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SHORT COMMUNICATION

Combination of Drugs Elevating Extracellular Adenosine with Granulocyte Colony-Stimulating Factor Promotes Granulopoietic Recovery in the Murine Bone Marrow after 5-Fluorouracil Treatment

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Summary

Combined administration of drugs elevating extracellular adenosine, namely dipyridamole and adenosine monophosphate, together with granulocyte colony-stimulating factor was shown to enhance granulopoietic recovery in the bone marrow of mice treated with 5-fluorouracil. Enhanced regeneration was found both at the level of hematopoietic progenitor cells for granulocytes and macrophages and in the compartment of morphologically recognizable granulocyte precursors. The results might have positive clinical impact. The adjunct use of drugs elevating extracellular adenosine might reduce the cost expenditure of therapy with granulocyte colony-stimulating factor.

Key words

5-fluorouracil • Granulopoiesis • Extracellular adenosine • Adenosine monophosphate • Dipyridamole • Granulocyte colony-stimulating factor

Adenosine and adenine nucleotides have specific signaling action in the regulation of a variety of functions including autoregulation of blood flow, neurotransmission, and several aspects of cell metabolism (Abbracchio and Burnstock 1998). Many of these actions are mediated by specific cell surface receptors. Signaling through adenosine receptors has also been found to modulate proliferation, differentiation and viability of cell renewal populations (Abbracchio 1996). It has been shown previously in our laboratory that drugs elevating extracellular adenosine and thus acting through adenosine receptor signaling synergize with the granulocyte colony-stimulating factor (G-CSF) to enhance granulopoiesis in normal mice (Pospíšil *et al.*1995). This drug combination has also been found to increase hematopoietic recovery when administered to gamma-irradiated mice (Pospíšil *et al.* 1998, Hofer *et al.* 1999). The results given in this report demonstrate the possible therapeutic potential of

the combination of G-CSF with drugs elevating extracellular adenosine in the treatment of myelosuppression induced in mice by 5-fluorouracil (5-FU).

Male B10CBAF1 mice aged 3 months and weighing 30 g on the average were obtained from AnLab Ltd. They received an intraperitoneal injection of 5-fluorouracil (Sigma, St. Louis, MO, USA) in a single dose of 3 mg per mouse. Based on our earlier experience (Pospíšil *et al.* 1995, 1998, Hofer *et al.* 1999), the elevation of extracellular adenosine was induced by the combined administration of dipyridamole (DP), a drug inhibiting the cellular uptake of adenosine, and adenosine monophosphate (AMP), serving as a source of exogenous adenosine. Dipyridamole (Sigma) was injected subcutaneously in a dose of 2 mg per mouse and 20 min later intraperitoneal injection of adenosine monophosphate from yeast (Sigma) was followed by a dose of 5 mg free acid per mouse. In the combined treatment, recombinant human G-CSF (Neupogen, Hoffman-LaRoche Ltd., Switzerland) was injected subcutaneously in a dose of 1.5 μg per mouse 30 min after AMP. Corresponding vehicles were injected to the controls. On repeated administration, drugs were injected at 24-hour intervals. Indices of granulopoiesis were ascertained in the femoral marrow. Progenitor cells involved in granulocyte-macrophage development (GM-CFC) were assayed using a semisolid plasma clot technique. Differential counts were assessed in smear preparations, and proliferative (myeloblasts through myelocytes) and nonproliferative (metamyelocytes through segmented stages) granulocytic cells were calculated. Details of the respective techniques are given in our previous papers (Pospíšil *et al.* 1995, 1998, Hofer *et al.* 1999).

In the first experiment, we investigated the short-term effects of the drugs given in a two-day regimen starting 2 h after 5-FU administration. The results are summarized in Table 1. Two days after 5-FU injection, damage in control mice was manifested by a reduction of GM-CFC by about 86 % and a reduction of total granulocytic cells in the femoral marrow by about 71 % compared to intact controls. Administration of G-CSF alone or DP+AMP did not induce significant effects when compared to 5-FU-injected controls, with the exception of the decrease of nonproliferative granulocytic cells after G-CSF, probably indicating their release into the circulation (Lord *et al.* 1991). On the other hand, significant stimulatory effects on bone marrow granulopoiesis were found in mice treated with the combination of DP+AMP+G-CSF. The effects of the drug combination were clearly synergistic given that neither G-CSF alone nor DP+AMP enhanced GM-CFC or granulocytic cells significantly. After the combined therapy, there was a 2.2-fold increase of GM-CFC values and 2.8-fold rise in the number of proliferative granulocytic cells compared to the 5-FU-injected control group.

Table 1. Indices of granulopoiesis in femoral marrow of mice treated with various drugs after 5-FU administration

	Counts of granulocytic cells (x 10^3)			
Treatment	**GM-CFC**	**Total**	**Proliferative**	**Nonproliferative**
Control	2 277±711	3 093±322	375±67	2 718±277
G-CSF	1 688±307	2 300±277	551±78	1 739±240[a]
DP+AMP	3 043±603	3 019±322	683±118	2 336±232
DP+AMP+G-CSF	4 962±363[abc]	4 999±388[abc]	1 035±338[a]	3 464±395[bc]

Drugs were injected in a 2-day treatment regimen starting 2 h after 5-FU, indices of granulopoiesis were ascertained 2 days after 5-FU, i.e. 24 h after the final drug treatment. Data are given as means±SEM from groups of 5-6 mice. Statistical analysis was performed using Kruskal-Wallis ANOVA followed by Mann-Whitney rank sum test. Significance ($P<0.05$): [a] - compared to control, [b] - compared to G-CSF alone, [c] - compared to DP+AMP. The mean counts of GM-CFC in femurs of 10 intact mice were 16 566±816 x 10^3, those of proliferative and nonproliferative granulocytic cells were 2 050±265 x 10^3, and 8 453±650 x 10^3, respectively.

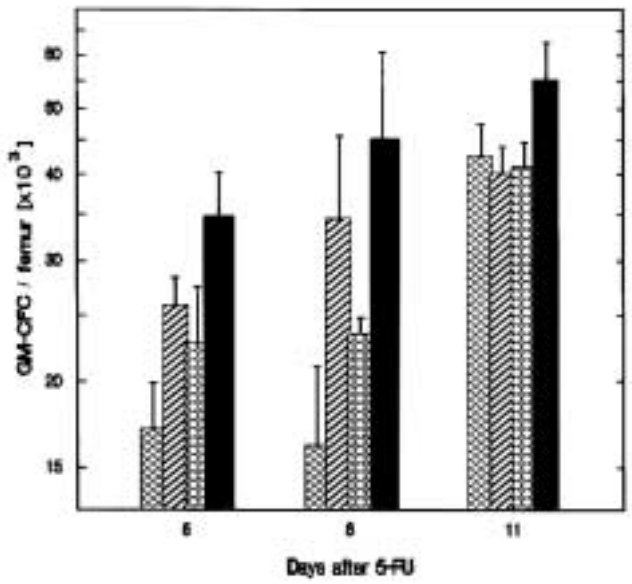

Fig. 1. *Counts of GM-CFC in femoral marrow of differently treated mice determined 5, 8 and 11 days after 5-FU administration. Drugs were injected in a 4-day treatment regimen starting 24 h after 5-FU injection. The four columns in each time interval denote values of the controls, G-CSF alone, DP+AMP, and DP+AMP+G-CSF combination and are given as means of GM-CFC counts from 10 mice per group (error bars represent S.E.M.). Data were logarithmically transformed and statistical significance of the differences was evaluated for the sum of all intervals using two-way ANOVA (time and drug effects as variables) followed by multiple comparisons using Tukey's test. C – control mice. Significance ($P<0.05$) was found when comparing the controls with the effects of drugs given either alone or in combination, as well as when comparing DP+AMP+G-CSF group with all other groups.*

The second experiment examined the effects of the drugs given in a four-day regimen starting 24 h after 5-FU administration. As is shown in Figure 1, the counts of GM-CFC in 5-FU-injected controls recovered to the initial values on days 5 and 8 and attained supranormal levels on day 11. In the mice treated with the drugs given either alone or in combination, an overshoot of the norm was already observed on day 5. The highest effectiveness of the drug combination to increase GM-CFC counts was again evident. In contrast to the short-term effects of the drugs, in this experiment both G-CSF alone or DP+AMP exhibited significant stimulatory effects and their combined administration seemed to act subadditively.

The possible mechanisms of these observed drug effects have been discussed elsewhere (Pospíšil *et al.* 1995, 1998, Hofer *et al.* 1999) and deserve further investigation. Hypothetically, the interaction of elevated extracellular adenosine with G-CSF can be due to coalescence of initially differing mitogenic signals into a common transduction pathway. Such a role may be played by the adenylate cyclase system, which is linked to both adenosine (Pospíšil *et al.* 1995) and G-CSF (Matsuda *et al.* 1989) cell receptors. As far as the practical aspects are concerned, the results of the presented experiments indicate a promising possibility to enhance the effects of G-CSF in the treatment of chemotherapy-induced myelosuppression. The adjunct use of drugs elevating extracellular adenosine might also reduce the cost of G-CSF therapy.

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Reprint requests

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