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Effect of exposure to radiation on the inflammatory process and its influence by diclofenac

M. El-Ghazaly, S. Kenawy, M.T. Khayyal[1], H. Roushdy & S. Saleh

Department of Pharmacology, Faculty of Pharmacy, Cairo University, and National Centre for Radiation Research and Technology, Cairo, Egypt

1 The effect of radiation exposure on the inflammatory process was studied in rats using the carrageenan-induced paw oedema and adjuvant-induced arthritis tests.

2 Irradiation (0.5,1 and 2 Grays) resulted in a significant augmentation of the tissue response to carrageenan and the early phase of adjuvant-induced arthritis, but suppressed the late phase.

3 Diclofenac ($1-5\ mg\ kg^{-1}$) effectively reduced the exaggerated inflammatory response in irradiated animals in both the carrageenan paw oedema and adjuvant-induced arthritis tests. The drug also had a prophylactic value in guarding against the induction of radiation damage.

4 The inflammatory responses produced by irradiation and the benefits obtained by drug treatment may be related to changes in tissue prostaglandin levels and/or changes in the immune system.

Introduction

It has been shown that irradiation of mammalian skin with ionizing radiation causes a sequence of inflammatory reactions in which increased capillary permeability constitutes a major component. Moreover, following irradiation of animal skin proteolytic enzymes, particularly the proteases, are activated and this in turn causes the liberation of biologically active kinins. These changes lead to an increase in both capillary permeability and the tissue response to injurious stimuli (Jolles & Harrison, 1966). Whole body irradiation was also found to enhance the synthesis and leakage of enzymes from lysosomes (Snyder, 1977). The increase in the activity of lysosomal hydrolases and proteinases to abnormally high levels in many mammalian tissues after radiation exposure was said to be related to an increase in cyclic nucleotide synthesis (Trocha & Catravas, 1980). In addition, there is evidence to suggest that ionizing radiation affects prostaglandin levels in animal tissues (Eisen & Walker, 1976; Trocha & Catravas, 1980). Thus, prostaglandin levels have been found to increase within several hours after exposure of mice to X-rays and to remain elevated for several days, especially in radiosensitive tissues such as the spleen and thymus (Eisen & Walker, 1976). Both prostaglandins and lysosomal enzymes are known mediators of cellular injury and inflammation. The present work was undertaken in order to study the effect of exposure to radiation on experimentally induced inflammatory processes and to assess the potential use of a non-steroidal anti-inflammatory agent (NSAID), diclofenac, in controlling the effects of radiation.

[1] Author for correspondence.

Methods

Male albino rats (150-180g) were irradiated using a Cs-137 Cell-40 at a radiation dose rate of $0.018\ Gy\ s^{-1}$, to attain radiation dose levels of 0.5, 1 or 2 Grays (Gy) per animal as required. The maximal time of animal exposure was 1.8 min to attain a radiation dose level of 2 Gy.

To study the effect of the intensity of radiation on the sensitivity of animals to acute inflammation, 0.05 ml of 1% carrageenan (BDH) suspension (Winter *et al.*, 1962) was injected subplantar into groups ($n = 6$) of normal and irradiated animals exposed to 0.5, 1 or 2 Gy each. The carrageenan was injected immediately after radiation exposure. The change in the size of the injected paw before and 3 h after the carrageenan injection was taken as an index of inflammation.

The paw volume was measured by an air displacement method using an apparatus devised by the Institute of Pharmacology, University of Muenster, F.R.G. This consists of a small metal cylindrical

matchbox device which is connected to a galvanometer, reading in μA. The zero reading is adjusted with the cylindrical matchbox device closed. Unanaesthetized rats were held tightly and the injected paw was then placed in the cylindrical device so that the air displaced by the paw causes a measurable change in electric resistance which can be read on a scale. Results were expressed in arbitrary units, each unit corresponding to a change in electric current of 1 μA.

To investigate the time course of radiation effects, three groups of animals ($n = 6$) exposed to 2 Gy were challenged with carrageenan 4 h, 3 days and 7 days after radiation exposure. In all experiments, the paw size was measured 3 h after carrageenan injection. In another set of experiments, the irradiated animals were treated with diclofenac sodium (Ciba-Geigy) one hour before the carrageenan injection. The drug was administered intraperitoneally at 3 different dose levels (1, 3 and 5 mg kg^{-1}) to both irradiated and normal control animal groups.

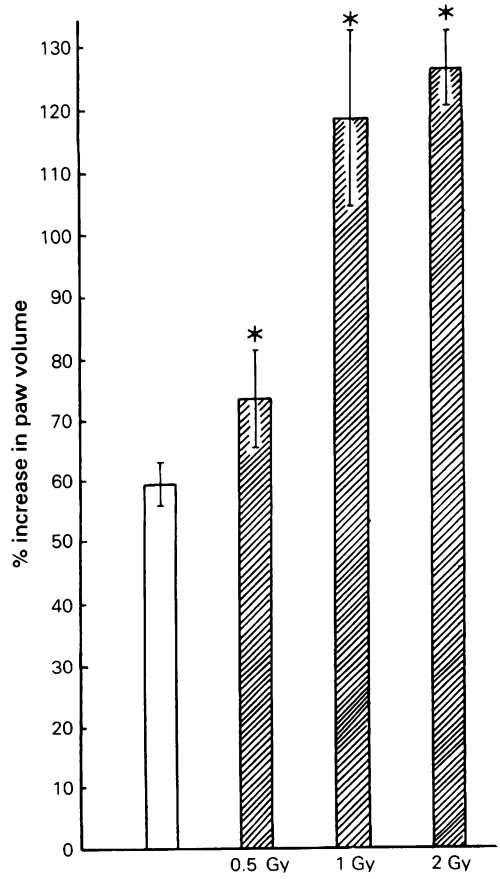

Figure 1 The effect of carrageenan-induced oedema in normal (□) and irradiated (0.5, 1 and 2 Gy) rats (▩). Results are expressed as percentage increase in paw volume, and the vertical lines indicate s.e.mean. Values given are means of 6 observations. * $P < 0.05$, significant changes compared to non-irradiated rats.

To study the possible protective value of the drug against radiation effects, it was given in a dose of 3 mg kg^{-1} intraperitoneally, 1 h before exposure to the lowest radiation level of 0.5 Gy and the animals were challenged with carrageenan immediately after irradiation.

The effect of radiation exposure on chronic inflammation was studied using the adjuvant-induced arthritis model in the rat (Pearson, 1964). A single subcutaneous injection of 0.1 ml of Freund's complete adjuvant (Behringwerke AG, Marburg) was inoculated into the right hind paw of groups ($n = 8$) of normal and irradiated rats (exposed to 0.5, 1 and 2 Gy). The adjuvant was injected 4 h after irradiation. The size of the injected paw was measured every 3 days for a period of 4 weeks.

The effect of diclofenac was investigated by injecting the drug intraperitoneally at a dose of 0.3 mg kg^{-1} both 1 h before and then daily following inoculation of the adjuvant and subsequent exposure of animals to radiation (2 Gy). Drug treatment was continued for 4 weeks and the paw size was again measured every 3 days.

Calculations

The results are expressed as % inhibition of oedema according to the formula

$$\% \text{ inhibition} = 100\left(1 - \frac{V_t}{V_c}\right)$$

where V_t and V_c are the increases in volume of the carrageenan-injected paws of the drug-treated and control groups, respectively. Statistical analysis of the data was made using Student's t test, and the 0.05 level of probability was regarded as significant.

Results

Figure 1 shows that the inflammatory response induced by carrageenan in irradiated rats was markedly higher than that induced in normal animals. In these experiments, carrageenan was injected immediately after irradiation and oedema was assessed in rat paws 3 h after injection of carrageenan, as described in Methods. The extent of oedema following irradiation was dependent on both the level of radiation exposure as well as on the time elapsed after exposure. With an exposure level of 1 and 2 Gy, the extent of oedema was more than doubled compared to the control non-irradiated animals (Figure 1).

To demonstrate the influence of time on radiation

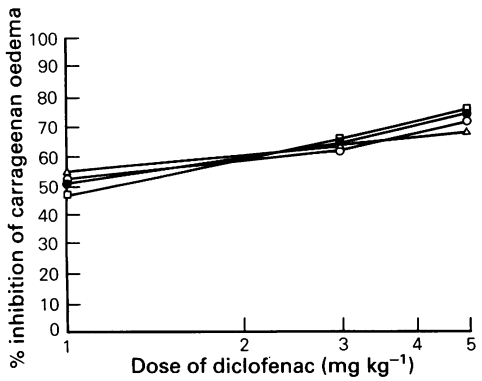

Figure 2 The effect of various doses of diclofenac administered intraperitoneally 1 h before carrageenan injection into the paws of rats treated as follows: non-irradiated controls (○); previously irradiated with 2 Gy at 4 h (□), 3 days (●), 7 days (△) before the carrageenan. Results show mean values of 6 observations; for clarity the s.e.means have been omitted.

effects, an experiment was devised in which animals exposed to 2 Gy were challenged with carrageenan 4 h, 3 and 7 days after exposure and the oedema measured after 3 h in each case. In this experiment, the animals showed a progressive increase in the oedema response to carrageenan with time. With a delay of 4 h between radiation exposure and carrageenan administration, the oedema was 126.0 ± 6.0% that of oedema in control animals reaching 147.0 ± 8.7% with a delay of 3 days and rising to 164.0 ± 15.2% 7 days post-irradiation. When such animals were treated, after irradiation, with diclofenac (1, 3 and 5 mg kg^{-1}) one hour before carrageenan injection, there was a dose-dependent inhibition of the inflammatory response (Figure 2). The percentage inhibition was quantitatively similar regardless of the time elapsed between irradiation and testing and ranged from nearly 50% with the smallest to 74% with the largest dose.

The effect of diclofenac given before irradiation showed that the drug was also capable in this case of reducing the inflammatory response to carrageenan. The administration of 3 mg kg^{-1} diclofenac one hour before irradiation at 0.5 Gy reduced the oedema induced by carrageenan, injected immediately after irradiation, to nearly half that induced in the control irradiated group (Figure 3). This experiment also confirmed that irradiation, 0.5 Gy, significantly augmented the oedema response to carrageenan within 3 h (compare with Figure 1).

In the adjuvant induced-arthritis model, exposure of the rats to γ-radiation at dose levels of 0.5, 1 and 2 Gy increased the intensity of inflammation during the first phase (Figure 4). The peak effect which was sharp and short-lived in normal animals, was larger and more sustained in irradiated rats. However, during the later stages of the condition (7–19 days), the inflammatory oedema was markedly suppressed (Figure 4) in a manner which depended on the dose of irradiation. When given 1 h before inoculation of the adjuvant, diclofenac (0.3 mg kg^{-1}) was effective in inhibiting the arthritic paw swelling in non-irradiated rats during both the early and late phases (Figure 5). The percentage inhibition was 52.0 ± 4.8% 8 days post-treatment and reached 63.8 ± 4.2% on day 28.

In irradiated rats, the drug was also effective in protecting against the exaggerated oedema response of the early phase (Figure 5); the percentage inhibition was 60.0 ± 11.2%, and 55.0 ± 28.5% on the fourth and eighth days, respectively, after irradiation and treatment.

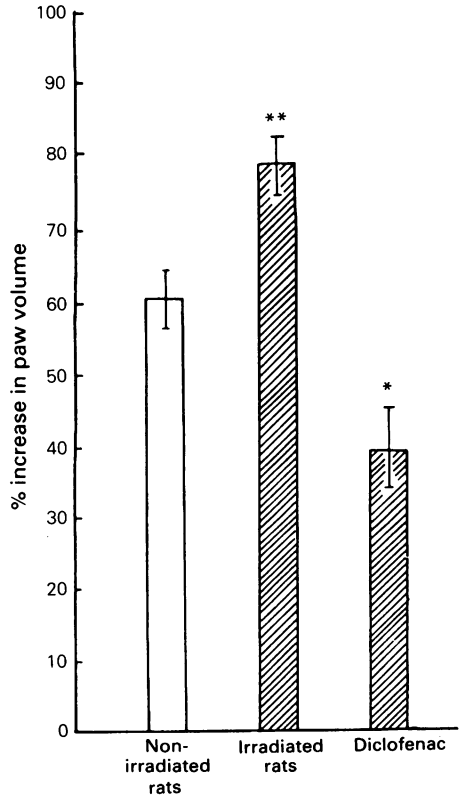

Figure 3 The effect of pretreatment with diclofenac (3 mg kg^{-1} i.p.) 1 h before carrageenan on the increase in paw volume induced by carrageenan administered immediately after exposure to irradiation, 0.5 Gy. Results are expressed as percentage increase in paw volume, and the vertical lines indicate s.e.mean. Values given are means of 6 observations. * $P < 0.05$, significant changes compared to irradiated rats. ** $P < 0.05$, significant changes compared to non-irradiated rats.

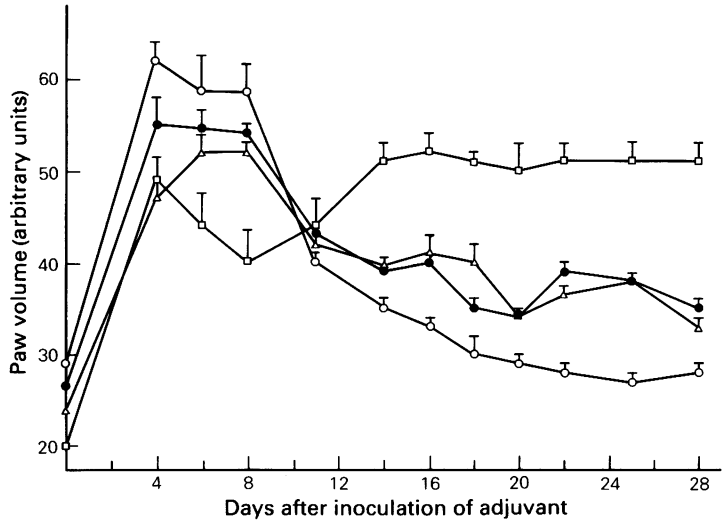

Figure 4 The effect of irradiation of adjuvant-induced arthritis in rats. Freund's adjuvant, 0.1 ml, was injected 4 h after irradiation into right hind paw of rats. Non-irradiated control (□), irradiated at 0.5 Gy (△), 1 Gy (●) and 2 Gy (○). Each point represents the mean of 8 observations; vertical lines indicate s.e.mean.

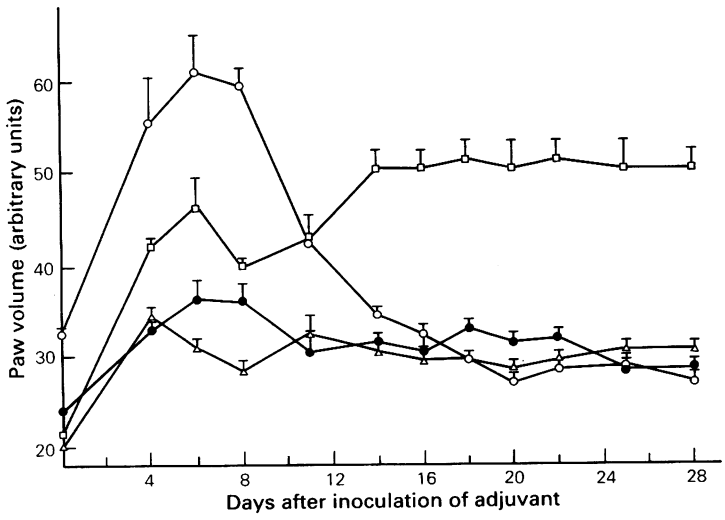

Figure 5 The effect of diclofenac (0.3 mg kg^{-1}, 1 h before adjuvant inoculation) on adjuvant-induced arthritis in normal and irradiated (2 Gy) rats. Non-irradiated controls (□), irradiated controls (○), non-irradiated drug-treated (△), irradiated drug-treated (●). Each point represents the mean of 8 observations and vertical lines indicate s.e.mean.

Discussion

Our results show that whole body exposure of rats to γ-radiation caused a significant increase in the inflammatory response to carrageenan. This exaggerated response was dependent on the dose of radiation and it increased progressively with the time elapsed between irradiation and carrageenan treatment.

The present results also show that the intensity of inflammation in the early phase of adjuvant-induced arthritis was much higher in irradiated rats than in normal ones, but that the later phase of the arthritis was markedly suppressed.

The administration of diclofenac, either before or after irradiation of animals, successfully reduced the intensity of the induced inflammatory response.

The oedema induced in rats by injection of carrageenan has been shown to be mediated by histamine and 5-hydroxytrypramine (5-HT) during the first hour (Willis, 1969; Di Rossa *et al.*, 1971), after which time the increased vascular permeability is maintained by kinin release (Van Arman *et al.*, 1965; Di Rosa *et al.*, 1971). At later stages of the inflammatory reaction to carrageenan, the response is mainly due to the potentiating action of prostaglandins on the effects of the other mediators (Di Rosa & Willoughby, 1971; Ferreira *et al.*, 1974; Lewis *et al.*, 1975; Bonta *et al.*, 1977). The implication of many mediators in carrageenan-induced oedema was confirmed by the findings of Willis (1969) who, by using a cascade superfusion technique, was able to detect the release of histamine, 5-HT, kinins and prostaglandins in carrageenan-induced exudates.

The exaggerated inflammatory response observed in irradiated rats could be attributed to the increased levels of prostaglandin E found in tissues following radiation exposure (Eisen & Walker, 1976) and/or to the release of lysosomal enzymes (Trocha & Catravas, 1980). Irradiation would accordingly result in an increase in prostaglandin levels and promotion of the release of lysosomal enzymes as a result of disruption of the cell membranes. This disruption may occur due to a direct interaction of cellular membranes with γ-rays, or through an action of the free radicals produced by radiation exposure on them. Breakage of the membranes following radiation exposure could affect enzymes and lipids associated with prostaglandin synthesis and eventually increase their concentration. Formation of prostaglandins might then destabilize lysosomal membranes, resulting in the dispersal of lysosomal enzymes throughout the cell. Furthermore, the increase in cyclic nucleotide synthesis, which has been observed after irradiation of animals, might contribute to lysis of the lysosomes and the release of enzymes that cause cellular injury (Trocha & Catravas, 1980).

In adjuvant-induced arthritis, the primary inflammatory response observed during the first week is due to the irritant quality of the adjuvant and the release of inflammatory mediators. The second phase of the inflammatory response is chronic and systemic and is known to be mediated through an immunological reaction (Waksman *et al.*, 1960; Watnick, 1975). The decrease in paw volume observed in the second phase of the adjuvant-induced arthritis following whole body irradiation could be explained by the immunosuppressant effect of ionizing radiation. Radiation can affect the immune system at three main points of cell proliferation (Till & McCulloch, 1961; Patt & Quastler, 1963). These are the formation of stem cells in the bone marrow, the early diffrentiation of cells in the thymus and the proliferation of immunocompetent cells.

The phenomenon of immunosuppression by radiation has been made use of in medical practice for suppressing immune responses in organ and tissue transplantation as well as in the treatment of patients with severe rheumatoid arthritis (Kotzin *et al.*, 1981, Trenthan *et al.*, 1981). It has also been used in the treatment of adjuvant arthritis (Schurman *et al.*, 1981) and collagen arthritis (McCune *et al.*, 1982) in rats.

The successful effect of diclofenac in suppressing the inflammatory responses produced in both normal and irradiated animals might perhaps be attributed, at least in part, to its potent inhibitory effect on prostaglandin synthetase which would result in inhibition of the vasodilatation and exudation potentiating effects due to prostaglandins (Krupp *et al.*, 1973; Ku *et al.*, 1975); an effect common to all non-steroidal anti-inflammatory drugs (Vane, 1971). Menassé *et al.* (1978) also showed that diclofenac stabilizes lysosomal membranes and thus inhibits the release of lysosomal enzymes, which are probably responsible for the tissue destruction occurring in inflammatory rheumatic diseases. This effect of diclofenac may further contribute to its protective effect against the radiation-induced changes observed in the present study. More work is currently in progress to investigate the nature of the released mediators following radiation exposure and to show how these are affected by drugs.

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