Original Article

EFFECT OF DEXAMETHASONE ON SOME MARKERS FOR CYTOTOXICITY AND PROLIFERATION IN RAT INTESTINE AFTER TOTAL BODY GAMMA IRRADIATION

Violeta Y. Dancheva, Milen P. Karaivanov[1], Veneta L. Shopova, Lyudmil G. Terziev[2], Galya T. Stavreva[3]

Sector of Disaster Medicine
[1]Department of Pathology
[2]Sector of Clinical Immunology and Allergology
[3]Sector of Experimental and Clinical Pharmacology
Medical University-Pleven

Summary

The effect of dexamethasone on some markers for cytotoxicity and proliferation in rat intestine after total body gamma irradiation was studied. Seventy-two male rats were divided into three groups: group 1 (controls); group 2 (receiving a single 6 Gy total body irradiation); group 3 (receiving dexamethasone and 6 Gy ionizing radiation). The animals in group 3 were injected *i.p.* with dexamethasone at a dose of 3 mg/kg four hours before irradiation, as well as on days 2 and 3 after exposure. The levels of IL-6 and CINC-1 were determined in the plasma by the ELISA method. Immunohistochemical and histological studies were performed in rat intestine. Ionizing radiation increased the levels of IL-6 and CINC-1 considerably in comparison to that in controls on day 3. Dexamethasone significantly decreased the level of IL-6 on day 7, as compared to both controls and irradiated group. The level of CINC-1 in group 3 was significantly lower on days 3 and 7 than that in the control group. Immunohistochemical testing with the marker for proliferative activity Ki-67 in group 2 showed a total suppression of the proliferative activity, in contrast to the controls. In group 3, the same testing showed a decrease, though the activity was still present. Dexamethasone produced moderate anti-inflammatory protection from radiation injury.

Key words: cell proliferation, dexamethasone, inflammation, interleukins, ionizing radiation

Introduction

Inflammation is a classical pathophysiological response to ionizing radiation. Inflammatory lesions have been found locally in a number of tissues, such as skin, intestine or lung, and in cases of a wide range of doses (between 5 and 40 Gy). Recent studies have shown early changes such as an increase in the number of adherent and emigrated leucocytes [1, 2]. An early and persistent cytokine production following local exposure of rat intestine [3] or lung [4] has been established and related to fibrosis, occurring later in time.

Endothelial cells play a crucial role in the initiation, development and maintenance of the

Corresponding Author:
Violeta Y. Dancheva
Sector of Disaster Medicine
Medical University-Pleven
1, Kliment Ohridski str.
5800, Pleven
e-mail:vilidana@abv.bg

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inflammatory response. During the inflammatory process, leucocytes accumulate in the damaged tissue after transendothelial migration mediated by a cascade of events involving pro-inflammatory cytokines, chemokines and adhesion molecules. The major mediators of these processes are IL-1 and TNF-α. They appear to be produced mainly by activated monocytes/macrophages and, in turn, activate both these cells and vascular endothelial cells. This activation involves different steps, at which adhesion molecules are expressed and chemoattractants are released thus allowing the migration of transendothelial leucocytes into the underlying tissue [5, 6].

Dexamethasone is a long-acting florinated glucocorticoid. It is faster acting with longer duration than the intermediate oral agents prednisone and prednisolone. Dexamethasone stimulates synthesis of enzymes required to decrease the inflammatory response. It also suppresses the immune response, stimulates bone marrow, and influences fat, protein, and carbohydrate metabolism. Corticosteroids have effects on several important biochemical pathways and cellular transport mechanisms, including cellular sodium transport, glycogen synthesis and anti-inflammatory responses [7]. Corticosteroids inhibit nuclear factor-kB and activator protein-1 by blocking nuclear factor-kB-dependent proinflammatory gene expression [8, 9].

The main aim of this study was to investigate the anti-inflammatory effect of Dexamethasone on some markers for cytotoxicity and cell proliferation, as well as morphological changes in rat intestine after total body gamma irradiation at a dose of 6 Gy.

Material and methods

Experimental animals and solutions

Male Wistar rats (n=72, 220-250 g, aged 4 months) were purchased from the Research and Laboratory Animal Breeding Center of Slivnitsa (Bulgaria) and were raised at the University vivarium for 1 month at a temperature 22±2C and a humidity of 50±10%. The animals were given a normal pelleted diet and water *ad libitum.* The animals were divided into three experimental groups: group 1 (controls); group 2 (receiving a single 6 Gy total body irradiation at a rate of 0.91Gy/min, using an IGUR-1 apparatus with radioactive cesium), and group 3 (treated with dexamethasone and 6 Gy ionizing radiation).

The animals in group 3 were injected *intraperitoneally* with dexamethasone at a dose of 3 mg/kg, dissolved in water, four hours before irradiation, as well as on days 2 and 3 after exposure. Dexamethasone was purchased from Sopharma, Bulgaria. The experiment was performed in accordance with Animal Welfare Regulations and was approved by the University Ethics Committee.

Investigations

Eight animals from each group were sacrificed on post-treatment days 1, 3 and 7 to measure the following parameters:

Measurement of inflammatory mediators in the plasma

Blood was collected by intracardiac puncture in a syringe containing EDTA 0.5 M after Thiopental anesthesia with (50 mg /kg). Interleukin-6 (IL-6) and Rat Cytokine-Induced Neutrophil Chemoattractant-1 (CINC-1) levels were determined in the plasma in pg/ml by specific ELISA method according to assay protocol, R&D Systems, UK.

Immunohistochemical and histological studies

Immunohistochemistry: We studied formalin-fixed and paraffin-embedded biopsy specimens from small intestine of 18 rats, obtained on days 1, 2 and 7 after irradiation. Haematoxylin-Eosin (H&E)-stained sections were evaluated for any pathologic changes. Immunohistochemical investigation with anti-Ki-67 (MIB-1) antibody was performed using an avidin-biotinylated enzyme complex kit (DAKO LSAB® 2), following manufacturer's instructions (DAKOCytomation). Computerized morphometric analysis was applied to calculate Ki-67-proliferating index *(percentage of Ki-67-positive cells /1000 cells)*, using image analysis system (Olympus BX40), a digital camera (Olympus C-5050 ZOOM) and UTHSCSA image processing and analysis program, version 2.03 (University of Texas Health Science Center at San Antonio, Texas). The histological changes were examined on histological slides stained with Haematoxylin-Eosin.

Statistical analysis

Experimental data were analyzed using Statgraphics plus for Windows 5.0. Statistical analysis was based on parametric methods using

theStudent's *t*-test (analysis of variance) with $p<0.05$. Data are presented as a percentage of the control level. Asterisk (*) stands for reliability in comparison with the control group, and Δ designates reliability in comparison with the irradiated group.

Results

Determination of plasma cytokine/chemokine levels

Ionizing radiation increased the levels of IL-6 and CINC-1 considerably in comparison to that in controls on day 3. In the group treated with dexamethasone, the increase was smaller but without a reliable difference from the irradiated group. Dexamethasone significantly decreased the level of IL-6 on day 7 as compared to both control and irradiated groups (Fig. 1).

The level of CINC-1 in the group treated with dexamethasone and ionizing radiation was significantly lower on days 3 and 7, as compared to that in the control group (Fig. 2).

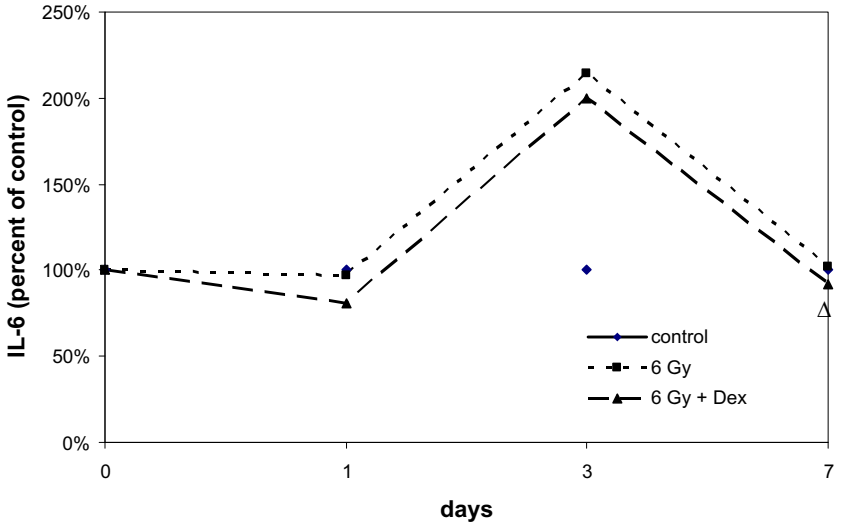

Figure 1. IL-6 level in plasma. Data are presented as a percentage of the control level for six animals. Asterisk (*) – reliability in comparison with the control group; Δ – reliability in comparison with the irradiated group.

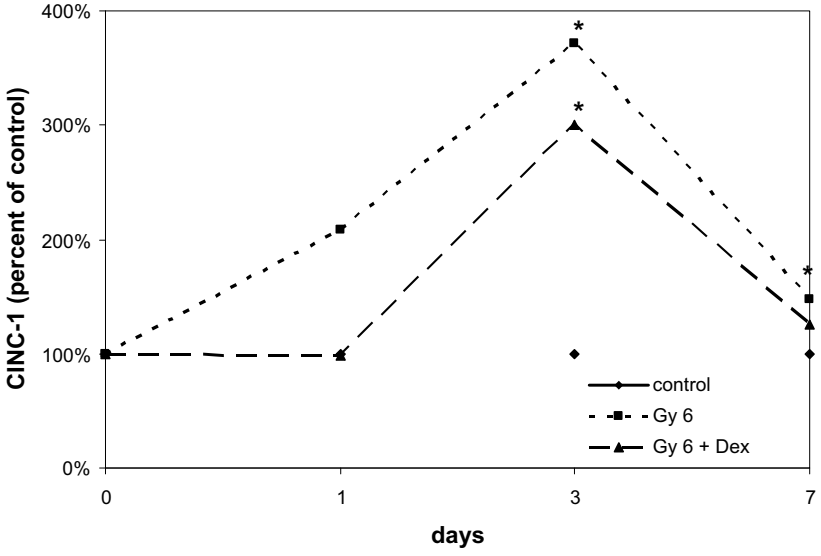

Figure 2. CINC-1 level in plasma. Data are presented as a percentage of the control level for six animals. Asterisk (*) – reliability in comparison with the control group; Δ – reliability in comparison with the irradiated group.

Histological studies

Group of laboratory animals, treated with ionizing radiation only

On the first day after exposure, marked atrophy was seen in the small intestine (the villi were shortened) (Fig. 3b). No such changes were found in the controls (Fig. 3a). The villi also looked thicker, deformed and had areas of adhesions between them. Marked oedema and inflammatory infiltrate was seen in the chorion (Fig. 3c). The crypts were atrophied and decreased in number. In a few of the histological slides, on day 7 after exposure, only single abortive crypts could be seen. On day 1, the earliest changes in the small intestine were seen in the blood vessels of the submucosa and subserosa. The changes included marked venous hyperaemia, perivascular oedema, haemorrhages and dystrophic changes in the ganglionic cells of the myenteric plexus (Fig. 3d).

Immunohistochemical testing of the laboratory animals with the marker for proliferative activity Ki-67 in the small intestine showed a total suppression of the proliferative activity (<10%), much lower than in the control the group (Fig. 3f), very noticeable in the epithelium of the crypts (single positive cells) and zones with total absence of expression of Ki-67 antigen (Fig. 3g).

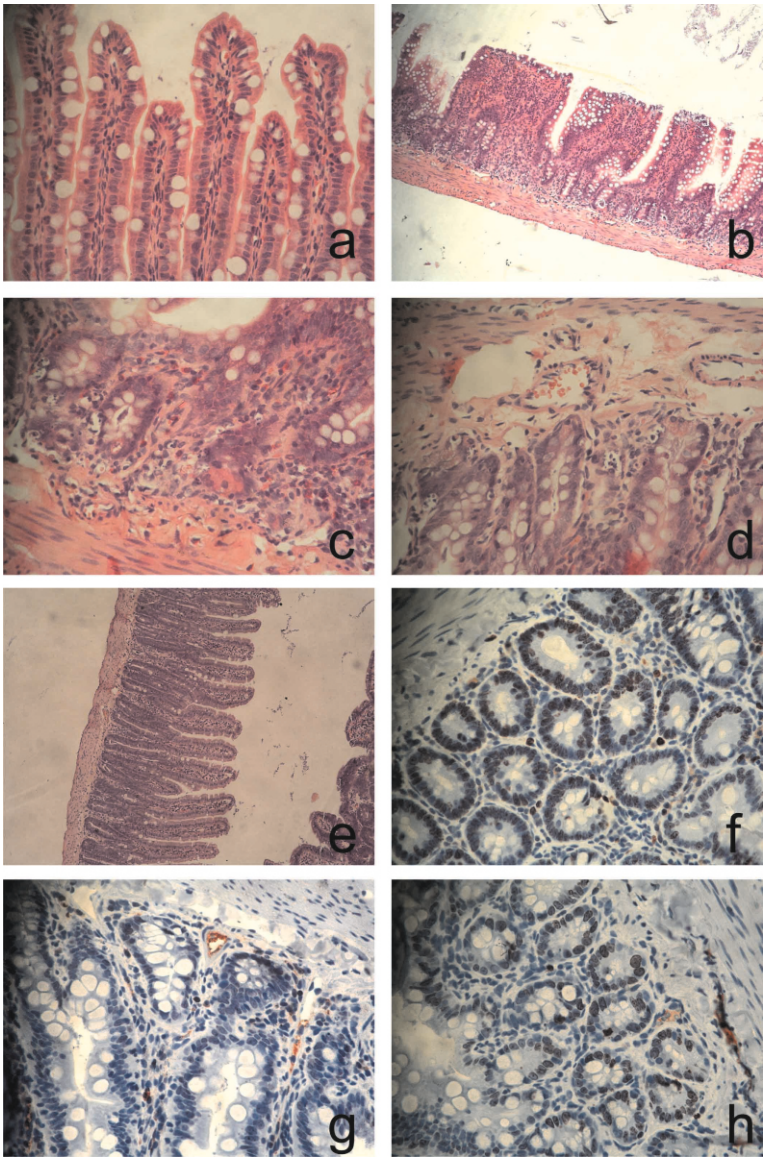

Figure 3. Histology (3a, 3b,3c, 3d and 3e) and immunohistochemistry (3f, 3g, 3h) of small intestine

Group of laboratory animals, treated with dexamethasone and ionizing radiation

The pathological changes seen on the histological slides from this group, stained with Haematoxylin-Eosin were poorly expressed, as compared to those in the group treated with ionizing radiation only. In the small intestine of the laboratory animals of the same group, very mild degree of shortening of the villi was observed. They villi had normal morphology without any deformation and desquamation of the surface epithelium. Adhesion of villi to each other was rare. The intestinal crypts were to a great extent preserved and almost same in number as compared to the group of controls. In the submucosa and subserosa, no severe oedema and hyperaemia were seen, similarly to the previous group (Fig. 3e).

Immunohistochemical testing of the laboratory animals with the marker for proliferative activity Ki-67 in the epithelium of the intestinal crypts showed a decrease of the proliferative activity, as compared to the group of controls, but it was generally preserved (>50%) and was greater than the activity seen in the group treated with ionizing radiation only (Fig. 3h).

Discussion

Medically, ionizing radiation may be used as the sole treatment or as adjuvant to chemotherapy or surgical management of neoplasia in different parts of the human body, e.g. breast, cervix, bones, kidneys and lungs amongst others [10]. Total body radiation is also included in preparing patients for bone marrow transplantation, as part of the conditioning regime. Actively dividing cells are known to be extremely sensitive to radiation [11].

Evidence exists for the involvement of endothelial cells in the radio-induced inflammatory response. An increase in leucocyte adhesion to irradiated endothelium has been observed *in vitro*, and is at least partly explained by the induction of some adhesion molecules, such as ICAM-1 in dermal microvasculature endothelial cells [12, 13]. Data obtained by other investigators have shown the release of chemoattractants by irradiated bovine endothelial cells [14, 15] and suggest that a biphasic response involving a lipid chemoattractant shortly after irradiation, could involve dysregulation of the vascular endothelium [16].

IL-6 is a multifunctional cytokine that plays important roles in host defence, acute phase reactions, immune responses, nerve cell functions and haematopoiesis [17, 18, 19]. IL-6 is expressed by a variety of normal and transformed lymphoid and non-lymphoid cells. It is a typical pleiotropic cytokine. For instance, IL-6 induces the differentiation of B cells to antibody-producing plasma cells [20].

Chemokines regulate inflammation, leukocyte trafficking, and immune cell differentiation [21]. CINC-1, otherwise known as CINC, rat GRO and rat KC, is a member of the alpha, or CXC family of chemokines [22]. Functionally, CINC-1 is described as a major neutrophil chemoattractant and activator [23] induced by IL-1β, TNF-α and bacterial products, promotes both neutrophil rolling and adhesion, probably through the up-regulation of surface integrins. CINC belongs to the interleukin 8 (IL-8) family [24]. An acute inflammatory response of the intestine after abdominal irradiation has been demonstrated through the occurrence of a neutrophil influx [25] and local proinflammatory cytokine production [26].

The potency of dexamethasone anti-inflammatory activity has been quantified to be 25-30 times higher than that of hydrocortisone [27]. Glucocorticoids also appear to stabilize lysosomal membranes in affected cells by preventing the release of vasoactive kinins and destructive enzymes [28]. This is an important fact since lysosomes are the most radiosensitive organelles. Lysosomal membrane damage may lead to disruption and subsequent lysis in radiation injury via lipid peroxidative attack by free radicals.

In order to evaluate the systemic radiation-induced inflammatory response, the measurements of inflammatory mediators in the plasma were carried out on days 1, 3 and 7 following radiation exposure. Il-6 and CINC-1 were increased in the plasma of irradiated rats on day 3, and the increase was significantly higher in CINC-1 level as compared to the control group. The increase in combined group (dexamethasone + 6 Gy) was smaller but without a reliable difference from the irradiated group. Dexamethasone decreased significantly the level of Il-6 on day 7 as compared to both control and irradiated groups. In addition to inflammatory cytokines, the induction of chemokines and endothelial adhesion receptors is a key process to inflammatory-induced organ injury. In such

cases, i.e. after irradiation, the injured gut has been cited as a cytokine-generating organ. The mediators released from the gut may reach other tissues after release in mesenteric lymph or general circulation. Such agents have a capacity to induce dysfunction across tissues and are to be considered as markers of a generalized inflammatory response. As a result to chemokine production, granulocyte accumulation and subsequent vascular leakage occur.

On the first day after exposure, marked atrophy was seen in the small intestine in the irradiated group. The villi were shortened, deformed, and thickened. Marked oedema and inflammatory infiltrate was seen in the chorion. The crypts were atrophied and decreased in number. On the seventh day after exposure, only single abortive crypts could be observed.

In the group treated with Dexamethasone and 6 Gy ionizing radiation, a mild degree of shortening of the villi was observed, with preserved morphology. Adhesion of villi was not present. The intestinal crypts were to a great extent unaffected. In the submucosa and subserosa severe oedema and hyperaemia were not seen.

Immunohistochemical testing of the irradiated animals with the marker of proliferative activity Ki-67 showed total suppression of the proliferative activity (10%) in the small intestine, and zones with total absence of expression of Ki-67 antigen were observed. In the combined group (Dexamethasone + 6 Gy) the proliferative activity in the epithelium of the intestinal crypts was decreased as compared to the group of controls, but generally preserved (50%), and was greater than the activity seen in the group treated with ionizing radiation alone.

Conclusion

The morphological patterns seen in our study make it reasonable to conclude that in the group of laboratory animals treated with dexamethasone and ionizing radiation, the pathological changes in the small intestine were mildly developed, as compared to the group of laboratory animals treated with ionizing radiation alone. The proliferative activity was preserved to a great extent preserved. Dexamethasone injected *intraperitoneally* four hours before irradiation, as well as on days 2 and 3 after irradiation at a dose of 3 mg/kg body weight, produced moderate anti-inflammatory protection from radiation injury.

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