

## Meloxicam, an Inhibitor of Cyclooxygenase-2, Increases the Level of Serum G-CSF and Might Be Usable as an Auxiliary Means in G-CSF Therapy

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Received March 8, 2007

Accepted March 27, 2007

On-line available May 30, 2007

### Summary

Hematopoiesis-modulating action of meloxicam, a cyclooxygenase-2 inhibitor, has been evaluated in mice. Increased serum level of granulocyte colony-stimulating factor (G-CSF) after meloxicam administration has been found in sublethally  $\gamma$ -irradiated animals. In further experiments hematopoiesis-stimulating effects of meloxicam and G-CSF given alone or in combination have been investigated. Granulocyte/macrophage progenitor cells counts were used to monitor these effects. Meloxicam and exogenous G-CSF did not act synergistically when given in combination, but could be mutually substituted during their repeated administration. The results suggest a promising possibility of using meloxicam as an auxiliary drug reducing the high costs of G-CSF therapy of myelosuppression.

### Key words

Hematopoiesis • Cyclooxygenase-2 inhibition • Meloxicam • Granulocyte colony-stimulating factor • Treatment of myelosuppression

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Since prostaglandins-E play an inhibitory role in hematopoiesis (Kurland and Moore 1977), suppression of

prostaglandin production by non-steroidal anti-inflammatory drugs (NSAIDs) has been widely explored in attempts to enhance hematopoiesis in myelosuppressive states (for review see Hofer and Pospíšil 2006). The undesirable side effects of the classical NSAIDs on the gastrointestinal tract represent a disadvantage of their use. Evidence that cyclooxygenase (COX) carrying out prostaglandin synthesis exists in two isoforms, i.e. COX-1, which is expressed constitutively in various tissues and whose inhibition is responsible for the side effects, and COX-2, which is inducible and responsible for prostaglandin production under inflammatory states, led to the development of new drugs inhibiting selectively COX-2 and exhibiting a low incidence of gastrointestinal side effects (Frölich 1977, Crofford *et al.* 2000). We have recently found that a representative of these drugs, meloxicam, stimulates hematopoiesis in sublethally irradiated mice and thus retains this favorable activity of the classical NSAIDs (Hofer *et al.* 2006). The aim of the experiments presented here was to ascertain the relationship between the meloxicam action and the granulocyte colony-stimulating factor (G-CSF) inducing proliferation and differentiation effects in the granulocytic cell system and accelerating regeneration after radiation-induced myelosuppression (Welte *et al.* 1996, Patchen *et al.* 1990). Two important findings are presented: i) that meloxicam increases the serum levels of G-CSF, and ii) that meloxicam can be profitably combined with exogenously administered

G-CSF. The model of radiation-induced myelosuppression in mice was employed. Counts of bone marrow hematopoietic progenitor cells were used to evaluate the investigated effects.

B10CBAF<sub>1</sub> male mice weighing on average 30 g were used. Their use and treatment followed the European Community Guidelines. The experiments were performed with the approval of the Institute's Ethical 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. G-CSF (Neupogen, Amgen Europe, Breda, The Netherlands) was diluted with 5 % glucose and administered subcutaneously (s.c.) in injections of 3 µg/mouse in a volume of 0.1 ml. This s.c. dose is known to induce an elevation of serum G-CSF levels about 1 or 2 hours after administration with a subsequent decline to nearly control levels by 4 hours (Kuwabara *et al.* 1996). The corresponding drug vehicles were used for control injections. The doses of the drugs, based on our earlier experiments (Pospíšil *et al.* 1995, Hofer *et al.* 2006), were chosen with the intention of inducing the effect of both drugs with an approximately similar influence on hematopoiesis. The mice were whole-body irradiated with a sublethal dose of 4 Gy of <sup>60</sup>Co gamma-rays (dose rate of 0.25 Gy/min). Concentrations of murine G-CSF in mouse serum were determined using an ELISA kit (R&D Systems, Minneapolis, USA). The assay had the sensitivity of 4.5 pg/ml. Total nucleated cells per femur were determined by means of a Coulter Counter (Model ZF, Coulter Electronics, UK). Granulocyte-macrophage colony-forming cells (GM-CFC) were assayed using a semisolid plasma clot technique (see e.g. Weitwrová *et al.* 2007). GM-CFC were scored after 7-day incubation. The numbers of GM-CFC *per* femur were calculated. The statistical 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.

Table 1 demonstrates the effects of meloxicam on serum levels of G-CSF. Mice were given meloxicam or saline in a single injection on day 3 after 4 Gy irradiation and the serum was sampled 3, 6, and 9 hours later. Interestingly, the irradiation *per se* induced a significant increase of G-CSF levels as compared to values in non-irradiated controls. This effect is not surprising. An increase of G-CSF mRNA expression in murine splenocytes has been reported even at a very low

radiation dose (Li *et al.* 2004). Meloxicam given after the irradiation further increased the serum levels of G-CSF. Such an effect has not yet been observed and may suggest a mechanism responsible for the stimulatory effects of meloxicam on hematopoiesis. The elevation of serum G-CSF up to the last sampling time interval of 9 hours after meloxicam administration is probably more protracted than that induced by external administration of G-CSF (Kuwabara *et al.* 1996). It is difficult to relate the serum levels of G-CSF to its biological activity. However, it seems that for obtaining an optimum effectiveness of G-CSF the decisive factor is the duration time of G-CSF serum level above a particular concentration and not its absolute concentration (Kuwabara *et al.* 1996). Thus, our observations allow us to assume that the elevated serum levels of G-CSF obtained after meloxicam administration play a role of a factor which is responsible for the effects induced in the compartment of hematopoietic progenitor cells.

**Table 1.** Serum concentrations of G-CSF (pg/ml) in non-irradiated and 4 Gy irradiated mice.

Unirradiated control mice		
158.4 ± 15.2		
Irradiated mice		
Time interval after injection (hours)	Saline-treated controls	Meloxicam-treated mice
3	444.6 ± 107.3*	1207.2 ± 127.3 <sup>##</sup>
6	739.3 ± 146.1*	1416.3 ± 112.6 <sup>#</sup>
9	664.1 ± 106.3*	1450.6 ± 55.6 <sup>##</sup>

Mice were administered saline or meloxicam in a single dose on day 3 after irradiation. Serum concentration of G-CSF was determined 3, 6, and 9 hours 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, P<0.01, respectively, vs. irradiated saline-treated mice.

Further experiments were performed to investigate the effects of endogenously and exogenously induced elevation of the serum levels of G-CSF on the hematopoietic progenitor cell (GM-CFC) counts, which had been reduced by irradiation (Table 2). In the first experiment, meloxicam and G-CSF were administered to mice in four doses on days 3, 4, 5, and 6 after the 4 Gy irradiation, either as monotherapy or in combination. In the combined treatment G-CSF was injected 6 hours after

**Table 2.** Numbers of GM-CFC in femoral bone marrow of mice determined on day 7 after 4 Gy irradiation.

<b>Experiment 1. Mice treated on days 3, 4, 5, and 6 after irradiation with meloxicam, G-CSF, or a combination of meloxicam + G-CSF; meloxicam was given at 8:00 h, G-CSF at 14:00 h</b>				
	<b>Solvent-treated controls</b>	<b>Meloxicam-treated mice</b>	<b>G-CSF-treated mice</b>	<b>Meloxicam + G-CSF-treated mice</b>
<i>GM-CFC per femur x 10<sup>3</sup></i>	4.48 ± 0.82	9.75 ± 1.05*	9.71 ± 0.90**	10.73 ± 0.81**
<b>Experiment 2. Mice treated on days 3 and 5 after irradiation with meloxicam, on day 4 and 6 with G-CSF, or a combination of meloxicam given on days 3 and 5, plus G-CSF given on days 4 and 6, always at 8:00 h</b>				
	<b>Solvent-treated controls</b>	<b>Meloxicam-treated mice</b>	<b>G-CSF-treated mice</b>	<b>Meloxicam + G-CSF-treated mice</b>
<i>GM-CFC per femur x 10<sup>3</sup></i>	3.38 ± 0.50	6.29 ± 0.43**	5.41 ± 0.68*	9.80 ± 0.67***, ##, ++

Data are given as means ± SEM. Both experiments were performed twice and altogether 10 animals per group were used. \*, \*\*, \*\*\*, P<0.05, P<0.01, and P<0.001, respectively, vs. solvent-treated controls; ##, P<0.01 vs. meloxicam-treated mice, ++, P<0.01 vs. G-CSF-treated mice. Numbers of GM-CFC per femur (x 10<sup>3</sup>) in unirradiated untreated control mice: 17.98 ± 0.82.

meloxicam administration. Initiation of therapy was delayed 3 days post-exposure to allow some post-irradiation repair and regeneration of the hematopoietic progenitor cells (Patchen *et al.* 1990). As shown on day 7 after the irradiation, both meloxicam and G-CSF given alone significantly increased the numbers of GM-CFC by a factor of 2.1 in comparison with saline-treated irradiated controls. Fortunately, both the drugs induced quantitatively similar stimulatory effects on GM-CFC indicating the success in the chosen doses. However, no mutual potentiation of the effects was observed when the two drugs were administered in combination. The absence of the synergistic interaction of the drugs can be explained by a common induction mechanism. As stated by Rozengurt (1986), “agents sharing a common signalling system cannot act synergistically”.

In the second experiment, numbers of doses of both drugs were reduced from four to two. Meloxicam was given on days 3 and 5, G-CSF on days 4 and 6 after irradiation, the combined therapy included two doses of meloxicam and two doses of G-CSF in the time intervals mentioned. Both the drugs given as monotherapy still induced significantly higher GM-CFC counts as compared to the irradiated controls, even though lower in comparison with the 4-dose regimen used in the first experiment. When meloxicam and G-CSF were

administered in combination with intervals of 24 hours between injections of the two drugs, significantly higher effects were observed in comparison with the effects induced by any of the drugs given alone. The sum of effects induced by two doses of meloxicam and two doses of G-CSF yielded a similar value to that obtained by the action of four doses of meloxicam or four doses of G-CSF shown in the first experiment.

To summarize, the presented experiments have suggested that the selective inhibitor of COX-2, meloxicam, can stimulate hematopoiesis of irradiated mice *via* increasing the serum level of G-CSF. Under proper dosage and timing, meloxicam can be profitably combined with exogenously administered G-CSF. If these effects are verified in clinical conditions, they could be utilized in attempts to reduce the frequency of G-CSF administration and the high costs of such a treatment.

### Conflict of Interest

There is no conflict of interest.

### Acknowledgements

This work was financially supported by the Academy of Sciences of the Czech Republic (projects No. 1QS500040507 and AV0Z50040507) and by the Grant Agency of the Czech Republic (project No. 305/08/0158).

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