Physiol. Res. 41: 157-161, 1992

Low Survival of Mice Following Lethal Gamma – Irradiation after Administration of Inhibitors of Prostaglandin Synthesis

M. HOFER, M. POSPÍŠIL, L. TKADLEČEK, Š. VIKLICKÁ, I. PIPALOVÁ, J. HOLÁ

Institute of Biophysics, Czechoslovak Academy of Sciences, Brno

Received January 24, 1992
Accepted February 26, 1992

Summary

An impairment of the survival of mice subjected to whole-body gamma-irradiation with a lethal dose of 10 Gy and treated with a repeated postirradiation administration of prostaglandin synthesis inhibitors (PGSIs), indomethacin or diclofenac, was observed. Morphological examination of the gastrointestinal tract and the estimation of blood loss into its lumen in animals treated with diclofenac did not show serious damage such as haemorrhages or perforation, but revealed structural injury to the intestinal mucosa indicating inflammatory processes. The lesions found are supposed to be connected with increased intestinal permeability which leads to endotoxin escape from the gut and a subsequent increased mortality rate of irradiated animals. It may be concluded that PGSIs are not suitable for the management of radiation sickness after an exposure to lethal doses of ionizing radiation.

Key words

Gamma-irradiation – Indomethacin – Diclofenac – Gastrointestinal toxicity

Introduction

Prostaglandins, especially those of the E group, were shown to inhibit the proliferation of haemopoietic progenitor cells and to reduce the cellularity of haemopoietic organs (Gentile *et al.* 1983). On the other hand, the administration of the prostaglandin synthesis inhibitor (PGSI), indomethacin, to mice led to an increase in bone marrow granulocyte-macrophage progenitor cells and splenic erythropoiesis (Boorman *et al.* 1982). The conclusions from these experiments were utilized for the treatment of postirradiation haemopoietic failure by PGSIs. Favourable effects, manifested in the enhancement of postirradiation haemopoietic recovery, were observed in sublethally irradiated mice not only after a preirradiation application of PGSIs (Furuta *et al.* 1988, Kozubík *et al.* 1989, Nishigushi *et al.* 1990), but also when PGSIs were given after irradiation (Pospíšil *et al.* 1986, Serushago *et al.* 1987, Pospíšil et al. 1989), and when their administration followed lethal irradiation and syngeneic bone marrow transplantation (Kozubík *et al.* 1987). Some stimulation of haemopoietic recovery by indometacin was also found in continuously irradiated rats (Mišúrová *et al.* 1989).

As haemopoietic dysfunction is closely linked to the lethal consequences of radiation damage, experiments were performed to modify the survival of lethally irradiated mice by using postirradiation PGSI administration. A whole-body dose of 10 Gy was used which is considered to be the upper limit of doses leading to death caused by damage of the haemopoietic system (Yarmonenko *et al.* 1988). Due to the possibility that also gastrointestinal radiation damage could contribute to the overall lethal response of the organism, examination of the gastrointestinal tract was included into the experimental protocol.

Material and Methods

Experimental animals. Conventional male $(CBAxC57BL/10)F_1$ mice, aged 3-4 months and weighing 27-32 g at the start of the experiment were used. The animals were kept in cages containing 20 individuals. Controlled lighting conditions (LD 12:12 h) and a temperature of 22 ± 1 °C were maintained throughout the experiment. Pelleted sterilized diet (DOS 2ST – Velaz) and HCl-treated tap water (pH 2-3) were given *ad libitum*. Control and experimental procedures were carried out concurrently in groups of mice from the same cage.

Irradiation. The mice were singly, whole-body irradiated with ^{60}Co gamma-rays (Chisostat, Chirana). The dose rate was 0.5 Gy/min. The mice were placed individually in chambers in a slowly rotating circular perspex container during the irradiation.

Table 1

Arrangement and results of experiments aimed at evaluating the survival of lethally irradiated (10 Gy) mice treated with indomethacin (IND) or diclofenac (DIC)

Group		n[a]	Dose (mg), route of administration (time after irradiation in hours)	Statistical significance [b]	LT50 (days) /95 % conf. limits/
I	IND	10	0.1, s.c. (2,24,48)	P<0.01	10.0 /8.5-11.8/
	Control	30	-	-	12.6 /11.5-13.7/
II	IND	10	0.02, s.c. (24,48,72)	NS[c]	12.1 /11.3-13.0/
	IND	10	0.06, s.c. (24,48,72)	NS	10.5 /9.5-11.7/
	IND	10	0.1, s.c. (24,48,72)	P<0.01	9.8 /8.8-10.9/
	Control	30	-	-	15.6 /14.6-16.8/
III	DIC	20	0.15, s.c. (24,48,72)	P<0.01	9.7 /9.2-10.2/
	Control	30	-	-	10.9 /10.4-11.4/
IV	DIC	40	0.15, s.c. (2,24)	P<0.05	9.1 /8.7-9.5/
	DIC	30	0.15, s.c. (48,72)	P<0.01	8.6 /8.0-9.2/
	Control	69	-	-	9.6 /9.3-10.0/
V	DIC	20	0.15, p.o. (2,24,48)	P<0.01	8.6 /7.6-9.6/
	Control	20	-	-	10.7 /10.2-11.1/
VI	DIC	30	0.15, p.o. (24,48,72)	P<0.01	8.2 /7.9-8.6/
	Control	29	-	-	9.4 /8.9-10.0/

[a] - number of animals; [b] - comparison to control (distribution test); [c] - not significant

PGSIs. Indomethacin (Léčiva) was dissolved in 95 % ethanol (10 mg per 1 ml), diluted with an isotonic phosphate buffer (pH 7.4), and injected s.c. in doses of 0.67, 2.00, or 3.33 mg/kg (0.02, 0.06, or 0.10 mg per mouse) in a volume of 0.2 ml. Control mice received a phosphate buffer solution with the corresponding ethanol concentration s.c. Diclofenac sodium salt ("Voltaren inj.", Pliva, Lic. Ciba-Geigy) was diluted with isotonic phosphate buffer and injected s.c. in a dose of 5.00 mg/kg (0.15 mg per mouse) in a volume of 0.2 ml. In some experiments, peroral diclofenac administration was used. In this case, diclofenac was given by a probe on the base of the tongue in a dose of 0.15 mg per mouse in 0.3 ml of 1 % starch gel. Control animals received either the phosphate buffer s.c. or starch gel p.o. In mice, the 14 day LD 50 estimate for a single dose (p.o.) of indomethacin was reported to be 66 mg/kg, that of diclofenac 109 mg/kg (Maier 1984). The highest single doses of indomethacin or diclofenac used in our experiments thus represent about one twentieth of their acute LD 50 and are comparable with respect to toxicity.

Survival. Deaths of mice were recorded in daily intervals after irradiation together with determination of blood loss into the gastrointestinal tract. Normal mice received an i.p injection of ^{59}Fe citrate in a dose of 1.85×10^5 Bq per mouse. After 72 hours, heparinized blood was collected in ether narcosis from the orbital sinus. Erythrocytic mass was obtained by means of centrifugation and repeated rinsing and injected i.v. into experimental animals in a volume of 0.2 ml per mouse. After another 2 hours, the experimental animals were sacrificed by cervical dislocation and their stomachs, duodena, and colons were removed. The radioactivity of the organs (including the content) was ascertained by using a Nuclear Chicago Automatic Well Gamma Counting System apparatus. Then, the organs were repeatedly rinsed until complete cleaning from the content was

achieved and their radioactivity was measured again. The difference between the activities found equalled the radioactivity of the organ content. The number of erythrocytes in 1 μl peripheral blood was also determined in the frame of this experiment by means of a Coulter Counter.

Morphology of the gastrointestinal tract. For the light microscopy examination, the organs (stomach, duodenum, and colon) were fixed withs 3.3 mol/l formaldehyde containing Ca^{2+} ions (pH 7). After dehydration, the organs were embedded in paraffin. The sections were stained with haematoxylin-eosin. For electron microscopy, 1 mm thick sections of the organs studied were fixed by a mixture of 0.2 mol/l glutaraldehyde, 1.33 mol/l formaldehyde, $8.7x10^{-4}$ mol/l picric acid, and $1.5x10^{-3}$ mol/l calcium chloride in 0.1 mol/l cacodylate buffer, postfixed with $7.9x10^{-2}$ mol/l OsO_4 in 0.1 mol/l cacodylate buffer and $1.2x10^{-2}$ mol/l uranyl acetate in distilled H_2O, rinsed in 0.1 mol/l ammonium acetate, dehydrated with an ascending acetone series, and embedded in Durcupan ACM (Fluka). Suitable parts of semithin sections stained with methylene blue and basic fuchsin were chosen. Ultrathin sections were prepared on an Ultrotome 3 (LKB) ultramicrotome, stained with uranyl acetate and lead citrate, and studied in an Opton EM 109 electron microscope.

Statistics. The statistical significance of the differences between arithmetic means was determined using the t-test. The significance of the differences in the survival of experimental mice was determined by means of the distribution test. In addition, the lethal time 50 % (LT50) including 95 % confidence limits was assessed (Roth *et al.* 1962).

Results

The arrangement and results of six experiments aimed at evaluating the survival of animals following a lethal dose of 10 Gy are compiled in Tab. 1. The findings indicate an impairment of survival in groups of animals receiving eihter of the two studied PGSIs, i.e., indomethacin and diclofenac after irradiation. The efficiency of PGSIs to shorten the survival of irradiated mice was manifested irrespective of whether 2 or 3 doses were administered, or of the time of the first postirradiation administration (2 or 24 hours). In the case of diclofenac, the effects were manifested irrespective of its subcutaneous or peroral route of administration, i.e., systemic or local action. An example of the death rate of mice treated with diclofenac p.o. (see Experiment V in Tab. 1) is shown in Fig. 1.

The blood loss into the gastrointestinal tract determined on day 7 after irradiation with the dose of 10 Gy (Tab. 2) was not found to be statistically different between the control group of mice and mice treated with three doses of 0.15 mg diclofenac p.o. 2, 24, and 48 hours after the irradiation. The blood content in the colon of diclofenac-treated animals is even lower (though insignificantly) as compared to the controls. The erythrocyte counts signify an equally pronounced degree of postirradiation anaemia in both groups of mice (erythrocyte concentration in the blood of nonirradiated control animals being $10.2 \pm 0.2x10^6$ per μl).

The macroscopic, light microscopic, and electron microscopic examination of the gastrointestinal tract (stomach, duodenum, and colon) performed in mice treated according to the same experimental protocol (day 6 after 10 Gy-irradiation) led to the following findings: The combination of irradiation and diclofenac administration (dose of 0.15 mg given p.o. 2, 24 and 48 hours after the irradiation) caused local swelling of lamina propria in the apical parts of the duodenal mucosa villi. When the above

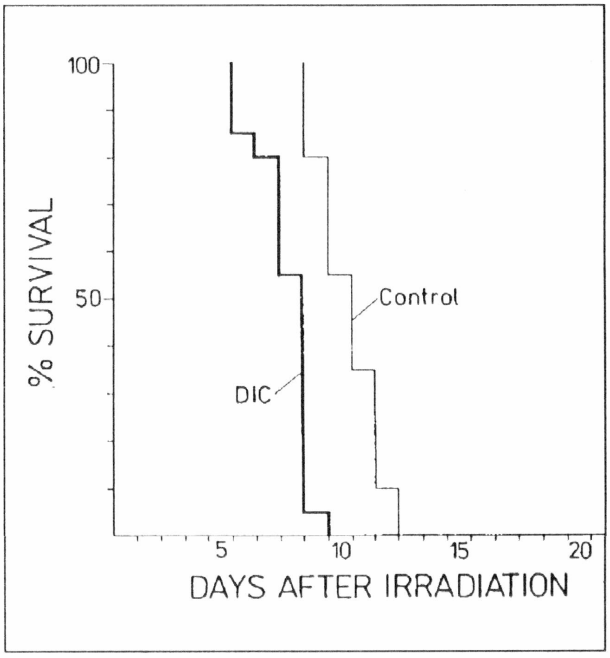

Fig. 1
Survival of lethally (10 Gy) irradiated mice. DIC - mice treated with 0.15 mg diclofenac p.o. 2, 24, and 48 hours after the irradiation. Control - mice only irradiated.

mentioned combination was used, the most marked pathological findings in the colon mucosa were observed; these consisted of irregularities of the mucosa surface and of bending and cystic dilation of the crypts. As an example of the morphological findings in the gastrointestinal tract, the differences in colon morphology between control nonirradiated, irradiated, and irradiated and diclofenac-treated mice are shown in Figs. 2, 3 and 4. No ulcerations and

Table 2

Blood loss into the gastrointestinal tract (radioactivity of the content in cpm) and numbers of erythrocytes in 1 μl of peripheral blood determined on day 7 after the irradiation (10 Gy) of control mice and mice treated with diclofenac (DIC) p.o. (0.15 mg 2,24, and 48 hours postirradiation)

	Content radioactivity (cpm)			Erythrocytes (x 10^6)
	stomach	duodenum + jejunum + ileum	colon	
DIC	452 ± 84	1569 ± 220	1276 ± 414	7.23 ± 0.34
Control	644 ± 131	1328 ± 455	2563 ± 735	7.61 ± 0.21

8-9 animals per group were used; values are means ± S.E.M.; differences between the control and the treated groups are not statistically significant.

bleeding symptoms in the gastric or intestinal mucosa were found in any of the groups studied.

Discussion

The results presented show an impaired survival of mice irradiated with a lethal dose of 10 Gy and treated postirradiation with indomethacin or diclofenac. As follows from Tab. 1, different results concerning the LT50 values of mice of the control groups in the individual experiments were obtained in spite of using the same strain and sex of mice and the same radiation dose. As the experiments were performed throughout the whole year period, the reasons of this variability may be seasonal or be due to other uncontrollable (such as the "cage effect") conditions of the experiments known to participate in the manifestations of individual radiosensitivity (Pospíšil and Vácha 1983). The doses of PGSIs used were below doses provoking an acute toxicity (see Material and Methods). The three-day treatment of normal (nonirradiated) mice with indomethacin (3 times 0.1 mg s.c.) or diclofenac (3 times 0.15 mg s.c.) did not induce death or body weight decrease during 14 days of observation (data not shown).

The fact that both indomethacin and diclofenac, i.e., structurally unrelated non-steroidal anti-inflammatory drugs, induce similar effects in terms of an increase in the death rate of lethally irradiated mice suggests the involvement of an inhibition of prostaglandin synthesis which is the common mechanism of action of both drugs (Menassé *et al.* 1978). The decrease in prostaglandin production is not only the basis of the anti-inflammatory action of these drugs, but also of their side effects which are induced by the loss of prostaglandin-mediated cytoprotection in the gastrointestinal tissues. The lessions of the gastrointestinal mucosa belong to the most important side effects of PGSIs. They include not only ulceration with haemorrhages and perforation, but also increased interenterocytic permeability (Auer *et al.* 1987, Bjarnason *et al.* 1991, Rask-Madsen 1987). Our experiments did not reveal serious side effects such as haemorrhage and perforation, but brought evidence of enteropathy, manifested by the swelling of lamina propria, irregularities of the mucosal surface, and bending and cystic dilation of the crypts. These effects could suggest inflammation and increased intestinal permeability which might allow luminal toxins and bacterial invasion of the mucosa (Bjarnason *et al.* 1991). According to Walker (1978), a postirradiation escape of endotoxin from the gut and successive endotoxaemia are important pathogenic mechanisms of the lethal radiation syndrome. Walker (1978) states that endotoxins are especially toxic to compromised hosts because essential components of their peripheral clearance system are missing (i.e., granulocytopenia in irradiated animals). In these conditions, the unbalanced host response may cause the damage associated with endotoxin toxicity to many host tissues. Enhancement of the leakage of endotoxin from the intestine may account for the impaired survival of lethally irradiated mice treated with PGSIs.

The results reported here enable us to conclude that PGSIs, though effective in the treatment of haemopoietic postirradiation syndrome after exposure to sublethal radiation doses (see

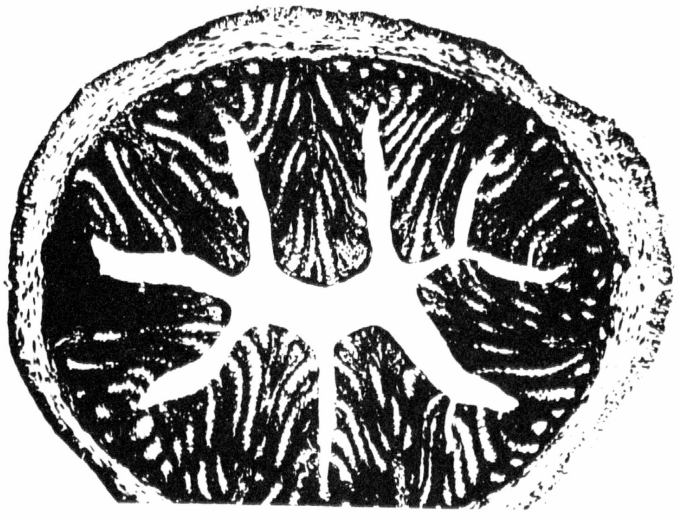

Fig. 2
Transversal colon section of a control, unirradiated mouse. The mucosa surface is smooth, the crypts are not dilated. (x 66)

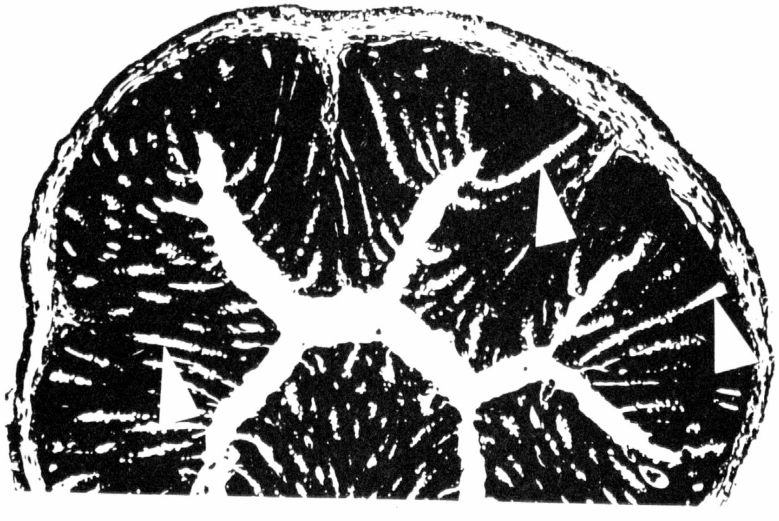

Fig. 3
Transversal colon section on day 6 after the irradiation with the dose of 10 Gy. The mucosa surface is irregularly dented, some crypts are dilated (arrows). (x 66)

Fig. 4
Transversal colon section on day 6 after 10 Gy irradiation followed by three doses of 0.15 mg diclofenac p.o. 2, 24, and 48 hours postirradiation. The mucosa surface is roughly dented, most of the crypts are dilated. (x 66)

Introduction), are not suitable for the management of the radiation sickness induced by higher lethal doses. In the conditions of serious radiation damage, i.e. at the level of highly depleted stem cell populations, the haemopoiesis stimulating effects of PGSIs lose their efficacy and the side effects of PGSIs, operating through the mechanism of intestinal damage, prevail.

References

AUER I.O., HABSCHIED W., MILLER S., GERHARDS W., EILLES C.: Nicht-steroidale Antiphlogistika erhöhen die Darmpermeabilität. *Dtsch. med. Wschr.* **112**: 1032-1037, 1987.

BJARNASON I., FEHILLY B., SMETHURST P., 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.

BOORMAN G.A., LUSTER M.I., DEAN J.H., LUEBKE R.W.: Effect of indomethacin on the bone marrow and immune system of the mouse. *J. Lab. Clin. Immunol.* **7**: 119-126, 1982

FURUTA Y., HUNTER N., BARKLEY T., HALL E., MILAS L.: Increase in radioresponse of murine tumors by treatment with indomethacin. *Cancer Res:* 3008-3013, 1988.

GENTILE P., BYER D., PELUS L.M.: In vivo modulation of murine myelopoiesis following intravenous administration of prostaglandin E_2. *Blood* **62**: 1100-1107, 1983.

KOZUBÍK A., POSPÍŠIL M., NETÍKOVÁ J.: The stimulatory effect of single-dose pre-irradiation administration of indomethacin and diclofenac on haemopoietic recovery in the spleen of gamma-irradiated mice. *Studia biophys.* (Berlin) **131**: 93-101, 1989.

KOZUBÍK A., POSPÍŠIL M., VIKLICKÁ Š., TKADLEČEK L., NETÍKOVÁ J.: Effect of indomethacin on the proliferation and differentiation activity in the haemopoietic system of lethally irradiated mice after syngeneic bone marrow transplantation. *Folia biol.* (Praha) **33**: 418-423, 1987.

KURLAND J.I., BROXMEYER H.E., PELLUS L.M., BOCKMAN R.S., MOORE M.A.S.: Role for monocyte-macrophage-derived colony-stimulating factor and prostaglandin E in the positive and negative feedback control of myeloid stem cell proliferation. *Blood* **52**: 388-407, 1978.

MAIER R.: Preclinical findings with pirprofen. In: Pirprofen (RRengasil) - an Update, V.A. NASSONOVA (ed). H. Huber Publ., Bern, 1984, pp. 13-20.

MENASSÉ R., HEDWALL P.R., KRAETZ J., PERICIN C., RIESTERER L., SALLMAN A., ZIEL R., JAQUES R.: Pharmacological properties of diclofenac sodium and its metabolites. *Scand. J. Rheumatol., Suppl.* b22: 5-16, 1978.

MIŠÚROVÁ E., KROPÁČOVÁ K., CHLEBOVSKÝ O., PADO D.: The effect of indomethacin on nucleic acids in blood, hemopoietic and lymphoid tissues in continuously irradiated rats. *Neoplasma* **36**: 541-547, 1989.

NISHIGUSHI I., FURUTA Y., HUNTER N., MURRAY D., MILAS L.: Radioprotection of haematopoietic tissues in mice by indomethacin. *Radiat. Res.* **122**: 188-192, 1990.

POSPÍŠIL M., NETÍKOVÁ J., KOZUBÍK A.: Enhancement of haemopoietic 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., VÁCHA J.: Individual Radiosensitivity. Academia, Praha, 1983.

RASK-MADSEN J.: The role of eicosanoids in the gastrointestinal tract. *Scand. J. Gastroenterol.* **22**: 1-4, 1987.

ROTH Z., JOZÍFKO M., MAL V., TRČKA V.: Statistical Methods in Experimental Medicine (In Czech). St. zdrav. nakl., Praha, 1962.

SERUSHAGO B.A., TANAKA K., KOGA Y., TANIGUCHI K., NOMOTO K.: Positive effects of indomethacin on restoration of splenic nucleated cell population in mice given sublethal irradiation. *Immunopharmacology* **14**: 21-26, 1987.

WALKER R.I.: The contribution of intestinal endotoxin to mortality in host with compromised resistance: A review. *Exp. Hematol.* **6**: 172-184, 1978.

YARMONENKO S.P.: Radiobiology of Humans and Animals. Mir Publishers, Moscow, 1988.

Reprints Requests

Dr. M. Hofer, Institute of Biophysics, Czechoslovak Academy of Sciences, CS-612 65 Brno, Czechoslovakia