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ROLE OF ADENOSINE SIGNALING IN HEMATOPOIESIS – A SHORT REVIEW

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MINI-REVIEW

ABSTRACT. HITHERTO OBTAINED DATA on the action of adenosine receptor signaling in the control hematopoiesis are summarized and discussed. Four subtypes of adenosine membrane receptors, designated as A₁, A_{2A}, A_{2B}, and A₃, are known to exist. Their pharmacologically induced activation can be either non-selective, elicited through elevation of extracellular adenosine levels, or by administration of synthetic adenosine analogs, more or less specific for individual receptor subtypes. The data acquired mostly from in vivo experiments on mice show evidence about an important role of adenosine receptor signaling in the hematopoietic regulatory network. Non-selective activation of adenosine receptors evoked by combined administration of dipyridamole, preventing the cellular uptake of adenosine, and adenosine monophosphate, an adenosine prodrug, has been found to stimulate hematopoiesis in normal mice, as well as in animals in which myelosuppression was induced by ionizing radiation or cytotoxic drugs. Studies using synthetic adenosine analogs have shown that activation of adenosine A₃ receptors is responsible for this stimulation and has curative effects. Directions for further research aimed at revealing exact mechanisms of adenosine receptor signaling in hematopoiesis are briefly delineated.

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1. BACKGROUND

Hematopoiesis is under the control of a number of regulatory factors acting on stem and progenitor cells of the hematopoietic system resulting in production of mature functional cells. Action of these factors is often pleiotropic, mutually overlapping, and final effects result from their interactions in a complex regulatory network. Clarification of the role of the individual factors and studies on their interactions may contribute not only to better understanding of mechanisms of the control of hematopoietic processes but also to setting optimal therapeutical procedures targeted on stimulating hematopoiesis suppressed, e.g., by cytostatic chemotherapy or ionizing radiation [1].

It has been recognized that adenosine, an ubiquitous purine nucleoside released into the extracellular environment from metabolically active or stressed cells, acts as a paracrine regulator of many cellular functions including those of cell proliferation and differentiation [2-5]. The regulatory role of extracellular adenosine is based on the activation of specific receptors located on the cell surface. Functional and molecular studies have demonstrated that adenosine receptors can be classified as A₁, A_{2A}, A_{2B}, and A₃ subtypes [6]. Adenosine receptors are coupled to several different G proteins which modulate the activity of various enzymes like adenylyl cyclase, phospholipase, mitogen-activated protein kinases, etc. [7]. More than one adenosine receptor can be expressed on a single cell and a mutual antagonism of the receptors cannot be excluded. Activation of adenosine receptors can be achieved either non-selectively, by the action of the natural agonist, i.e. adenosine itself, or by the activation of individual adenosine receptor subtypes by synthetic adenosine analogs having various proportions of selectivity for a particular receptor [8,9]. Even when the main application domains of the concept of adenosine receptor signaling are the areas of cardiovascular and nervous physiology, it is possible to mention some earlier suggestions explaining adenosine actions on hematological functions. These include the findings demonstrating the participation of adenosine receptor signaling in the control of margination, O₂ generation, and phago-

cytic activities of blood neutrophils [10] and, more generally, also in the response of the cells of the adaptive immune system [11]. Furthermore, it has been shown that the addition of adenosine to murine long-term bone marrow cultures results in an increased production of granulocytes [12]. Such an accumulated knowledge motivated the attempts to investigate the possible role of adenosine receptor signaling in the regulation of hematopoiesis. This paper summarizes the hitherto obtained data reflecting the importance of the regulatory role of adenosine and of its receptors in hematopoiesis and points out open questions in this field.

2. NON-SELECTIVE ACTIVATION OF ADENOSINE MEMBRANE RECEPTORS BY ELEVATED EXTRACELLULAR ADENOSINE

Pharmacologically evoked elevation of extracellular adenosine leads to an enhancement of non-selective receptor-mediated adenosine action. Dipyridamole, a drug inhibiting the cellular uptake of adenosine [13,14], has been used for obtaining higher extracellular adenosine levels, for discrimination between receptor-mediated and non-receptor-mediated mechanisms of adenosine action, and/or for potentiation of the receptor-mediated mode of adenosine functioning, e.g., in studies carried out with normal and transformed keratinocytes [15], neuroblastoma cells [16], adenocarcinoma and bladder carcinoma cells [17], or fibrosarcoma cells [18]. Less attention has been paid to the evaluation of the effects of elevated extracellular adenosine levels on various populations of the hierarchically organized hematopoietic cell system which is under the control of a complex regulatory network. In the published studies on mice, elevated extracellular adenosine levels and thereby evoked enhancement of non-selective activation of adenosine membrane receptors have been achieved by combined administration of dipyridamole and adenosine monophosphate. Adenosine monophosphate (AMP), which is rapidly metabolized extracellularly to adenosine by cell-surface ectonucleotidase activity [19], has served as an adenosine prodrug having a higher solubility in water as com-

pared with adenosine, and, therefore, being more convenient for *in vivo* administration. It should be noted that doses of dipyridamole and AMP used in the experiments described in the following text exhibit significant pharmacological effects at low toxicity [20]. The efficiency of the combined action of the drugs compared to their action when given alone has been verified [21].

First suggestions of the role of extracellular adenosine in modulation of hematopoietic functions under *in vivo* conditions when using the above-described approach for increasing its level were brought by radiobiological experiments. The combination of dipyridamole and AMP administered several minutes before whole-body gamma-irradiation of mice decreased cellular damage as revealed by the thymidine level in plasma and the amount of free polynucleotides in the thymus and spleen [22]. Similar effects of the drug combination were found when administering the drugs immediately after irradiation [23]. In addition it has been ascertained that preirradiation administration of the drugs decreases the formation of micronucleated polychromatic erythrocytes, an indicator of the genetic radiation damage [24]. Mechanisms of the effects of adenosine receptor action on these early indices of cell radiosensitivity are not clear. However, it seems that the short-term reduction of the blood pressure due to bradycardia and vasodilation, which are known effects of the receptor action of extracellular adenosine and lead to a transient systemic hypoxia [2], can be responsible for this effect; hypoxia-induced radioprotection is a classical radiobiological phenomenon. Another alternative can be the enhancement of the mechanisms of the intracellular repair.

Further experiments investigated the manifestations of the postirradiation bone marrow syndrome, *i.e.*, suppression of hematopoiesis in sublethally irradiated mice, and survival of animals irradiated with absolute lethal doses. These experiments confirmed the protective action of dipyridamole and AMP combination under conditions of single [20,25,26] and also fractionated irradiation [27,28]. These effects are most probably due to the modulation of regulatory functions, *i.e.* to the stimulatory action of adenosine receptor signaling on hemato-

poiesis. This has been suggested by experiments utilizing administration of noradrenaline which reduces cardiovascular and thus hypoxia-inducing effects of the combination of dipyridamole plus AMP [25] and, furthermore, by experiments prolonging the time interval between the administration for the drug combination and irradiation so as to avoid the operation of hypoxia during the radiation exposure [28]. In both these situations protective action of adenosine receptor signaling occurring in hematopoiesis and postirradiation survival of animals was preserved.

Several studies focused attention to the action of extracellular adenosine in normal and myelosuppressed mice. It has been found that administration of dipyridamole and AMP to normal mice induces pleiotropic amplification effects in cell compartments of the bone marrow and spleen [21,29], enhances cycling of committed hematopoietic progenitor cells [30], and mobilizes these cells into peripheral blood [31]. The possibility that elevation of extracellular adenosine activates mechanisms of the positive control of hematopoiesis was supported also by findings demonstrating curative effects of the drugs administered repeatedly under conditions of myelosuppression induced in mice by irradiation [32], cytostatic drugs [33,34], and combination of both these myelosuppressive actions [35]. Enhancement of the regeneration of hematopoiesis by the combination of drugs elevating extracellular adenosine has been found in all these experiments.

Previously it has been shown that adenosine signaling can act in concert with conventional growth factors, cytokines and other growth regulatory molecules to modulate cell functions in various non-hematopoietic cell systems [4]. Such effects were observed also in studies investigating the effects of combined administration of drugs elevating extracellular adenosine with granulocyte colony-stimulating factor (G-CSF) on hematopoiesis. Mutual potentiation of granulopoiesis-stimulating effects of these agents has been observed in normal mice [29,31], and in mice treated under conditions of myelosuppression induced by radiation [32,36], cytostatic drugs [34], or a combination of both these myelosuppressive actions [35]. These results may have clinical impact, especially in oncology.

3. SELECTIVE ACTIVATION OF ADENOSINE MEMBRANE RECEPTORS BY SYNTHETIC ADENOSINE RECEPTOR AGONISTS

To obtain more detailed information about mechanisms of extracellular, receptor-mediated adenosine action on hematopoiesis and to uncover new therapeutic possibilities of treatment of hematopoietic suppression by activating adenosine receptors, further experimental steps should have been carried out taking into account the known adenosine receptor diversity. Discrimination between adenosine receptors was made possible when more or less selective adenosine receptor agonists became available. These agents, adenosine analogs, do not undergo facilitated uptake nor represent a substrate for cellular metabolism and are relatively resistant to extracellular adenosine-metabolizing enzymes [8,9]. In recent experiments on mouse hematopoiesis, selected synthetic adenosine analogs, namely 5'-N-ethyl-carboxamidoadenosine (NECA, non-selective adenosine receptor agonist), N⁶-cyclopentyladenosine (CPA, A₁-selective), 2-p-(2-carboxyethyl)-phenethylamino-5'-N-ethylcarboxamidoadenosine (CGS 21680, A_{2A}-selective), and N⁶-(3-iodobenzyl)adenosine-5'-N-methyluronamide (IB-MECA, A₃-selective) have been employed. The hitherto obtained results of experiments exploiting the model of hematopoietic damage induced by 5-fluorouracil (5-FU), a cycle-specific cytostatic drug, document the significant action of the above-mentioned adenosine receptor agonists on hematopoietic functions. When comparing the effects of different agonists, it has been shown that activation of some adenosine receptor subtypes induces cell cycle independent protective effects (A₂ receptors), while others modulate the cell cycle of hematopoietic progenitor cells in the stimulatory and inhibitory way and thus induce sensitization (A₃ receptors) or protection (A₁ receptors) towards the cytotoxic effects of 5-FU [37,38]. The evidence that the adenosine A₃ receptor agonist IB-MECA sensitizes the hematopoietic progenitor cells towards 5-FU indicates its stimulatory action in the cycling of these cells. The findings concerning the action of A₃ receptors are in accordance with those reported by Fishman's group,

which has demonstrated the stimulatory action of IB-MECA, an agonist of A₃ receptors, on hematopoietic recovery in mice myelosuppressed by cyclophosphamide [39,40]. It is evident that the activation of different adenosine receptor subtypes can induce multiple effects in hematopoiesis and that adenosine receptors represent a regulatory system with both positive and negative control action in some hematopoietic functions. The data on the positive effects of an A₃ receptor agonist on hematopoiesis are of special interest in view of findings demonstrating inhibitory effects of the agonists of A₃ receptors on tumor cell growth [41], a phenomenon observed also in experiments of the authors of this paper [42]. These findings can be of clinical importance because myelosuppression belongs to undesirable complications of chemo- and radiotherapy of tumors.

4. CONCLUSIONS AND DIRECTIONS OF FURTHER RESEARCH

The so far accumulated data suggest the significance of adenosine receptor signaling in hematopoiesis and point out the possibilities of practical utilization of pharmacologically evoked activation of adenosine receptors in clinical practice. Nevertheless, the topic is still far from complete understanding.

The first open question is whether the observed effects of adenosine signaling in hematopoiesis represent the consequence of a direct activation of adenosine receptors on hematopoietic progenitor cells or whether the effects on hematopoietic cells are secondary, mediated through cytokines and/or hematopoietic growth factors released from auxiliary cells of the hematopoietic system, like macrophages, which are stimulated primarily by adenosine or its analogs. Published detection of adenosine A₃ receptors on neutrophils and promyelocytic HL-60 cells [43] suggests indirectly that these receptors can be present also on hematopoietic progenitor cells and that their direct stimulation might be thus possible. Nevertheless, recent observations from the laboratory of the authors of this paper show that serum from mice administered adenosine

monophosphate shows colony-stimulating activity, i.e. the ability to stimulate the growth of hematopoietic progenitor cells *in vitro* (Weiterová et al., unpublished results). These findings bear an evidence of the presence in the serum of some stimulatory factor(s) released from auxiliary cells by the action of adenosine and, thus, they support the possibility of indirect action of adenosine on hematopoietic cells. Therefore, the problem of contribution of direct and indirect mechanisms of the action of adenosine receptor signaling on hematopoietic cells is still to be resolved. In addition, the problem of antagonism of different adenosine receptor subtypes in the regulatory network remains to be analysed.

In order to better understand the mechanisms of the observed effects, further methodical approaches have to be used. These can include the utilization of advances in adenosine receptor antagonist research [44], as well as the use of transgenic mice with genetically deleted or overexpressed adenosine receptor subtypes [45]. Up to now, neither of these approaches has been utilized in the reviewed field.

Mutually potentiating effects of G-CSF and drugs activating non-selectively adenosine receptors were confirmed in a number of studies mentioned above. Still open is the possibility to combine the drugs elevating extracellular adenosine or selected adenosine receptor agonists with other cytokines and/or growth factors known for their hematostimulatory effects. Recent study from the laboratory of the authors has shown that under conditions *in vitro*, adenosine potentiates significantly the effects of stem cell factor and interleukin-3 on the growth of mouse hematopoietic progenitor cells for granulocytes and macrophages (GM-CFC) [46]. An extension of this study to *in vivo* conditions is foreseen.

A potential problem of practical significance might arise when considering administration of adenosine analogs in human clinical practice because the results published so far have been obtained in experiments on animals or tissue cultures and their validity in human physiology remains to be ascertained. Recently a clinical study has been published which reports on tolerability and pharmacokinetics of CF101, an A₃ receptor agonist,

which may be administered *per os* [47]. From the point of view of contingent use of adenosine analogs in the clinics in hematological indications, A₃ adenosine receptor agonists represent one of the most promising groups of adenosine analogs because of their stimulatory effects on hematopoiesis.

Worth consideration are also the findings of desensitization of adenosine A₃ receptors after their activation in cell systems *in vitro* [48], especially from the point of view of possible repeated administration of the adenosine receptor agonists to the patients. The results of recent *in vivo* experiments on mice performed by the authors and comprising the administration of as much as eight IB-MECA doses in the course of 4 days (Hofer et al, unpublished results) do not, however, suggest any expressive influence of this phenomenon on the action of this A₃ adenosine receptor agonist *in vivo*.

To summarize, further investigations of the role of adenosine receptor signaling in hematopoiesis, especially those using the selective adenosine receptor agonists, seem to be well substantiated. Such studies can be important not only for fundamental research but also for clinical applications.

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