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Effects of adenosine A_3 receptor agonist on bone marrow granulocytic system in 5-fluorouracil-treated mice

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Abstract

The purpose of the experiments reported was to investigate effects of N^6-(3-iodobenzyl)adenosine-5'-*N*-methyluronamide (IB-MECA), a selective adenosine A_3 receptor agonist, on the granulocytic system in femoral marrow of mice depleted by the cytotoxic drug 5-fluorouracil. In the phase of the highest cell depletion IB-MECA was injected i.p. at single doses of 200 nmol/kg given either once or twice daily in 2- and 4-day regimens starting on day 1 after 5-fluorouracil administration; the effects were evaluated on days 3 and 5, respectively. The general effect of IB-MECA in all these experiments was an enhancement of the counts of morphologically recognizable proliferative granulocytic cells, interpreted as evidence of the differentiation of committed progenitor cells. A more expressive effect was observed after IB-MECA injected twice daily. It was found that the induction of the strong differentiation pressures by IB-MECA given twice daily shortly after 5-fluorouracil treatment can be counterproductive due to the preponderance of differentiaton processes over the proliferation control. In additional experiments, it has been shown that the use of the 2-day administration of IB-MECA given twice daily in the recovery phase, i.e., on days 5 and 6 after 5-fluorouracil administration, does not induce stimulatory effects. Thus, the dosing and timing of IB-MECA treatment determines its effectivity in stimulating granulopoiesis under conditions of myelosuppression.

Keywords: Adenosine A_3 receptor; Granulopoiesis; Myelosuppression; 5-Fluorouracil; (Mouse)

1. Introduction

The concept of adenosine receptor signalling is widely explored in various areas of physiology and pathophysiology including the regulation of the proliferation and differentiation of cells (Abbracchio, 1996; Schulte and Fredholm, 2003). So far, four adenosine receptors, i.e. A_1, A_{2a}, A_{2b}, and A_3 coupled to G proteins have been classified (Fredholm et al., 2000). These receptors can play a role also in haematopoiesis. In earlier studies we have shown that elevation of extracellular adenosine enhances haematopoiesis in normal and myelosuppressed mice and synergizes with effects of the granulocyte colony-stimulating factor (Hofer et al., 1997, 1999, 2002; Pospíšil et al., 1995, 1998). Moreover, we have demonstrated that elevation of extracellular adenosine increases cycling of haematopoietic progenitor cells as inferred from the cytotoxic effects of 5-fluorouracil (Pospíšil et al., 2001). Other authors have found that the selective agonist of adenosine A_3 receptors, N^6-(3-iodobenzyl)adenosine-5′-*N*-methyluronamide (IB-MECA), induces similar effects on granulopoiesis (Bar-Yehuda et al., 2002; Fishman et al., 2000a). Gessi et al. (2002) have shown that adenosine A_3 receptors are present in human neutrophils as well as in promyelocytic HL60 cells, thus suggesting that this receptor subtype can be functional at the early stages of myeloid differentiation. Thus, the adenosine A_3 receptor can be responsible for the above-mentioned findings. In our experiments we have demonstrated stimulatory effects of IB-MECA on the cycling of granulocytic progenitor cells (Pospíšil et al., 2004, 2005). Interestingly, it has been shown by Fishman et al. (2000b) and also in our laboratory (Hoferová et al., 2003), that IB-MECA induces cytostatic effects in various tumour cell

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systems both in vitro and in vivo. The association of the stimulatory action of IB-MECA on haematopoiesis with its inhibitory action on the growth of tumour cells is promising from practical point of view, because myelosuppression belongs to undesirable complications of chemo- and radiotherapy of tumours. Thus, effects of IB-MECA on haematopoiesis deserve further attention. Recently, there has appeared a clinical study on the tolerability and pharmacokinetics of IB-MECA in healthy men (Van Troosteburg et al., 2004).

The purpose of the presented experiments was to investigate the effects of IB-MECA administration on the suppressed bone marrow granulopoiesis in mice pretreated with the cytotoxic drug 5-fluorouracil. Attention was focused on the timing of IB-MECA administration with the aim to define principles of the optimum treatment schedules.

2. Materials and methods

2.1. Animals

B10CBAF$_1$ male mice aged 3 months and weighing in average 30 g 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 were available ad libitum. The use and treatment of the animals followed the European Community Guidelines as accepted principles for the use of experimental animals. The experiments were performed with the approval of the Institute's Ethics Committee.

2.2. Drugs

N^6-(3-iodobenzyl)adenosine-5′-*N*-methyluronamide (IB-MECA), the agonist of adenosine A_3 receptors, was dissolved initially in dimethyl sulphoxide, then diluted with sterile saline and injected i.p. at single doses of 200 nmol/kg in a volume of 0.2 ml. The final concentration of dimethyl sulphoxide was 2%. The schemes of administration of IB-MECA are given under Results. The choice of the dose of IB-MECA was based on our former experiments showing that this dose induces cycling of murine haematopoietic progenitor cells under in vivo conditions (Pospíšil et al., 2004, 2005). 5-Fluorouracil was diluted in saline and injected i.p. at a single dose of 100 mg/kg in a volume of 0.2 ml. The corresponding drug vehicles were used for control injections. All the drugs were obtained from Sigma (St. Louis, MO, USA).

2.3. Haematological methods

Blood samples were taken from the tail vein. Mice were then sacrificed by cervical dislocation. The femurs were dissected and marrow cells were flushed from the bone. Blood cell counts and numbers of nucleated cells of the bone marrow were determined using a Coulter Counter (model ZF; Coulter Electronics, UK). Differential counts were performed on blood and marrow smears stained with the May–Grünwald–Giemsa method. Based on the differentiation of marrow cells, the counts of proliferative (myeloblasts through myelocytes) and nonproliferative (metamyelocytes through segmented stages) granulocytic cells per femur were determined. Total number of granulocytic cells per femur represents the sum of the proliferative and nonproliferative cells. Bone marrow haematopoietic progenitor cells committed to granulocyte–macrophage development (granulocyte–macrophage colony-forming cells [CFC-GM]) were assayed using a semisolid plasma clot technique. Femoral marrow cell suspensions were plated in triplicate and incubated at 37 °C in humidified atmosphere containing 5% CO_2. CFC-GM were scored after 7-day incubation as colonies containing 50 or more cells. The numbers of CFC-GM per femur were calculated.

2.4. Statistics

The data are given as means ± S.E.M. Experiments were repeated twice to three times and the data were pooled. Mann–Whitney rank sum test was used for comparison of the effects and the Holm's method was applied to correct for multiple comparisons. The significance level was set at $P<0.05$.

3. Results

3.1. Effects of 5-fluorouracil alone on granulocytic cells

Fig. 1 demonstrates effects of 5-fluorouracil alone on the total number of granulocytic cells in femoral marrow compared to that obtained in untreated control mice. As shown, the cell system responded within the 7-day interval by phases of depletion and recovery. On the basis of such time course of the bone marrow cell response, the effects of IB-MECA were investigated separately in both these phases.

3.2. Effects of IB-MECA administered in the phase of cell depletion

IB-MECA at a dose of 200 nmol/kg per each injection was given i.p. on days 1 and 2 after 5-fluorouracil administration in

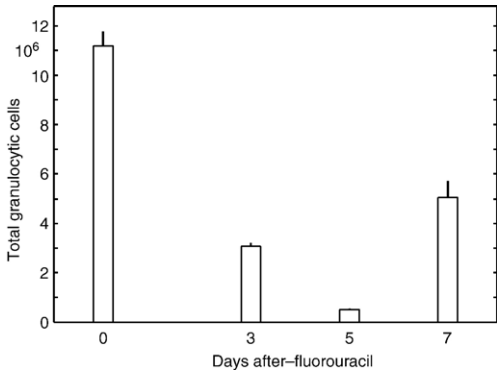

Fig. 1. Effects of 5-fluorouracil (100 mg/kg) alone on the counts of total granulocytic cells in femoral marrow of mice. Baseline values found in untreated control mice are given on day 0. Data are given as means ± S.E.M; 10 to 30 mice per group were used.