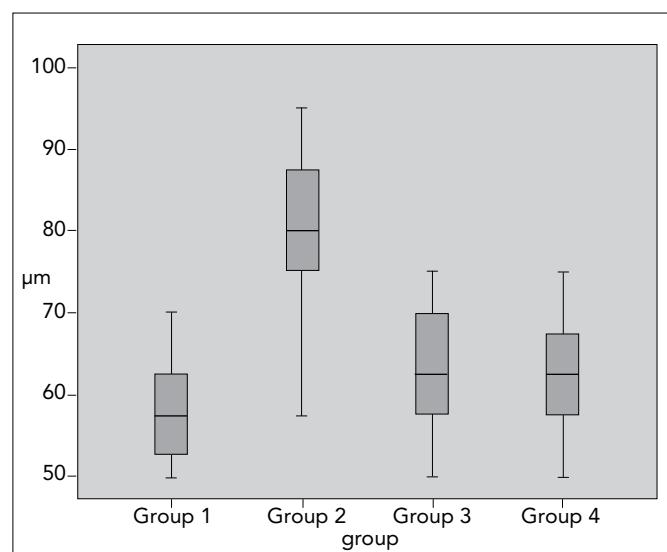


Figure 3. Length of basal lamina of 10 enterocytes

Group 1: control; group 2: irradiated-control group; 3: irradiated + ibuprofen group; group 4: irradiated + L-carnitine group

Table 2. Effects of ibuprofen and L-carnitine pretreatment on histomorphometric and histopathologic parameters

Parameters	Group 1 Median (Min-Max)	Group 2 Median (Min-Max)	Group 3 Median (Min-Max)	Group 4 Median (Min-Max)	p
Height of enterocytes on the top of villus (μm)	27.50 (23-30) ^{‡#}	17.50 (13-25) ^{‡#}	22.50 (18-33) ^{‡#}	25.00 (20-33) ^{‡#}	0.000 ^{*#}
Length of basal lamina of 10 enterocytes (μm)	57.50 (50-70) ^{‡#}	80.00 (58-95) ^{‡#}	62.5 (50-75) ^{‡##}	62.5 (50-75)	0.000 ^{*#}
Radiation injury score	0.00 (0-0) ^{‡#}	4 (3-5) ^{‡#}	1 (1-2) ^{‡##}	2 (1-3)	0.000 ^{*#}

Group 1: control; group 2: irradiated-control group; 3: irradiated + ibuprofen group; group 4: irradiated + L-carnitine group
*:Kruskal Wallis test, ‡: Mann Whitney U test
#:p<0,01; Group 1-2, Group 1-3, Group 1-4, Group 2-3, Group 2-4, #:p>0,05; Group 3-4

Studies point out that both ibuprofen and L-carnitine protect normal tissues against injurious effects of cancer treatments such as radiotherapy and chemotherapy, without an inhibitor effect against their therapeutic effects (17-19). Administration of ibuprofen along with radiation therapy to animals bearing LNCaP tumors results in a 2-fold increase in tumor growth delay compared with radiation alone.^[30] Previous studies have shown that L-carnitine and its derivatives provide protection against radiation damage by inhibiting radiation-induced increasing nitric oxide and malondialdehyde levels and by modulating radiation-induced changes in the antioxidant defense mechanisms in rat tissues (16, 31). From all these investigations, we have determined that ibuprofen and L-carnitine have a lot of beneficial effects against cancer treatment-related toxicities. Both ibuprofen and L-carnitine also have a role as an antioxidant and anti-inflammatory and a cytoprotective agent. Dokmeci et al. (12, 16) found that ibuprofen and L-carnitine were effective in the prevention of radiation-induced injury in hamsters by ameliorating disturbances in antioxidant balance. These studies show that administration of ibuprofen and L-carnitine preceding gamma radiation exposure significantly decreases the thiobarbituric acid-reactive substance level and increases the activity of superoxide dismutase and catalase enzymes in the plasma.

In this study, we have found that, after irradiation, whole body with sublethal (8 Gy) doses in hamster duodenum, the height of the enterocytes on the top of the villus was significantly decreased. Our results also indicate that irradiation with sublethal doses influences the length of basal lamina of 10 enterocytes. Twenty-four hours after irradiation with the dose of 8 Gy, the length became significantly increased. According to Fajardo (32), the main microscopic sign of radiation-induced intestine injury is flattening of enterocytes on the surface of villi. The height of the enterocyte is dependent on the dose of irradiation and might be considered as a very sensitive and suitable biodosimetric marker after irradiation (33). The flattening of enterocytes in intestinal radiation-induced pathology is a compensatory mechanism enabling a lower amount of cells to cover a larger surface of villus (32).

Driak et al. (22) reported that, after whole body gamma-irradiation with subralethal doses in rat jejunum, the length of basal lamina of 10 enterocytes was significantly increased and the height of the enterocyte on the top of the villus was decreased, although irradiation with sublethal doses did not significantly change either the length of basal lamina of 10 enterocytes or the height of the enterocyte on the top of the villus.

In conclusion, a significant decrease in the severity of histopathological and morphometric changes induced by radiation was observed in hamster duodenum treated with ibuprofen and L-carnitine. Ibuprofen and L-carnitine administrated by the oral route may be a good radioprotector against small intestinal damage in patients undergoing radiotherapy.

Conflict of Interest

No conflict of interest was declared by the authors.

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