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Effects of Stable Adenosine Receptor Agonists on Bone Marrow Hematopoietic Cells as Inferred from the Cytotoxic Action of 5-Fluorouracil

M. POSPÍŠIL, M. HOFER, A. VACEK, V. ZNOJIL[1], I. PIPALOVÁ[1]

Institute of Biophysics, Academy of Sciences of the Czech Republic, and [1]Medical Faculty, Masaryk University, Brno, Czech Republic

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Summary

The aim of the study was to investigate the effects of stable adenosine receptor agonists on bone marrow hematopoiesis by utilizing the model of hematopoietic damage induced by 5-fluorouracil (5-FU), a cycle-specific cytotoxic agent. Effects of a non-selective agonist NECA activating all the known adenosine receptors (A_1, A_{2A}, A_{2B}, A_3) and of the selective agonists for A_1 (CPA), A_{2A} (CGS 21680), and A_3 (IB-MECA) adenosine receptors were investigated. Experiments were performed with B10CBAF1 mice under *in vivo* conditions. Adenosine receptor agonists were given in single injections before 5-FU administration and the effects were determined 4 days later. The numbers of femoral marrow nucleated cells and hematopoietic progenitor cells (CFC-GM and BFU-E) were taken as indices of the effects. The non-selective agonist NECA given at a dose of 200 nmol/kg induced biphasic time-dependent effects, i.e. protection and sensitization, when given 10 h and 22 h before 5-FU administration, respectively. The use of isomolar doses of selective receptor agonists indicated that the protective effects of NECA were induced by activation of A_{2A} and A_{2B} receptors, while the sensitizing action of NECA was mediated *via* A_3 receptors. In addition, it was observed that A_1 receptors induced protection when activated by administration of CPA 22 h before 5-FU. These findings are discussed with respect to the action of adenosine receptor agonists on the cell cycle state and on the cell cycle-independent cellular protective mechanisms.

Key words
Adenosine receptor agonists • Hematopoiesis • 5-Fluorouracil

Introduction

In previous studies, we have shown that the elevation of extracellular adenosine induced by the combined administration of dipyridamole, a drug inhibiting the cellular uptake of adenosine, and adenosine monophosphate (AMP), an adenosine prodrug, enhances hematopoiesis in normal and myelosuppressed mice and synergizes with the effects of the granulocyte colony-stimulating factor (Pospíšil *et al.* 1995, 1998, Hofer *et al.* 1999, 2001, 2002, Weiterová *et al.* 2000). Moreover, we have demonstrated that co-administration of dipyridamole and AMP to mice increases the cycling of hematopoietic progenitor cells as inferred from the cytotoxic effects of 5-fluorouracil (Pospíšil *et al.* 2001). These results have suggested that, in addition to the well known

Fax +420 241 062 164
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

participation of adenosine in the regulation of cardiovascular and nervous functions (Abbracchio and Burnstock 1998, Poulsen and Quinn 1998), adenosine also plays an important role in the control of hematopoiesis.

Generally, extracellular adenosine is regarded as a regulatory metabolite and an endogenous activator of defense mechanisms at various levels of the biological system (Newby 1984, Bruns 1991). It has been recognized that its effects are mediated *via* a family of cell surface G protein-coupled receptors designated as A_1, A_{2A}, A_{2B}, and A_3. Adenosine receptor subtypes have been characterized pharmacologically, structurally and functionally. They are expressed in various organs and tissues and sometimes more than one adenosine receptor subtype is expressed in a single cell (Fredholm *et al.* 2000, 2001, Linden 2001). Adenosine receptors are an interesting target for pharmacological interventions. Such a possibility is supported by the development of metabolically stable adenosine derivatives, which provide agonists with a more or less selective binding activity to different receptor subtypes (Klotz 2000, Jacobson 2002). Thus, our above mentioned experiments, based on the action of the endogenous agonist, adenosine, had to be completed by utilizing stable adenosine analogs. Such a study could lead to a better understanding of the role of different adenosine receptors in hematopoiesis, and to the proposal of new ways of controling hematopoietic functions.

The purpose of the experiments presented here was to investigate the hematopoiesis-modulating effects of the stable non-selective adenosine receptor agonist and of stable selective agonists for A_1, A_{2A}, and A_3 adenosine receptors. The experimental procedure investigating *in vivo* the cytotoxic effects of 5-fluorouacil (5-FU) on hematopoietic progenitor cells in the femoral marrow of mice was employed (Molineux *et al.* 1994, Neta *et al.* 1996, Pospíšil *et al.* 2001). Such an approach could reveal not only the effects of adenosine receptor agonists on the cycling status of cells, because of the preferential sensitivity of cells in S-phase to 5-FU, but also a possible cell cycle-independent action of these drugs on the survival of cells exposed to the cytotoxic action.

Methods

Mice

Male B10CBAF1 mice aged 3 months and weighing 30 g on the average were obtained from the breeding facility of the Medical Faculty, Masaryk University, Brno, Czech Republic. The mice were kept under controlled conditions; standardized pelleted diet and HCl-treated tap water (pH 2-3) were available *ad libitum*.

Drug administration

The following adenosine receptor agonists were used: non-selective agonist 5‘-(N-ethylcarboxamido)-adenosine (NECA), selective A_1 receptor agonist N^6-cyclopentyladenosine (CPA), selective A_{2A} receptor agonist 2-p-(carboxyethyl)-phenethylamino-5‘-N-ethyl-carboxamidoadenosine (CGS 21680), and selective A_3 receptor agonist 1-deoxy-1-(6-[([3-iodophenyl]methyl)-amino]-9H-purin-9-yl)-N-methyl-β-D-ribofuranuron-amide (IB-MECA). NECA, CPA and CGS 21680 were obtained from Sigma (St. Louis, MO, USA), IB-MECA from Tocris (Bristol, UK). NECA was dissolved in 0.1 N HCl, CPA and CGS 21680 in water, IB-MECA in dimethyl sulfoxide. All drug solutions were further diluted with saline and injected intraperitoneally in a volume of 0.2 ml. The corresponding drug vehicles were used for control injections. The drug doses used were based on the previous *in vivo* studies on mice (Ueno *et al.* 1988, Haskó *et al.* 1998) and on results obtained by preliminary assays in our laboratory. 5-Fluorouracil (Sigma) was diluted in saline and injected intraperitoneally at a dose of 100 mg/kg in a volume of 0.2 ml to mice pretreated in different time intervals with either adenosine receptor agonists or control injections.

Hematological methods

The mice were sacrificed by cervical dislocation on day 4 after 5-FU administration. This time interval was also used in other studies investigating the cytotoxic effects of 5-FU on murine hematopoiesis (Molineux *et al.* 1994, Neta *et al.* 1996, Pospíšil *et al.* 2001) and is based on the fact that active cytotoxic metabolites of 5-FU persist in tissues of mice for 3 days (Chadwick and Rogers 1972) and that after 4 days the regeneration processes are not yet manifested. The femurs were removed and the marrow cells were harvested by standard procedures. The numbers of nucleated cells of the femoral marrow were determined using a Coulter Counter (Model ZF, Coulter Electronics, UK). Differential counts were performed on smear preparations stained with May-Grünwald-Giemsa method. Standard procedures were used for the *in vitro* assays of the femoral marrow clonogenic cells, as described in detail

earlier (Pospíšil *et al.* 2001). Briefly, CFC-GM were assayed using a semisolid plasma clot technique, BFU-E were cultivated in methylcellulose. Femoral marrow cell suspensions were plated in triplicate for both assays and incubated at 37 °C in a humidified atmosphere containing 5 % CO_2. CFC-GM were scored after 7-day incubation as colonies containing 50 or more cells. Hemoglobinized colonies were counted as BFU-E after 8-day incubation. The numbers of each hematopoietic population per femur were calculated.

Statistics

Each individual experiment included investigation of 5-8 mice treated with adenosine receptor agonists together with the same number of control animals. Some experiments were repeated 2 to 4 times and the data were pooled. Effects of the drug treatment were expressed as percent changes compared to values of the respective controls (100 %) and were given as geometric means because of asymmetric distributions. The sets of experiments investigating the time or dose dependences of the effects as well as the comparisons of responses to different adenosine receptor agonists were subjected to nonparametric Kruskal-Wallis ANOVA. Two-group data based on absolute values were compared using the Mann-Whitney rank sum test. The significance level was set at $p<0.05$. Data were expressed as mean ± SEM.

Results

5-FU alone at a dose of 100 mg/kg induced a suppression of the bone marrow hematopoiesis as revealed after 4 days by the counts of nucleated cells, CFC-GM and BFU-E in the femur. Compared to the values of these indices obtained in 20 control mice that were not treated with 5-FU (17.84±1.58 x 10^6 for nucleated cells, 17304±1465 for CFC-GM, 18796±1023 for BFU-E), counts of nucleated cells decreased to 4.60± 0.16 x 10^6, those of CFC-GM to 2257±112, and those of BFU-E to 3775±137, as calculated for the pooled data from all respective groups investigated. The effects of adenosine receptor agonists were expressed as percent changes according to the control levels obtained in mice treated with 5-FU alone (100 %). To eliminate seasonal and other uncontrolled sources of variability, each experimental group treated with the agonist was compared with the simultaneously investigated control mice treated with 5-FU alone.

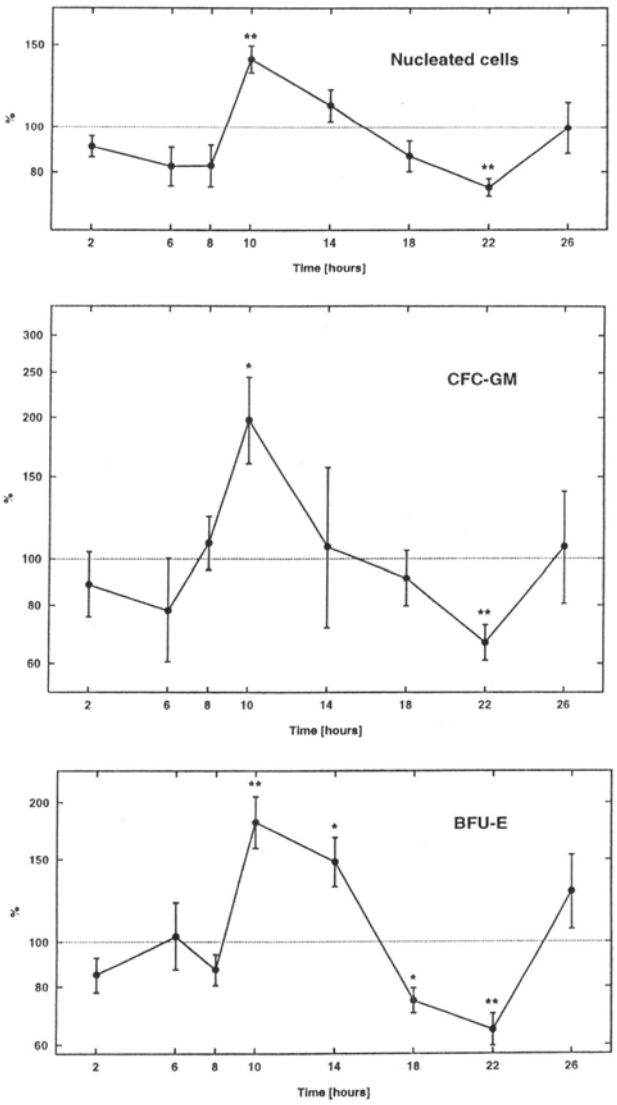

Fig. 1. Dependence of the counts of femoral marrow nucleated cells, CFC-GM and BFU-E on time differences between the administration of 200 nmol/kg of NECA and 5-FU. The data are expressed as percentage changes compared to values obtained in the simultaneously examined mice treated with 5-FU alone (100 %). The points represent mean ± S.E.M for groups of 7-30 mice. Significant differences: $^{*}p<0.05$, $^{**}p<0.01$.

Results of experiments investigating the effects of the non-selective adenosine receptor agonist NECA given before 5-FU administration (Figs 1 and 2) demonstrate the time and dose dependence of the induced effects. The significant influence of the time and dose factors on the observed effects was confirmed by the use of Kruskal-Wallis ANOVA. The time-dependent effects of NECA can be characterized as biphasic with protective responses followed by sensitization (Fig. 1). Bell-shaped relationships have been found for the dose dependence of the protective effects in all indices used (Fig. 2). The

most striking phenomenon concerned the protection induced by 200 nmol/kg of NECA administered 10 hours before 5-FU. In a separate experiment using this dose and time of NECA administration morphologically recognizable erythroid, granulocytic and lymphoid cells of the femoral marrow were also determined. As shown in Table 1, only nonproliferative granulocytic cells were increased significantly by the NECA treatment and are thus responsible for the increased bone marrow cellularity.

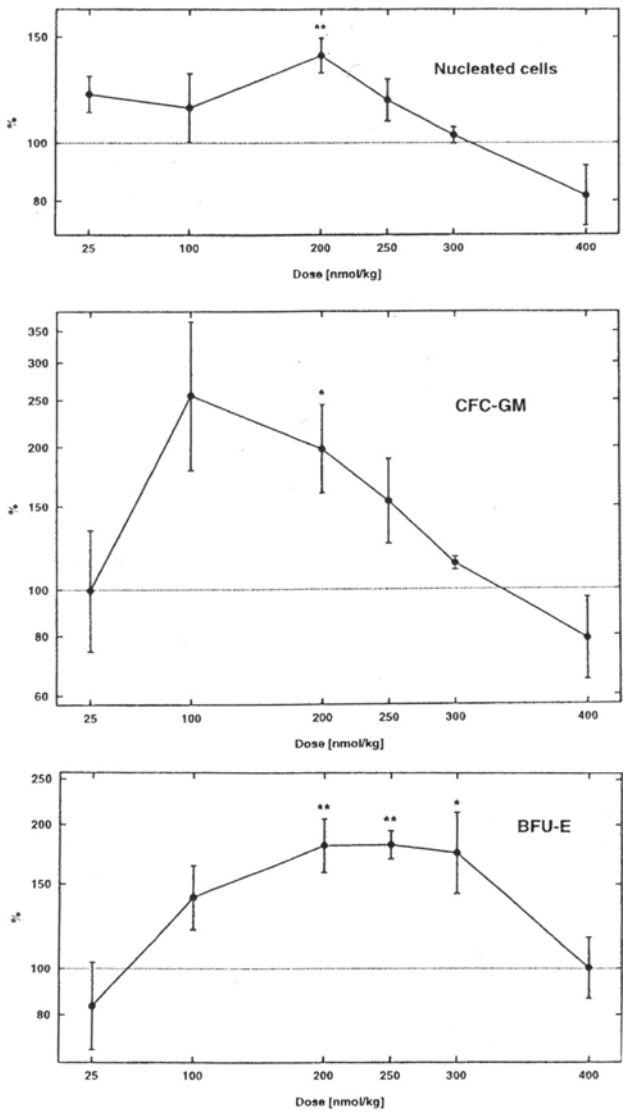

Fig. 2. Effects of different doses of NECA administered 10 h before 5-FU on the counts of nucleated cells of the femoral marrow, CFC-GM, and BFU-E. The data are expressed as percentage changes compared to values obtained in the simultaneously examined mice treated with 5-FU alone (100 %). The points represent mean ± S.E.M for groups of 7-30 mice. Significant differences: $^{*}p<0.05$, $^{**}p<0.01$.

Effects of isomolar 200 nmol/kg doses of selective agonists for A_1, A_{2A}, and A_3 receptors (CPA, CGS 21680, and IB-MECA, respectively) given either 10 or 22 h before 5-FU administration were compared with those induced by NECA. Kruskal-Wallis ANOVA proved significant differences in the action of the agonists in all comparisons (Table 2). They indicate that the protective effects of NECA induced after 10 h can be mimicked in part by the action of CGS 21680 in case of BFU-E counts. When looking at the activity observed 22 h after drug administration, two selective agonists induced significant effects. While the sensitizing action of NECA was reproduced by the action of IB-MECA in all the indices investigated, a significant opposite action, expressed as protection, was induced by CPA in CFC-GM and BFU-E counts.

Discussion

Experiments using the adenosine derivative NECA provided the basic information concerning the time- and dose-dependent action of this stable and universal adenosine receptor agonist on the hematopoietic damage as measured by femoral marrow cellularity and counts of CFC-GM and BFU-E on day 4 after 5-FU administration in mice. An approximately optimum dose of 200 nmol/kg of NECA could be defined, which induced the protective action at 10 h and the sensitizing action at 22 h after its administration. Because of the preferential role of murine bone marrow in granulopoiesis, the protective effects of NECA were reflected mainly in the granulocytic cells of the femoral marrow.

Calabrese (2001) summarized examples of the non-linear bell-shaped and often biphasic dose responses induced by adenosine and its stable analogs in numerous physiological systems and deduced that they can be explained by the interaction of two adenosine receptors differing in their affinities for the agonist and in the direction of their response. Similarly, time-dependent biphasic responses after NECA administration observed in our experiments can be explained by activation of two receptors differing in the kinetic profile of their counteracting actions. NECA was originally considered to be an A_2-selective compound but it also turned out to be a non-selective agonist with high affinity to other receptors (Klotz 2000, Jacobson 2002). Therefore, the identification of the adenosine receptors responsible for the effects of NECA depends on the use of selective

agonists. For this reason isomolar doses of 200 nmol/kg of CPA, CGS 21680, and IB-MECA, i.e. selective A_1, A_{2A}, and A_3 receptor agonists, respectively, were administered at the intervals of 10 or 22 h before 5-FU injection, and their effects were compared with those of the reference agonist NECA.

Table 1. Numbers of erythroid, granulocytic and lymphoid cells in femoral marrow of mice treated either with 5-FU alone or NECA administered at a dose of 200 nmol/kg 10 h before 5-FU.

	5-FU	NECA + 5-FU
	Cells per femur x 10^3	
Erythroid proliferative cells	182 ± 25	299 ± 84
Erythroid nonproliferative cells	1121 ± 176	1172 ± 189
Granulocytic proliferative cells	141 ± 32	252 ± 61
Granulocytic nonproliferative cells	1908 ± 154	2988 ± 158**
Lymphocytes	316 ± 31	390 ±58

Data are given as mean ± SEM for 7 mice per group. Statistical significance: **, $p<0.01$ compared to group treated with 5-FU alone.
Note: proliferative cells were proerythroblasts, macroblasts, basophilic erythroblasts, myeloblasts, promyelocytes, myelocytes; nonproliferative cells were polychromatic and orthochromatic erythroblasts, metamyelocytes, band and segmented neutrophils.

Table 2. Effects of different adenosine receptor agonists administered at a dose of 200 nmol/kg either 10 or 22 h before 5-FU treatment on the counts of femoral marrow nucleated cells, CFC-GM and BFU-E

	Nucleated cells (%)	CFC-GM (%)	BFU-E (%)
	Agonists given 10 hours before 5-FU (protection)		
NECA	140 ± 10**	198 ± 46*	181 ± 26**
CGS 21680	117 ± 8	110 ± 13	156 ± 12**
IB-MECA	96 ± 5	91 ± 9	120 ± 15
CPA	105 ± 8	91 ± 14	94 ± 11
	Agonists given 22 hours before 5-FU (sensitization)		
NECA	74 ± 3**	65 ± 5**	66 ± 6**
IB-MECA	75 ± 3**	67 ± 7**	57 ± 6**
CGS 21680	99 ± 6	116 ± 18	129 ± 8
CPA	118 ± 5	158 ± 24*	154 ± 18**

Data are expressed as percent changes (mean ± SEM) compared to values obtained in the simultaneously examined mice treated with 5-FU alone (100 %). Fourteen to 30 mice per group were used. Statistical significance: *, $p<0.05$, **, $p<0.01$. Note that selective agonists are ordered with regard to their potency to induce protection or sensitization.

It has been shown that only NECA given 10 h prior to 5-FU induced a significant protection in all indices investigated. Neither CPA, nor IB-MECA mediated any significant effect 10 h after their administration. CGS 21680 induced significant protection only in case of BFU-E counts. These actions indicate the role of A_2 receptors and probably the interplay of A_{2A} and A_{2B} receptor subtypes. NECA is one of the agonists with the highest potency at the A_{2B} receptors so far known (Klotz 2000). Because selective A_{2B} agonists are still missing, it is now accepted that the effects of NECA surpassing those of the selective agonists acting through A_1, A_{2A}, and A_3 receptors indicate the participation of A_{2B} receptors (Brackett and Daly 1994, Feoktistov *et al.* 1998, Jacobson 2002). Since CGS 21680 does not protect CFC-GM, A_{2A} receptors play a specific role only in the

protection of the progenitors of the erythroid cell lineage. It is worth noting that A_{2B} receptors exhibit a very low affinity for endogenous adenosine. Fredholm *et al.* (2001) reported that in terms of the adenosine effects on cyclic AMP accumulation in cells, adenosine was approximately equipotent at A_1, A_{2A}, and A_3 receptors, but about 50 times higher concentrations were needed at A_{2B} receptors to achieve comparable effects. The low affinity of A_{2B} receptors to endogenous adenosine can explain the lack of the early protective effects against 5-FU toxicity in our former experiments (Pospíšil *et al.* 2001) using the co-administration of dipyridamole and AMP, which is believed to act through the elevation of the natural agonist, adenosine. Two mechanisms might be responsible for the protective action mediated by NECA and CGS 21680. Either the activated receptors induce a decrease of G_1 to S transition and thus increase the resistance of cells to 5-FU, or by some other cell cycle-independent mechanisms of cytoprotection. It is known that apoptotic cell death can be evoked by the oxidative stress, i.e. by enhanced formation of reactive oxygen species damaging cellular structures (Buttke and Sandstrom 1994). It has been shown that activation of adenosine receptor signaling may counter the oxidative stress by increasing the activities of antioxidant enzymes and thus decreasing the cell damage (Ramkumar *et al.* 2001). A support for such an interpretation is provided by Walker *et al.* (1997) who reported that NECA and CGS 21680 delayed apoptosis in human neutrophils and proposed that these effects were mediated by the elevation of cyclic AMP. Both A_{2A} and A_{2B} receptors are coupled to Gs proteins and activate adenylyl cyclase, but they differ in their coupling to other proteins and in the activation of different transduction pathways (Fredholm *et al.* 2000, 2001).

As compared to the cytoprotective action induced by NECA, the sensitizing action occurring after a time period of 22 h seems to be mediated by an other receptor subtype. This effect has been mimicked by IB-MECA, an agonist of A_3 receptors, and can be attributed to enhanced cell cycling and thus to the induction of increased 5-FU sensitivity. Such an action of IB-MECA is consistent with recent findings of Bar-Yehuda *et al.* (2002) demonstrating the stimulatory action of this adenosine receptor agonist on the murine bone marrow colony forming cells *in vivo*. Furthermore, it has to be noted that the lag period of the occurrence of this effect is similar to that obtained when hematopoietic progenitor cells *in vivo* were triggered into the S phase by interleukin 1 (Neta *et al.* 1987) or isoproterenol, which acts through β_1-adrenergic receptors (Nečas *et al.* 1976). Interestingly, CPA, a selective agonist for A_1 receptors, mediated an opposite action compared to the agonist for A_3 receptors, i.e. enhanced resistance of hematopoiesis to 5-FU at this time interval. This effect can be due to decreased cell cycling. The fact that both these selective agonists induce significant effects suggests the action of A_1 and A_3 receptors in the compartments of hemato-poietic progenitor cells. In terms of their signaling pathways, both these receptors inhibit adenylyl cyclase (Fredholm *et al.* 2000), but it has been reported that they differ in their coupling to phospholipases C and D (Parsons *et al.* 2000). It can be the ratio of the expression of these two receptors, which determines the resulting effect on cell cycling. From this point of view, the sensitizing effects of the non-selective agonist NECA as well as of those mediated by the natural agonist adenosine, described by us previously (Pospíšil *et al.* 2001), might reflect the preponderance of A_3 receptor activity.

Further studies are needed to understand the mechanisms underlying the observed results. Even if not yet proved directly, the presence of adenosine receptors on hematopoietic progenitor cells is highly probable because they were detected in the mature blood cells (Cronstein *et al.* 1990, 1992, Walker *et al.* 1997, Gessi *et al.* 2000). However, the observed effects need not be mediated only by signaling through receptors located on the target cells, but also by indirect effects, such as the ability of adenosine agonists to modulate production of various cytokines and growth factors (Bouma *et al.* 1994, Haskó *et al.* 1998, Bar-Yehuda *et al.* 2002). In spite of these interpretation difficulties, the presented data provide evidence demonstrating the effects of stable adenosine analogues on the control processes in the compartments of hematopoietic progenitor cells *in vivo* and imply the possible therapeutic potential of these agents.

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References

ABBRACCHIO MP, BURNSTOCK G: Purinergic signalling: pathophysiological roles. *Jpn J Pharmacol* **78**: 113-145, 1998.

BAR-YEHUDA S, MADI L, BARAK D, MITTELMAN M, ARDON E, OCHAION A, COHN S, FISHMAN P: Agonists to the A_3 adenosine receptor induce G-CSF production via NF-κB activation: a new class of myeloprotective agents. *Exp Hematol* **30**: 1390-1398, 2002.

BOUMA MG, STAD RK, VAN DEN WILDENBERG FAJM, BUURMAN WA: Differential regulatory effects of adenosine on cytokine release by activated human monocytes. *J Immunol* **153**: 4159-4168, 1994.

BRACKETT LE, DALY JW: Functional characterization of the A_{2B} adenosine receptor in NIH 3T3 fibroblasts. *Biochem Pharmacol* **47**: 801-814, 1994.

BRUNS RF: Role of adenosine in energy supply/demand balance. *Nucleosides Nucleotides* **10**: 931-941, 1991.

BUTTKE TM, SANDSTROM PA: Oxidative stress as a mediator of apoptosis. *Immunol Today* **15**: 7-10, 1994.

CALABRESE EJ: Adenosine: biphasic dose responses. *Crit Rev Toxicol* **31**: 539-551, 2001.

CHADWICK M, ROGERS WI: The physiological disposition of 5-fluorouracil in mice bearing solid L1210 lymphocytic leukemia. *Cancer Res* **32**: 1045-1056, 1972.

CRONSTEIN BN, DAGUMA L, NICHOLS D, HUTCHISON AJ, WILLIAMS M: The adenosine/neutrophil paradox resolved: human neutrophils possess both A_1 and A_2 receptors that promote chemotaxis and inhibit O_2 generation, respectively. *J Clin Invest* **85**: 1150-1157, 1990.

CRONSTEIN BN, LEVIN RI, PHILIPS M, HIRSCHHORN R, ABRAMSON SB, WEISSMANN G: Neutrophil adherence to endothelium is enhanced via adenosine A_1 receptors and inhibited via adenosine A_2 receptors. *J Immunol* **148**: 2201-2206, 1992.

FEOKTISTOV I, WELLS JN, BIAGGIONI I: Adenosine A_{2B} receptors as therapeutic targets. *Drug Dev Res* **45**: 198-206, 1998.

FREDHOLM BB, ARSLAN G, HALLDNER L, KULL B, SCHULTE G, WASSERMAN W: Structure and function of adenosine receptors and their genes. *Naunyn-Schmiedeberg's Arch Pharmacol* **362**: 364-374, 2000.

FREDHOLM BB, ARSLAN G, HALLDNER L, KULL B, SCHULTE G, ÅDÉN U, SVENNINGSON P: Adenosine receptor signaling in vitro and in vivo. *Drug Dev Res* **52**: 274-282, 2001.

GESSI S, VARANI K, MERIGHI S, ONGINI E, BOREA PA: A_{2A} adenosine receptors in human peripheral blood cells. *Br J Pharmacol* **129**: 2-11, 2000.

HASKÓ G, NÉMETH ZH, VIZI ES, SALZMAN AL, SZABÓ C: An agonist of adenosine A_3 receptors decreases interleukin-12 and interferon-γ production and prevents lethality in endotoxemic mice. *Eur J Pharmacol* **358**: 261-268, 1998.

HOFER M, POSPÍŠIL M, NETÍKOVÁ J, ZNOJIL V, VÁCHA J: Granulocyte colony-stimulating factor and drugs elevating extracellular adenosine act additively to enhance the hemopoietic spleen colony formation in irradiated mice. *Physiol Res* **48**: 37-42, 1999.

HOFER M, POSPÍŠIL M, WEITEROVÁ L, ZNOJIL V, VÁCHA J, HOLÁ J, VACEK A, PIPALOVÁ I: Combination of drugs elevating extracellular adenosine with granulocyte colony-stimulating factor promotes granulopoietic recovery in the murine bone marrow after 5-fluorouracil treatment. *Physiol Res* **50**: 521-524, 2001.

HOFER M, POSPÍŠIL M, ZNOJIL V, VACEK A, WEITEROVÁ L, HOLÁ J, VÁCHA J: Drugs elevating extracellular adenosine promote regeneration of hematopoietic progenitor cells in severely myelosuppressed mice: their comparison and joint effects with the granulocyte colony-stimulating factor. *Eur J Haematol* **68**: 4-11, 2002.

JACOBSON MA: Adenosine receptor agonists. *Expert Opin Ther Patents* **12**: 489-501, 2002.

KLOTZ K-A: Adenosine receptors and their ligands. *Naunyn-Schmiedeberg's Arch Pharmacol* **362**: 382-391, 2000.

LINDEN J: Molecular approach to adenosine receptors: receptor-mediated mechanisms of tissue protection. *Annu Rev Pharmacol Toxicol* **41**: 775-787, 2001.

MOLINEUX G, MIGDALSKA A, HALEY J, EVANS GS, DEXTER TM: Total marrow failure induced by pegylated stem-cell factor administered before 5-fluorouracil. *Blood* **83**: 3491-3499, 1994.

NEČAS E, POŇKA P, NEUWIRT J: Study on the proliferative state of haemopoietic stem cells (CFU). *Cell Tissue Kinet* **9**: 223-230, 1976.

NETA R, SZTEIN MB, OPPENHEIM JJ, GILLIS S, DOUCHES SD: The in vivo effects of interleukin 1. I. Bone marrow cells are induced to cycle after administration of interleukin 1. *J Immunol* **139**: 1861-1866, 1987.

NETA R, KELLER JR, ALI N, BLANCHETTE F, DUBOIS CM: Contrasting mechanisms of the myeloprotective effects of interleukin-1 against ionizing radiation and cytotoxic 5-fluorouracil. *Radiat Res* **145**: 624-631, 1996.

NEWBY AC: Adenosine and the concept of "retaliatory metabolites". *Trends Biochem Sci* **9**: 42-44, 1984.

PARSONS M, YOUNG L, LEE JE, JACOBSON KA, LIANG BT: Distinct cardioprotective effects of adenosine mediated by differential coupling of receptor subtypes to phospholipases C and D. *FASEB J* **14**: 1423-1431, 2000.

POSPÍŠIL M, HOFER M, ZNOJIL V, VÁCHA J, NETÍKOVÁ J, HOLÁ J: Synergistic effect of granulocyte colony-stimulating factor and drugs elevating extracellular adenosine on neutrophil production in mice. *Blood* **86**: 3692-3697, 1995.

POSPÍŠIL M, HOFER M, ZNOJIL V, NETÍKOVÁ J, VÁCHA J, HOLÁ J, VACEK A: Granulocyte colony-stimulating factor and drugs elevating extracellular adenosine synergize to enhance hematopoietic reconstitution in irradiated mice. *Eur J Haematol* **60**: 172-180, 1998.

POSPÍŠIL M, HOFER M, VACEK A, NETÍKOVÁ J, HOLÁ J, ZNOJIL V, WEITEROVÁ L: Drugs elevating extracellular adenosine enhance cell cycling of hematopoietic progenitor cells as inferred from the cytotoxic effects of 5-fluorouracil. *Exp Hematol* **29**: 557-562, 2001.

POULSEN S-A, QUINN RJ: Adenosine receptors: new opportunities for future drugs. *Bioorg Med Chem* **6**: 619-641, 1998.

RAMKUMAR V, HALLAM DM, NIE Z: Adenosine, oxidative stress and cytoprotection. *Jpn J Pharmacol* **86**: 265-274, 2001.

UENO M, BROOKINS J, BECKMAN B, FISHER JW: A_1 and A_2 adenosine receptor regulation of erythropoietin production. *Life Sci* **43**: 229-237, 1988.

WALKER BAM, ROCCHINI C, BOONE RH, IP S, JACOBSON MA: Adenosine A_{2a} receptor activation delays apoptosis in human neutrophils. *J Immunol* **158**: 2926-2931, 1997.

WEITEROVÁ L, HOFER M, POSPÍŠIL M, ZNOJIL V, VÁCHA J, VACEK A, PIPALOVÁ I: Influence of the joint treatment with granulocyte clony-stimulating factor and drugs elevating extracellular adenosine on erythropoietic recovery following 5-fluorouracil-induced haematotoxicity in mice. *Eur J Haematol* **65**: 310-316, 2000.

Reprint requests

Professor Milan Pospíšil, MD, PhD, Institute of Biophysics, Academy of Sciences of the Czech Republic, Královopolská 135, 612 65 Brno, Czech Republic; fax: +420-541211293, e-mail: hofer@ibp.cz