Meloxicam Elevates Serum Concentration of Erythropoietin and Numbers of Bone Marrow Erythroid Progenitor Cells in Sublethally Gamma-Irradiated Mice

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Abstract

Meloxicam, a non-steroidal anti-inflammatory drug selectively inhibiting cyclooxygenase-2, has been found to enhance the regeneration of erythroid progenitor cells (BFU-E) in the femoral bone marrow of mice when administered after sublethal irradiation (4 Gy gamma-rays). In mice treated with meloxicam once daily on days 3, 4, 5, and 6 after irradiation, the values of BFU-E per femur in meloxicam-treated mice were on days 7 and 14 after irradiation at the levels of 156 % and 191 %, respectively, related to those in irradiated saline-treated controls ($P < 0.01$ and $P < 0.001$, respectively). Moreover, it has been shown that these effects of meloxicam can be associated with its ability to stimulate erythropoietin production in irradiated mice. Six and 12 hours after one dose of meloxicam given on day 3 after irradiation, the serum level of erythropoietin was twofold higher in comparison with irradiated saline-treated controls ($P < 0.05$). These findings may have practical implications in the treatment of myelosuppression.

Cyclooxygenase-2 inhibition, erythropoiesis

Erythropoiesis is a complex process under the control of a number of factors like erythropoietin (EPO), stem cell factor (SCF), interleukin-11 (IL-11), and granulocyte colony-stimulating factor (G-CSF) (DeHaan et al. 1996). EPO is a crucial factor for the stimulation of erythropoiesis (Fisher 2003); for clinical treatment of anaemia, EPO (Kendall 2001) or synthetic darbepoietin-alpha are usually used, having a longer terminal half-life (Overbay and Manley 2002).

Inhibitors of prostaglandin production, known also as non-steroidal anti-inflammatory drugs (NSAIDs), which act on the principle of non-selective inhibition of cyclooxygenases, have been successfully tested as stimulators of suppressed haematopoiesis in a number of animal studies (for a review see Hofer and Pospíšil 2006). Although the vast majority of the findings concerns stimulatory effects of non-selective NSAIDs in the compartments of developing white blood cells, one study reports also an up-regulation of rat bone marrow erythropoiesis by these drugs (Kalaidjieva 1999). Recently we have published the results of a study testing the effectiveness of meloxicam, a NSAID belonging to selective cyclooxygenase-2 (COX-2) inhibitors developed for reducing the risks ensuing from undesirable gastrointestinal effects of non-selective NSAIDs (Lanas et al. 2003), in the treatment of radiation-suppressed mouse haematopoiesis. It follows from this study that meloxicam favourably influences not only granulopoiesis but also erythropoiesis of the experimental animals by increasing the numbers of granulocytic and erythroid progenitor cells (GM-CFC and BFU-E), as well as blood granulocytes and erythrocytes (Hofer et al. 2006). A consequential study aimed at clarifying the mechanism of the effects of meloxicam on granulopoiesis has revealed that meloxicam increases the blood serum levels of granulocyte colony-stimulating factor (G-CSF) in mice (Hofer et al. 2008). In this communication we show that post-irradiation administration of meloxicam to sublethally irradiated mice can enhance the counts of bone marrow erythroid progenitor cells and elevate serum concentration of EPO.

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Materials and Methods

B10CBAF$_1$ male mice weighing 30 g on average were used. Their use and treatment followed the European Community Guidelines. The experiments were performed with the approval of the Institute's Ethics Committee. Meloxicam (Sigma, St. Louis, MO, USA) was diluted with saline and administered intraperitoneally in injections of 0.6 mg/mouse in a volume of 0.2 ml. Saline was used for control injections. The mice were whole-body irradiated with a sublethal dose of 4 Gy of ^{60}Co gamma-rays (dose rate of 0.25 Gy/min). The total number of nucleated cells per femur was determined by means of a Coulter Counter (Model ZF, Coulter Electronics, UK). Erythroid progenitor cells (burst-forming units, BFU-E) were cultivated on methylcellulose. Haemoglobinized colonies were counted as BFU-E after 8-day incubation and the numbers of BFU-E per femur were calculated. Serum concentrations of EPO were determined using commercial ELISA kits (R&D Systems, Inc., Minneapolis, USA). The assay had the sensitivity of 18.0 pg/ml. The significance of differences was evaluated using the Mann-Whitney U test followed by the Holm's correction for multiple comparisons. The significance level was set at $P < 0.05$.

Results

The results of the two experiments evaluating the effects of meloxicam on numbers of bone marrow erythropoietic progenitor cells BFU-E are summarized in Table 1. In Experiment 1, sublethally (4 Gy) irradiated mice were treated with meloxicam in a four-dose (one dose per day) regimen; the drug was given on days 3, 4, 5, and 6 after irradiation. On days 7 and 14 after irradiation, the values of BFU-E per femur in meloxicam-treated mice were at 156% and 191%, respectively, related to those in irradiated saline-treated controls. Statistical processing revealed significance of both these differences. On day 21 after irradiation, the numbers of BFU-E per femur in the animals given meloxicam were still at 135% of control values but the difference was not significant. With the aim to assess whether meloxicam is able to enhance the numbers of bone marrow BFU-E also when the number of its doses is reduced, Experiment 2 was performed in which mice were administered two doses of the drug on days 3 and 5 after irradiation. It was found that the stimulatory effect of meloxicam on the numbers of BFU-E per femur is preserved; on day 7 after irradiation the values of BFU-E per femur in meloxicam-treated mice attained 159% of those in irradiated saline-treated controls ($P < 0.01$).

Table 1. Numbers of BFU-E in the femoral bone marrow of meloxicam-treated mice after 4 Gy γ-irradiation

BFU-E per femur × 10^3		
Day after irradiation	Irradiated saline-treated control mice	Meloxicam-treated mice
Experiment 1. Mice treated with meloxicam on days 3, 4, 5, and 6 after irradiation		
7	7.74 ± 0.52	12.11 ± 1.17**
14	10.53 ± 0.69	20.09 ± 1.34***
21	17.40 ± 2.82	23.49 ± 1.99
Experiment 2. Mice treated with meloxicam on days 3 and 5 after irradiation		
7	6.32 ± 0.53	10.04 ± 1.06**

Values are given as means ± SEM. The experiments were performed twice and the results were pooled. A total of 10 animals per group were used.
**, ***, $P < 0.01$, and $P < 0.001$, respectively, compared with irradiated saline-treated controls (Mann-Whitney U test). Number of BFU-E per femur in non-irradiated untreated controls: 23.73 ± 1.58 × 10^3.

For determination of possible modulatory effects of meloxicam on serum concentration of EPO, a treatment scheme consisting of one dose of meloxicam given on day 3 after irradiation of the mice by the dose of 4 Gy was used. Serum samples were taken 6 or 12 h after the administration of meloxicam. The results obtained on serum EPO are shown in

Table 2. Serum concentrations of EPO (pg/ml) in 4 Gy-irradiated mice after administration of meloxicam in a single dose on day 3 after irradiation

Non-irradiated control mice		
48.3 ± 18.1		
Irradiated mice		
Time interval after injection (h)	Saline-treated control mice	Meloxicam-treated mice
6	206.0 ± 86.6*	433.1 ± 87.6#
12	186.3 ± 16.1*	421.4 ± 82.6#

Mice were administered saline or meloxicam at a single dose on day 3 after irradiation. Serum concentration of EPO was determined 6 and 12 h after the injection. Data are given as means ± S.E.M. Five animals per group were used.
* - $P < 0.05$ vs. non-irradiated control mice; # - $P < 0.05$ vs. irradiated saline-treated mice.

Table 2. Irradiation itself caused an expressive and significant increase in the concentration of EPO in the serum to about 400% of that in untreated controls. This result is in agreement with previously reported findings (Krantz and Jacobson 1970). A single injection of meloxicam increased the EPO concentration to more than a double of those observed in irradiated saline-treated controls. This finding was obtained in both sampling time intervals and the differences between the meloxicam-treated and saline-treated mice were again significant.

Discussion

The findings presented in this communication extend our previously (Hofer et al. 2006) reported data on the protective and stimulatory effects of the COX-2 inhibitor meloxicam in irradiated mice and newly demonstrate the ability of meloxicam to increase the production of EPO and regeneration of erythroid progenitor cells when giving this drug under post-irradiation conditions. Mechanisms of these effects are yet to be analyzed. Thus, it remains to be established whether the originally postulated mechanism of stimulation of haematopoiesis by NSAIDs via suppression of production of negatively acting prostaglandins (Fontagné et al. 1980) includes the stimulation of G-CSF and EPO production, or whether the increased cytokine concentrations are independent of the influence of the drug on the metabolic pathway of prostaglandins. However, it should be noted that our pilot studies did not show any effects of meloxicam on the production of EPO in normal non-irradiated mice (data not given). Thus, it seems that meloxicam only supports the mechanisms of positive control of erythropoiesis activated by irradiation.

The fact that meloxicam stimulates both post-irradiation granulopoiesis and erythropoiesis, may be of interest also for clinical practice. Recently it has been emphasized by a panel of specialists that studies of radioprotectors for use prior to irradiation and of therapeutic agents for post-exposure treatment are highest priority research areas for radiological nuclear countermeasures (Pellmar et al. 2005). Utilization of the obtained results on the haematopoiesis-stimulating action of meloxicam may be also advisable in further studies aimed at alleviation of myelotoxicity resulting from cytotoxic chemotherapy during oncological treatments. The possibility of using NSAIDs devoid of their undesirable side effects for the treatment of both granulocytopaenia and anaemia might be of interest for medical practice.

Meloxicam zvyšuje hladinu erytropoetinu a počet erytroidních progenitorových buněk kostní dřeně u myší subletálně ozářených gama paprsky

Zjistili jsme, že meloxicam, nesteroidní antiflogistikum selektivně inhibující cyklooxygenázu-2, podaný po ozáření myší subletální dávkou (4 Gy gama paprsků), posiluje regeneraci erytroidních progenitorových buněk (BFU-E) v kostní dřeni femuru. U myší

léčených meloxicamem 3., 4., 5. a 6. den po ozáření se počty BFU-E na femur nacházely na úrovni 156% a 191% ve srovnání s hodnotami u ozářených kontrol, jimž byl podán fyziologický roztok ($P < 0,01$ a $P < 0,001$). Další pozorování ukázala, že zmíněný účinek meloxicamu může být spojen s jeho schopností stimulovat u ozářených myší produkci erytropoetinu. Šest a 12 hodin po jedné dávce meloxicamu podané 3. den po ozáření byla sérová koncentrace erytropoetinu dvojnásobná ve srovnání s ozářenými kontrolami ($P < 0,05$). Tyto nálezy mohou mít praktické uplatnění v léčbě útlumu kostní dřeně.

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