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Hematopoiesis-Stimulating and Anti-Tumor Effects of Repeated Administration of Diclofenac in Mice with Transplanted Fibrosarcoma Cells

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Summary

Positive effects of repeated administration of diclofenac, an inhibitor of prostaglandin synthesis, in terms of prevention of tumor development and stimulation of hematopoiesis have been observed in C3H mice transplanted subcutaneously with G:5:113 fibrosarcoma cells. Fourteen-day treatment with diclofenac (3.75 μg/kg/day) started from day 5 after tumor cell transplantation. Measurements of tumors and hematological examinations were performed on day 30. The results strongly suggest the possibility that inhibitors of prostaglandin synthesis (non-steroidal anti-inflammatory drugs) may be used in oncological practice where the observed effects are highly desirable.

Key words

Diclofenac • Prostaglandins • Hematopoiesis • Fibrosarcoma

Introduction

A number of articles have been published, both by our group (e.g. Pospíšil *et al.* 1986, Kozubík *et al.* 1989, Hofer *et al.* 1996) and by others (Serushago *et al.* 1987, Nishiguchi *et al.* 1990) on the hematopoiesis-stimulating effects of various non-steroidal anti-inflammatory drugs (NSAIDs), acting on the principle of inhibition of prostaglandin (PG) production. The drugs have been shown to be effective in mice with radiation-suppressed hematopoiesis when given in both protective and therapeutic regimens.

Various NSAIDs have been shown to suppress the growth of solid tumors in experiments on animals (e.g. Pollard and Luckert 1981, Suzuki *et al.* 1994), as well as in clinical practice (Klein *et al.* 1987, Rosemberg *et al.* 1991). We have documented recently that diclofenac, one of the most widely used NSAIDs, evokes distinct anti-tumor action on the growth of tumors arising from subcutaneously (s.c.) transplanted G:5:113 fibrosarcoma cells (Hoferová *et al.* 2002). This finding may be of significance because fibrosarcoma belongs to tumors that are often resistant to non-surgical therapy.

The aim of the present experiment was to ascertain whether diclofenac exerts its hematopoiesis-stimulating effects in mice with transplanted fibrosarcoma cells and, if so, whether it is possible to use both the anti-tumor and the hematopoiesis-stimulating

actions of this drug in the pharmacological treatment of the fibrosarcoma. The pharmacological treatment regimen was based on our previous results (Hoferová *et al.* 2002). The administered daily doses of 0.15 mg/mouse are equivalent to 6 mg/kg in our 25 g mice, i.e. roughly comparable (especially if probable differences in metabolic rate are taken into account) with the routinely used daily doses of 1 to 2.5 mg/kg in human clinical practice.

Methods

Animals

Male C3H/DiSn mice, 12-15 weeks old, weighing 25 g on the average, were obtained from VELAZ Ltd., Czech Republic. The 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 Science.

Tumor cell line

The N-methyl-N'-nitro-N-nitrosoguanidine-induced G:5:113 fibrosarcoma cell line was kindly provided by Dr. Margaret Kripke (University of Texas, M. D. Anderson Cancer Center, Houston, TX, USA) (Donawho and Kripke 1991). The cells were maintained in RPMI-1640 culture medium (Sebak, Germany) supplemented with 10 % heat-inactivated fetal calf serum (PAN Systems, Germany) in 75-cm^2 culture flasks (Nunc, Denmark), at 37 ^oC/5 % CO_2 atmosphere and were used for transplantation during the exponential growth phase.

Transplantation of tumor cells

The cells were harvested by trypsinization (0.25 % Trypsin/2 % EDTA, Sigma, USA), washed twice with serum-free medium. Animals were anesthetized intraperitoneally (i.p.) with Narcamon/Rometar solution (5 % Narcamon/2 % Rometar in the ratio of 2.63:1, Spofa, Czech Republic) and s.c. received an injection into the flank of 10^5 viable tumor cells per mouse in a volume of 0.125 ml.

Pharmacological treatment regimen

Diclofenac (ASW Alfa Wassermann, S.p.A., Italy) was dissolved in saline and i.p. injected in repeatedly administered individual doses of 0.15 mg/mouse given in volumes of 0.2 ml/dose in a 14-day regimen starting on day 5 after tumor cell transplantation. On day 30 after tumor cell transplantation, the tumor size was measured three-dimensionally using a calliper

Hematological methods

Numbers of leukocytes and erythrocytes per 1 μl peripheral blood, as well as the cellularity of femoral bone marrow and the spleen were determined using Coulter Counter (model ZF, Coulter Electronics, UK). The numbers of granulocytes and agranulocytes per 1 μl peripheral blood were assessed using blood smears and the number of hematopoietic progenitor cells for granulocytes and macrophages (GM-CFC) per femur and per spleen were determined by an *in vitro* technique according to Vacek *et al.* (1990) using a 10 % lung conditioned medium as a source of colony-stimulating activity. Progenitor cells for erythrocytes (BFU-E) were counted by the methylcelulose-based *in vitro* technique using erythropoietin and 5 % conditioned medium from the mouse IL-3-producing myeloma cell line (Karasuyma and Melchers 1988) as a source of colony-stimulating activity.

Statistics

Student's t-test that was preceded by the F-test was used for evaluating statistical significance of differences in hematological parameters. The significance level was set at $P<0.05$.

Results

The development of tumors from transplanted G:5:113 fibrosarcoma cells was suppressed in mice treated with diclofenac. Whereas none of the four mice treated with diclofenac showed a measurable tumor on day 30 after tumor cell transplantation, four out of five diclofenac-untreated mice that received tumor cells bore measurable tumors (mean tumor size ± S.E.M was 0.019 ± 0.014 cm^3).

The hematological results are summarized in Table 1. Fourteen-day administration of diclofenac from day 5 to day 19 after transplantation of tumor cells significantly elevated the numbers of GM-CFC and BFU-E per femur in comparison with pharmacologically untreated mice with transplanted fibrosarcoma cells. A prominent elevation of GM-CFC per spleen was also found in diclofenac-treated mice.

Assessment of blood smears showed that observed elevation of total leukocytes in mice treated with diclofenac was caused by a significant increase in blood granulocytes. No significant effects of the pharmacological treatment were observed on agranulocytes and erythrocytes.

Table 1. Hematological parameters of hematopoietic organs and peripheral blood in mice with implanted G:5:113 fibrosarcoma cells on day 30 after injecting tumor cells

Parameter	Control mice (no treatment) n=10	Mice administered	
		Fibrosarcoma cells plus diclofenac n=5	Fibrosarcoma cells no diclofenac n=5
Leukocytes per 1 μl peripheral blood (x 10^{-3})	3.94 ± 0.36	5.15 ± 0.68	4.52 ± 0.57
Granulocytes per 1 μl peripheral blood (x 10^{-3})	1.22 ± 0.13	1.97 ± 0.24*	1.26 ± 0.19
Agranulocytes per 1 μl peripheral blood (x 10^{-3})	2.46 ± 0.20	3.18 ± 0.46	3.26 ± 0.46
Erythrocytes per 1 μl peripheral blood (x 10^{-6})	6.50 ± 0.21	6.1 ± 0.04	6.66 ± 0.12
Femoral bone marrow cellularity (x 10^{-6})	10.86 ± 0.73	14.16 ± 0.42	10.51 ± 1.26
GM-CFC per femur (x 10^{-3})	11.04 ± 0.66	14.52 ± 0.21*	10.55 ± 1.21
BFUe per femur (x 10^{-3})	5.24 ± 0.59	8.79 ± 0.86*	4.58 ± 0.71
Spleen cellularity (x 10^{-6})	138.00 ± 22.51	144.00 ± 14.91	116.60 ± 5.12
GM-CFC per spleen (x 10^{-3})	2.98 ± 0.16	11.99 ± 0.67**	2.06 ± 0.19

*Values are means ± S.E.M.; n - number of animals; GM-CFC - granulocyte-macrophage colony-forming cells; BFUe - burst-forming units-erythroid; *, ** - P<0.05, P<0.01, respectively, in comparison with mice transplanted with fibrosarcoma cells which had not received diclofenac*.

Discussion

The results bear evidence that hematopoiesis-stimulating effects of diclofenac, a drug from the group of NSAIDs, acts potently in fibrosarcoma-bearing mice. The previously observed tumor-suppressing action of diclofenac in fibrosarcoma-bearing mice (Hoferová *et al.* 2002) has thus been confirmed.

Stimulation of hematopoiesis in oncological patients represents an important component of anti-tumor therapy. It is known that many types of tumors produce PGs (Maxwell *et al.* 1990, Marnett 1992). PGs are known to play a role in negative feedback control of GM-CFC proliferation (Pelus 1989). Thus, elevated levels of PGs in patients with some types of cancer may play a role in the suppression of their hematopoiesis and, consequently, their immune response. Therefore, administration of NSAIDs, acting on the principle of inhibition of PG production seems to be especially relevant for the purpose of granulopoietic stimulation in some groups of oncological patients.

Caution will, however, be necessary when testing the anti-tumor efficacy of NSAIDs in combination with radiotherapy. The side effects of NSAIDs, especially those on the gastrointestinal system, may be enhanced if a part of the gastrointestinal system is also irradiated. These assumptions are in accordance with our previous findings of intestinal damage evoked by combined exposure of mice to NSAIDs and high radiation doses (Hofer *et al.* 1992).

In conclusion, our results indicate the possibility of using diclofenac for stimulation of hematopoiesis in cancer patients. Further studies are needed to determine the indications (tumor types) in which the modulation of the metabolism of prostaglandins is especially desirable.

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