

Modulation of Haemopoietic Radiation Response of Mice by Diclofenac in Fractionated Treatment

M. HOFER, M. POSPÍŠIL, I. PIPALOVÁ, J. HOLÁ

Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic

Received October 19, 1995

Accepted March 12, 1996

Summary

The effects of diclofenac, an inhibitor of prostaglandin synthesis, were studied on the acute radiation syndrome elicited in mice by fractional irradiation. Several haematological parameters were evaluated in mice irradiated with 5x2 Gy and 3x, 4x, or 5x3 Gy (intervals between fractions 24 h) from a ^{60}Co gamma-ray source. The animals were treated with diclofenac either before each fraction or only once before the last fraction. The survival of mice was recorded after the irradiation regimen of 5x3 Gy followed by a "top-up" dose of 3.5 Gy given 24 h after the last radiation fraction. Statistically significant enhancement of the endogenous spleen colony formation and of leukopoiesis was found in mice treated with diclofenac repeatedly, as compared with both saline-treated irradiated controls and animals administered a single diclofenac dose, if a sublethal total radiation dose had been accumulated. However, following accumulation of a lethal total radiation dose, slightly impaired survival was observed in mice given diclofenac. It follows from the results that diclofenac is a suitable drug for enhancing leukopoiesis impaired by sublethal fractionated irradiation. Nevertheless, undesirable side effects of this drug negatively influence the survival of experimental animals following a lethal accumulated radiation dose.

Key words

Fractionated irradiation – Radiation protection – Prostaglandin synthesis inhibition – Diclofenac – Haemopoiesis

Introduction

Inhibitors of prostaglandin synthesis, such as indomethacin and diclofenac, have been reported to enhance postirradiation recovery of haemopoiesis if given alone to mice in protective (Kozubík *et al.* 1989, Nishiguchi *et al.* 1990) or therapeutic regimens (Kozubík *et al.* 1987, Pospíšil *et al.* 1986, 1989, Serushago *et al.* 1987), or if administered in combination with sulphhydryl compounds (Kozubík *et al.* 1990, 1991) or immunomodulators (Pospíšil *et al.* 1992, Fedoročko *et al.* 1994). The haemopoiesis-enhancing effects of inhibitors of prostaglandin synthesis in sublethally irradiated animals are supposed to be due to the fact that prostaglandins, especially those of the E series, inhibit the proliferation of haemopoietic precursor cells and play a role in the negative feedback control of myeloid cell proliferation (Fontagné *et al.* 1980, Kurland and Moore 1977, Pelus 1989).

The above-mentioned papers dealing with pharmacological interventions with inhibitors of prostaglandin synthesis were aimed at assessing the suitability of the treatment in animals irradiated with a single dose. Recently, we have shown that one of the widely clinically used inhibitors of prostaglandin synthesis, a non-steroidal anti-inflammatory drug diclofenac (Menassé *et al.* 1978) enhanced haemopoiesis in repeatedly irradiated mice (6x2 Gy delivered during three weeks), especially in combination with a soluble glucan derivative, carboxymethylglucan (Hofer *et al.* 1993).

The purpose of the experiments reported here was to investigate in more detail the ability of diclofenac to influence the response of mice to fractionated radiation treatment consisting of three to five radiation fractions given in 24-h intervals. Haemopoietic recovery and the survival of mice were used as indices of the radiation damage.

Methods

Male (CBA x C57BL/10)F1 mice, 3 months old of 25 g mean body weight, were used. The mice were caged under controlled lighting conditions (light/dark 12:12) and at a constant temperature of 22±1 °C. Standardized pelleted diet and HCl-treated tap water (pH 2–3) were given *ad libitum*.

The mice were exposed to fractional total-body irradiation from a ⁶⁰Co gamma-ray source at a dose rate of 0.4 Gy/min. The individual fractional doses were of 2 or 3 Gy each; they were given three to five times, the intervals between fractions being 24 h. Five fractions of 3 Gy were not lethal; no deaths of mice were recorded within 30 days after the completion of irradiation. In order to judge the radiation damage in terms of lethality, the mice were exposed to 5x3 Gy and an additional "top-up" dose of 3.5 Gy delivered 24 hours after the last radiation fraction. During irradiation the mice were placed individually in chambers in a circular ventilated Plexiglass container.

Diclofenac (diclofenac sodium salt, Sigma) was dissolved in saline and administered i.p. in individual doses of 0.6 mg per mouse in a volume of 0.2 ml 2 h before each fractional irradiation or only before the fifth fraction. In the survival experiments, no pharmacological treatment preceded the additional "top-up" dose.

Material was sampled on days 4, 7 and 10 after the fifth fractional irradiation dose. Blood samples were drawn from a fine incision in the tail vein. For tissue collection, the mice were sacrificed by cervical dislocation.

The numbers of erythrocytes and leukocytes in the peripheral blood and the cellularity of the femoral

bone marrow were determined using a Coulter counter.

Smears of peripheral blood were prepared and stained by the May-Grünwald and Giemsa-Romanowski methods. Relative and calculated absolute numbers of granulocytes and lymphocytes were assessed.

Bone marrow haemopoietic progenitor cells committed to granulocyte-macrophage differentiation (granulocyte-macrophage colony-forming cells, GM-CFC) were assayed by a semisolid plasma clot technique (Vacek *et al.* 1990). Briefly, femoral bone marrow cell suspensions were plated in quadruplicate using 10 % mouse lung-conditioned medium as the source of colony-stimulating factors. Colonies (>50 cells) were counted after 7 days of incubation in a humidified environment at 37 °C containing 5 % CO₂.

Numbers of surviving haemopoietic stem cells (CFU-S) were determined by the technique of endogenous spleen colonies on day 10 after the fifth 3-Gy radiation fraction. Grossly visible nodules larger than 0.4 mm in diameter were counted. The protection factor derived from the CFU-S survival was calculated as the equal-effect dose ratio.

Survival of mice irradiated with five 3-Gy fractions and the "top-up" dose of 3.5 Gy was recorded daily up to day 30 after the completion of irradiation.

Results of the experiments are given as means ± S.E.M. The statistical significance of differences in the haematological parameters was evaluated by the t-test and Dunnett's tables for multiple comparisons with the control (Dunnett 1964), where appropriate. The differences in survival of mice were assessed by means of the logrank test (Peto *et al.* 1977). The level of significance was set at P<0.05.

Table 1
Haemopoietic effects of repeated diclofenac administration before each of five 2-Gy fractional irradiations: effects in bone marrow and spleen

Day ^a	n	Treatment	GM-CFC per femur	Femoral bone marrow cellularity x 10 ⁶	Spleen weight (mg)
4	10	Diclofenac	465±9**	7.83±0.55*	28.2±0.6
	10	Saline	366±22	5.69±0.31	27.1±0.9
7	10	Diclofenac	2708±280***	12.77±0.69*	53.6±5.3*
	10	Saline	601±25	9.54±0.42	34.9±2.1
10	10	Diclofenac	2854±112***	17.77±0.95	128.3±6.6***
	10	Saline	1675±64	16.66±0.81	52.0±3.5

^a – day after irradiation with the fifth fractional dose; n – number of animals; *, **, *** – significantly higher values in comparison with saline-treated irradiated animals, P<0.05, P<0.01, P<0.001, respectively.

Results

Haematological findings in mice irradiated with five 2-Gy radiation fractions applied in 24-h intervals, obtained on days 4, 7, and 10 after the last irradiation, are summarized in Tables 1 and 2. The exposure of the experimental mice to this radiation regimen produced a marked decrease of the haematological parameters studied. In saline-treated mice, on the fourth day after the last irradiation, the values of GM-CFC per femur, femoral bone marrow cellularity, spleen weight, and counts of granulocytes, lymphocytes and erythrocytes in the peripheral blood represented 2.9 ± 0.2 , 19.9 ± 1.1 , 34.9 ± 1.2 , 49.4 ± 5.2 , 9.9 ± 1.2 , and 60.4 ± 1.4 % of the corresponding values found in non-irradiated control mice. At later postirradiation intervals, the values of these indices

increased suggesting the onset of recovery. It follows from the results that all three haematological indices in mouse haemopoietic organs studied, i.e. numbers of GM-CFC in the femur, femoral bone marrow cellularity and spleen weight, are significantly enhanced in mice administered diclofenac before each radiation fraction in comparison with saline-treated irradiated controls in most time intervals studied (Table 1). As far as the peripheral blood is concerned, granulocyte counts were found to be significantly higher in mice treated repeatedly with diclofenac compared with the controls on days 7 and 10, whereas the lymphocyte counts were higher only on day 10 (Table 2). On the other hand, erythrocytes exhibited significantly lower values on days 4 and 7 in mice which had repeatedly received diclofenac, as compared with the controls (Table 2).

Table 2
Haemopoietic effects of repeated diclofenac administration before each of five 2-Gy fractional irradiations: effects in peripheral blood

Day ^a	n	Treatment	Granulocytes in 1 µl of peri- pheral blood	Lymphocytes in 1 µl of peri- pheral blood	Erythrocytes in 1 µl of peri- pheral blood x 10 ⁶
4	10	Diclofenac	643 ± 99	325 ± 43	5.39 ± 0.17 ⁺⁺
	10	Saline	571 ± 60	447 ± 55	6.46 ± 0.15
7	10	Diclofenac	1928 ± 258 ^{**}	1282 ± 285	4.81 ± 0.19 ⁺
	10	Saline	780 ± 73	1101 ± 108	5.69 ± 0.19
10	10	Diclofenac	2164 ± 466 [*]	1466 ± 252 [*]	6.82 ± 0.20
	10	Saline	803 ± 124	790 ± 112	6.72 ± 0.20

^a – day after irradiation with the fifth fractional dose; n – number of animals; *, ** – significantly higher values in comparison with saline-treated irradiated animals, $P < 0.05$, $P < 0.01$, respectively. +, ++ – significantly lower values in comparison with saline-treated irradiated animals, $P < 0.05$, $P < 0.01$, respectively.

In order to find out whether repeated diclofenac administration before each radiation fraction is necessary for obtaining the haemopoietic effects observed or whether only one diclofenac dose would suffice, two further experiments were performed using irradiation with 4x3 Gy. Mice were given either diclofenac before each radiation fraction or in a single dose before the last fraction; estimations were performed on day 10 after the last irradiation. Values of four representative haematological parameters, i.e. endogenous spleen colonies, GM-CFC per femur, spleen weight and leukocyte counts in the peripheral blood are shown in Figure 1. Though the single diclofenac dose led to a significant increase in all the haemopoietic parameters in comparison with saline-

treated control animals, the repeated administration of diclofenac was followed by further significant enhancement in three of the four parameters. Another series of experiments was aimed at evaluating the haemopoiesis in mice given three, four or five 3-Gy radiation fractions in 24-h intervals, on day 10 after the last irradiation. It should be mentioned that, following the irradiation with the total dose of 15 Gy (5x3 Gy), 30 % of the mice treated repeatedly with diclofenac died before the day of sampling, i.e. before day 10, though this total dose was found to be sublethal in non-treated mice. Figure 2 shows the radiation inactivation curves for endogenous haemopoietic spleen colonies in mice treated repeatedly with diclofenac or saline before each radiation fraction. The

survival curve for the diclofenac-treated mice was significantly displaced by about 5.4 Gy to the right of the curve for the controls. The slopes of the curves did not differ, however. The protection factor derived from CFU-S survival and induced by the repeated treatment with diclofenac was calculated to be 1.4 within the range of the radiation doses used. These results suggest that the protective action of diclofenac does not lose its effectiveness by repeated administration. If the single protective actions had gradually lost their efficacy, this could have been observed as an increase in the slope of the survival curve for CFU-S.

Values of peripheral blood haematological parameters on day 10 after the last of three, four, or five 3 Gy fractional radiation doses are shown in Table 3. Repeatedly administered diclofenac significantly elevated the numbers of both granulocytes and lymphocytes. Erythrocytes were found to be decreased in diclofenac-treated mice in comparison with saline-treated controls after all the three total radiation doses; however, the difference did not reach the 0.05 level of significance.

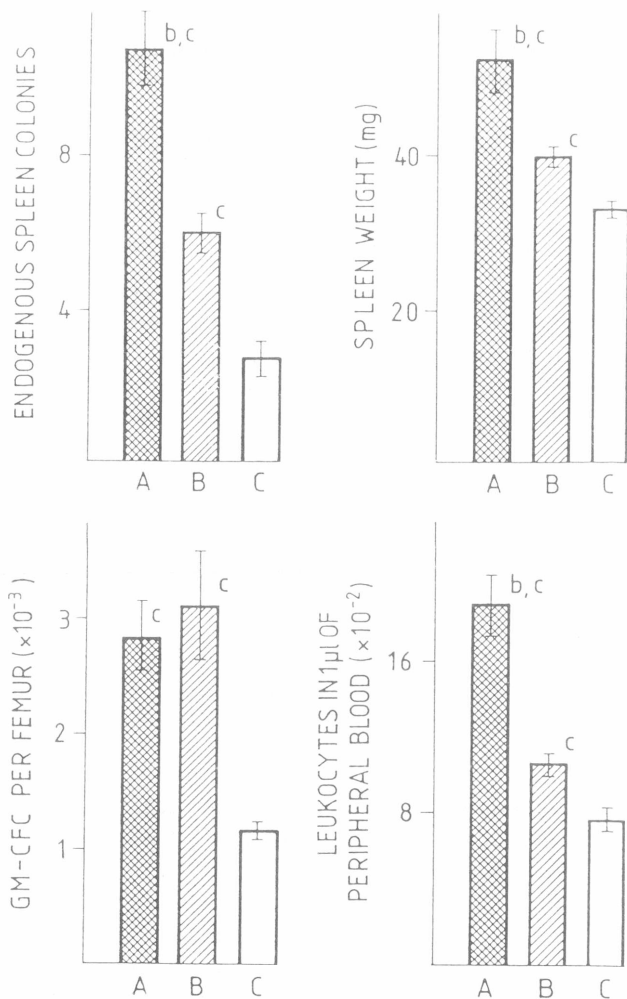


Fig. 1
Numbers of endogenous haemopoietic spleen colonies, numbers of femoral bone marrow GM-CFC, spleen weight, and numbers of leukocytes in peripheral blood on day 10 after completion of fractionated irradiation with 4 x 3 Gy in 24-h intervals. Fourteen mice per group were used. A – mice given diclofenac before each radiation fraction. B – mice given diclofenac before the last fraction and saline before the preceding three fractions. C – control mice given saline before each fraction. b, c – the values are significantly higher ($P < 0.05$) in comparison with those of the B or C group, respectively.

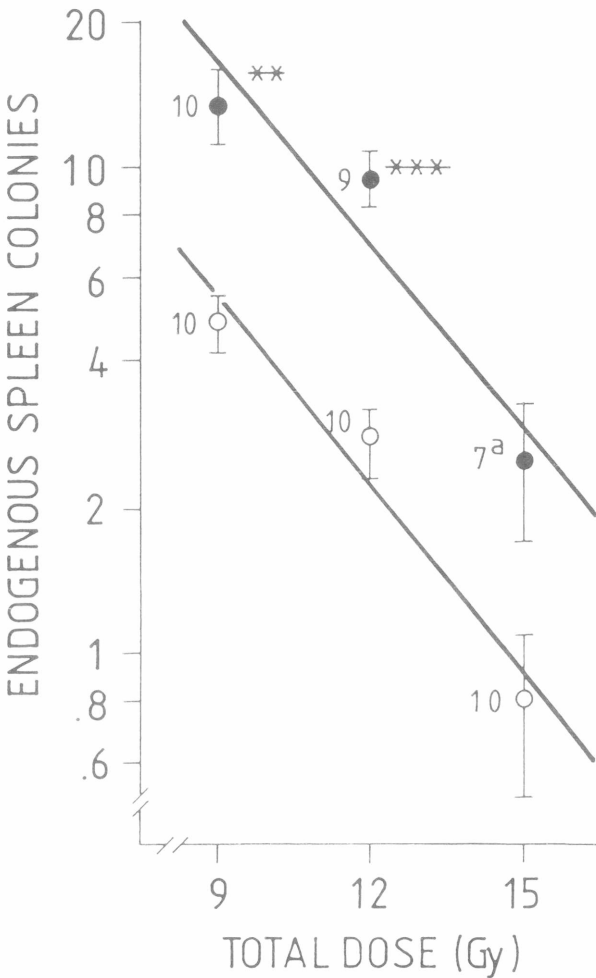


Fig. 2
Numbers of endogenous haemopoietic spleen colonies on day 10 after irradiation with the last of three, four, or five 3-Gy radiation fractions (interval between fractions 24 h). Closed circles – mice given diclofenac before each radiation fraction. Open circles – control mice given saline before each fraction. Numbers of animals in groups are given next to each circle. **, *** – the values are significantly higher in comparison with those of control mice ($P < 0.01$, $P < 0.001$, respectively). a – the group consisted originally of 10 mice; 3 of them died before the day of sampling.

Survival experiments were performed with the aim to assess the effects of repeated administration of diclofenac on the mortality of mice irradiated with five 3-Gy radiation fractions and the "top-up" dose of 3.5

Gy (Fig. 3). The mice which had been administered diclofenac before each of the five 3-Gy fractions exhibited slightly (insignificantly) impaired survival in comparison with the saline-treated controls.

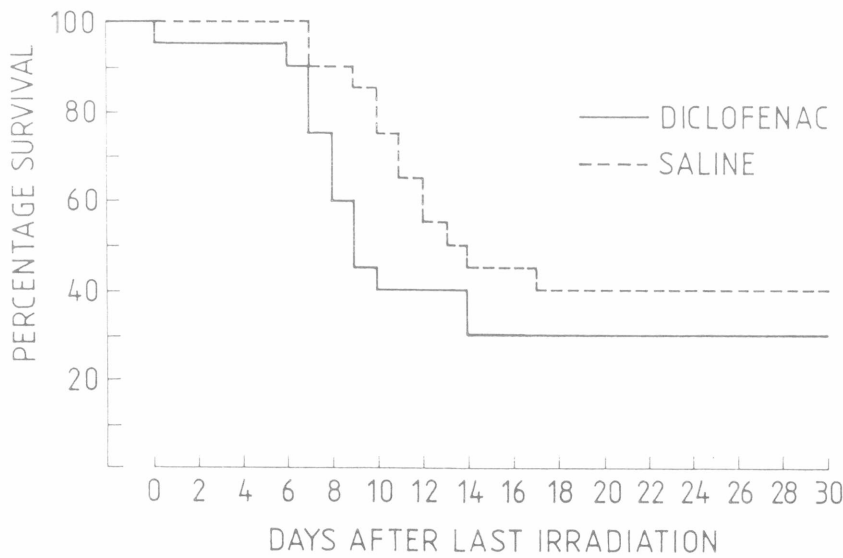


Fig. 3
Survival of mice irradiated with five 3-Gy radiation fractions and a "top-up" dose of 3.5 Gy 24 h after the last fraction. Mice were treated with diclofenac before each of the radiation fractions. Control irradiated mice were given saline in the respective time intervals. Each group consisted of 20 mice.

Table 3
Effects of repeated diclofenac administration before each of three, four or five 3-Gy fractional radiation doses on peripheral blood cells on day 10 after the last irradiation

Radiation dose	n	Treatment	Granulocytes in 1 µl of peripheral blood	Lymphocytes in 1 µl of peripheral blood	Erythrocytes in 1 µl of peripheral blood x 10 ⁶
3x3 Gy	10	Diclofenac	672±85***	566±85*	5.40±0.15
	10	Saline	291±21	371±29	5.61±0.13
4x3 Gy	9	Diclofenac	847±143**	557±69	4.94±0.23
	10	Saline	396±56	410±56	5.68±0.29
5x3 Gy	7 ^b	Diclofenac	685±97**	778±115**	3.66±0.70
	10	Saline	255±76	372±57	5.13±0.46

n – number of animals; *, **, *** – significantly higher values in comparison with saline-treated irradiated animals, $P<0.05$, $P<0.01$, $P<0.001$, respectively; ^b – the group consisted originally of 10 mice; 3 of them died before the day of sampling.

Discussion

The results of the present experiments have confirmed the previous findings of haemopoiesis-stimulating effects of inhibitors of prostaglandin synthesis in irradiated animals (see Introduction). Moreover, they suggest that these stimulatory effects also operate under conditions close to the standard fractionation schedules used in clinics. Concerning the mechanism of this protective action, inhibitors of

prostaglandin synthesis do not modify the radiosensitivity of haemopoietic cells, but enhance the proliferation of these cells in the early postirradiation interval and thus lead to earlier restoration of the counts of mature blood cells during the later postirradiation period (Kozubík *et al.* 1990, 1994). Thus, the effects of prostaglandin inhibitors cannot be considered as "radioprotection" in the traditional meaning, but merely as stimulation of postirradiation haemopoiesis. It was observed (Furuta *et al.* 1988) that

the administration of another inhibitor of prostaglandin synthesis, indomethacin, to irradiated tumour-bearing mice not only enhanced haemopoietic recovery, but also increased the tumour radioresponse. An augmented response of tumours to radiotherapy following the administration of cyclooxygenase inhibitors was also reported by others (Besa *et al.* 1993, Milas *et al.* 1990, Teicher *et al.* 1994). Especially in connection with these findings, our results showing enhanced recovery of haemopoietic stem cells and leukopoiesis by diclofenac in the 24-h fractionation radiation regimen may be of practical importance.

The parameters of peripheral blood presented in Table 3 did not show any dependence on the total radiation dose. The most reasonable explanation seems to be that the cells of the pertinent haemopoietic compartments respond to fractionated irradiation by sufficiently increasing their proliferative rate depending on the total dose thus overcoming the depletion caused by irradiation. An analogous phenomenon was observed by Chaffey and Hellman (1968) on endogenous spleen colony-forming cells.

Besides this favourable action, also undesirable side-effects of diclofenac treatment appear to participate in our experiments. Although the values of leukocytes in the peripheral blood were found to be enhanced in mice which had been given diclofenac repeatedly, erythrocytes were shown to be decreased at most samplings, often significantly. Since prostaglandin E₁ was reported to enhance mouse erythropoiesis *in vivo* (Hangoc *et al.* 1987), the inhibitory effect of diclofenac on prostaglandin production might explain this finding. The escape of erythrocytes through the damaged intestinal wall could also contribute to the decrease in blood erythrocytes. Nevertheless, we were not able to substantiate this phenomenon by means of radioactively labelled erythrocytes in our previous experiments with single lethal irradiation and postirradiation diclofenac administration (Hofer *et al.* 1992). Potential erythropoiesis-suppressing effects of inhibitors of prostaglandin synthesis would have to be taken into account if anaemia was supposed to be in the foreground of the clinical picture following radiation therapy.

Our finding of slightly impaired survival following diclofenac administration after accumulation of high total doses might be due to some undesirable side-effects of this drug on the gastrointestinal tract,

including ulceration with haemorrhages and perforation, and increased interenterocytic permeability (Auer *et al.* 1987, Rask-Madsen 1987, Bjarnason *et al.* 1991). Our earlier studies (Hofer *et al.* 1992) brought evidence of enteropathy manifested by the swelling of lamina propria, irregularities of the mucosal surface, and bending and dilation of the crypts, which were found in 10 Gy-irradiated mice and was further enhanced in animals treated after irradiation with indomethacin or diclofenac. These effects suggested inflammation and increased intestinal permeability which might allow luminal toxins and bacterial invasion of the mucosa (Bjarnason *et al.* 1991). According to Walker (1978), postirradiation escape of endotoxin from the gut and successive endotoxaemia are important pathogenetic mechanisms of the lethal radiation syndrome. Moreover, Pettipher and Wimberly (1994) have shown that inhibitors of prostaglandin synthesis enhance tumour necrosis factor production and mortality of mice induced by endotoxic shock. These negative effects of non-steroidal anti-inflammatory drugs counteract probably those enhancing haemopoietic recovery and, at higher accumulated radiation doses, might prevail and impair the survival of the animals. The group of potential protective and/or curative drugs which still enhance haemopoietic recovery at lethal radiation doses, and yet sensitize the intestinal tract to ionizing radiation, may include a wider spectrum of substances. This possibility follows from a recent report of Neta *et al.* (1994) describing experiments with IL-12. Contingent clinical utilization of such drugs is thus limited to sublethal radiation doses eliciting a "pure" bone marrow radiation syndrome. Newly appearing derivatives of common non-steroidal anti-inflammatory drugs with reduced toxicity could help in overcoming these limitations.

Acknowledgements

This work was supported by grant No. 604104 from the Grant Agency of the Academy of Sciences of the Czech Republic. The authors wish to thank Mrs. V. Reichmannová for her skillful technical assistance. Research was conducted according to the principles enunciated in the "Guide for the Care and Use of Laboratory Animals" issued by the Czech Society for Laboratory Animal Sciences.

References

- AUER I.O., HABSCHIED W., MILLER S., GERHARDS W., EILLES C.: Nichtsteroidale Antiphlogistica erhöhen die Darmpermeabilität. *Dtsch. Med. Wchschr.* 112: 1032–1037, 1987.
- BESA P.C., HUNTER N.R., MILAS L.: Improvement in radiotherapy for a murine sarcoma by indomethacin plus WR-2721. *Radiat. Res.* 135: 93–97, 1993.
- BJARNASON I., FEHILLY B., SMETHURST D., MENZIES I.S., LEVI A.S.: Importance of local versus systemic effects of non-steroidal anti-inflammatory drugs in increasing small intestinal permeability in man. *Gut* 32: 275–277, 1991.

- CHAFFEY J.T., HELLMAN S.: Studies on dose fractionation as measured by endogenous spleen colonies in the mouse. *Radiology* 90: 363–365, 1968.
- DUNNETT C.W.: New tables for multiple comparisons with a control. *Biometrics* 20: 482–491, 1964.
- FEDOROČKO P., MACKOVÁ O.N., BREŽÁNI P., KOPKA M.: Administration of the bacterial extract Broncho-Vaxom enhances radiation recovery and myelopoietic regeneration. *Immunopharmacology* 28: 163–170, 1994.
- FONTAGNÉ J., ADOLPHE M., SEMICHON M., LECHAT P.: Effects of *in vivo* treatment with indomethacin on mouse granulocyte-macrophage colony-forming cells in culture (CFUc). Possible role of prostaglandins. *Exp. Hematol.* 8: 1157–1164, 1980.
- FURUTA Y., HUNTER N., BARKLEY T., Jr., HALL E., MILAS L.: Increase in radioresponse of murine tumors by treatment with indomethacin. *Cancer Res.* 48: 3008–3013, 1988.
- HANGOC G., LU L., BROXMEYER H.E.: The comparative enhancing effects of prostaglandin E₁ on colony formation by erythroid progenitor cells from bone marrow of mice differing in the Fv-2 locus. *Leukem. Res.* 11: 501–507, 1987.
- HOFER M., POSPÍŠIL M., TKADLEČEK L., VIKLICKÁ Š., PIPALOVÁ I., HOLÁ J.: Low survival of mice following lethal gamma-irradiation after administration of inhibitors of prostaglandin synthesis. *Physiol. Res.* 41: 157–161, 1992.
- HOFER M., POSPÍŠIL M., VIKLICKÁ Š., VACEK A., PIPALOVÁ I., BARTONÍČKOVÁ A.: Hematopoietic recovery in repeatedly irradiated mice can be enhanced by a repeatedly administered combination of diclofenac and glucan. *J. Leukocyt. Biol.* 53: 185–189, 1993.
- KOZUBÍK A., POSPÍŠIL M., VIKLICKÁ Š., TKADLEČEK L., NETÍKOVÁ J.: Effect of indomethacin on proliferative and differentiation activity in the haemopoietic system of lethally irradiated mice after syngeneic bone marrow transplantation. *Fol. Biol. (Prague)* 33: 418–423, 1987.
- KOZUBÍK A., POSPÍŠIL M., NETÍKOVÁ J.: The stimulatory effect of single-dose pre-irradiation administration of indomethacin and diclofenac on hemopoietic recovery in the spleen of gamma-irradiated mice. *Stud. Biophys.* 131: 93–101, 1989.
- KOZUBÍK A., POSPÍŠIL M., NETÍKOVÁ J.: Enhancement of haemopoietic recovery in sublethally gamma-irradiated mice by the joint use of indomethacin and cystamine. *Fol. Biol. (Prague)* 36: 291–300, 1990.
- KOZUBÍK A., POSPÍŠIL M., NETÍKOVÁ J.: Possibilities of the combined use of non-steroidal anti-inflammatory drugs and sulfhydryl compounds in radioprotection. *Strahlenther. Onkol.* 167: 186–190, 1991.
- KOZUBÍK A., HOFMANOVÁ J., POSPÍŠIL M., NETÍKOVÁ J., HOLÁ J., LOJEK A.: Effects of drugs inhibiting prostaglandin or leukotriene biosynthesis on postirradiation haematopoiesis in mouse. *Int. J. Radiat. Biol.* 65: 369–377, 1994.
- KURLAND J., MOORE M.A.S.: Modulation of hemopoiesis by prostaglandins. *Exp. Hematol.* 5: 357–373, 1977.
- MENASSÉ R., HEDWALL P.R., KRAETZ J., PERICIN C., RIESTERER L., SALLMAN A., ZIEL R., JACQUES R.: Pharmacological properties of diclofenac sodium and its metabolites. *Scand. J. Rheumatol. Suppl.* 22: 5–16, 1978.
- MILAS L., FURUTA Y., HUNTER N., NISHIGUCHI I., RUNKEL S.: Dependence of indomethacin-induced potentiation of murine tumor radioresponse on tumor host immunocompetence. *Cancer Res.* 50: 4473–4477, 1990.
- NETA R., STIEFEL S.M., FINKELMAN F., HERMANN S., ALI N.: IL-12 protects bone marrow from and sensitizes intestinal tract to ionizing radiation. *J. Immunol.* 153: 4230–4237, 1994.
- NISHIGUCHI I., FURUTA Y., HUNTER N., MURRAY D., MILAS L.: Radioprotection of hematopoietic tissues in mice by indomethacin. *Radiat. Res.* 122: 188–192, 1990.
- PELUS L.M.: Modulation of myelopoiesis by prostaglandin E₂: demonstration of a novel mechanism of action *in vivo*. *Immunol. Res.* 8: 176–184, 1989.
- PETO R., PIKE M.C., ARMITAGE P., BRESLOW N.E., COX D.R., HOWARD S.V., MANTEL N., MCPHERSON K., PETO J., SMITH P.G.: Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II. Analysis and examples. *Br. J. Cancer* 35: 1–39, 1977.
- PETTIPHER E.R., WIMBERLY D.J.: Cyclooxygenase inhibitors enhance tumour necrosis factor production and mortality in murine endotoxic shock. *Cytokine* 6: 500–503, 1994.
- POSPÍŠIL M., NETÍKOVÁ J., KOZUBÍK A.: Enhancement of hemopoietic recovery by indomethacin after sublethal whole-body gamma irradiation. *Acta Radiol. Oncol.* 25: 195–198, 1986.
- POSPÍŠIL M., NETÍKOVÁ J., KOZUBÍK A., PIPALOVÁ I.: Effect of indomethacin, diclofenac sodium and sodium salicylate on peripheral blood cell counts in sublethally gamma-irradiated mice. *Strahlenther. Onkol.* 165: 627–631, 1989.

- POSPÍŠIL M., HOFER M., PIPALOVÁ I., VIKLICKÁ Š., NETÍKOVÁ J., ŠANDULA J.: Enhancement of hematopoietic recovery in gamma-irradiated mice by the joint use of diclofenac, an inhibitor of prostaglandin production, and glucan, a macrophage activator. *Exp. Hematol.* 20: 891–895, 1992.
- RASK-MADSEN J.: The role of eicosanoids in the gastrointestinal tract. *Scand. J. Gastroenterol.* 22: 1–4, 1987.
- SERUSHAGO B.A., TANAKA K., KOGA Y., TANIGUCHI K., NOMOTO K.: Positive effect of indomethacin and interleukin-2 on murine hemopoietic stem cells. *Immunopharmacology* 14: 21–26, 1987.
- TEICHER B.A., KORBUT T.T., MENON K., HOLDEN S.A., ARA G.: Cyclooxygenase and lipoxygenase inhibitors as modulators of cancer therapies. *Cancer Chemother. Pharmacol.* 33: 515–522, 1994.
- VACEK A., ROTKOVSKÁ D., BARTONÍČKOVÁ A.: Radioprotection of hemopoiesis conferred by aqueous extract from chlorococcal algae (Ivastimul) administered to mice before irradiation. *Exp. Hematol.* 18: 234–237, 1990.
- WALKER R.I.: The contribution of intestinal endotoxin to mortality in host with compromised resistance. *Exp. Hematol.* 6: 172–184, 1978.

Reprint Requests

Dr. M. Hofer, Institute of Biophysics, Academy of Sciences of the Czech Republic, Královopolská 135, 612 65 Brno, Czech Republic.