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Combined pharmacological therapy of the acute radiation disease using a cyclooxygenase-2 inhibitor and an adenosine A_3 receptor agonist

Mini-Review

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Abstract: Combined approaches to the treatment of acute radiation disease are preferred to single-agent therapies due to proven or anticipated better outcomes comprising increased therapeutic efficacy and decreased incidence of undesirable side effects. Our studies on post-exposure treatment of mice irradiated by sublethal or lethal doses of ionizing radiation included testing the effectiveness of meloxicam, a cyclooxygenase-2 inhibitor, and IB-MECA, an adenosine A3 receptor agonist. The efficacy of meloxicam and IB-MECA to positively influence the progress of the acute radiation disease has been tested in situations of their combined administration with granulocyte colony-stimulating factor (G-CSF) or with each other. The results of our studies revealed a significantly improved regeneration of hematopoietic cell populations ranging from the bone marrow progenitor cells to mature blood cells following combined treatments. Also, survival of mice exposed to lethal radiation doses was highest in the animals treated with a combination of the two drugs. It can be inferred from the results that if the drug combinations employed were used in humans, *e.g.* in the treatment of victims of radiation accidents, a better therapeutic outcome could be expected. Therefore, further studies directed at clinical applications of meloxicam and IB-MECA in radiation victims is recommended.

Keywords: *Hematopoiesis • Radiation-induced myelosuppression • Post-radiation pharmacological approach • Cyclooxygenase inhibition • Adenosine receptor agonist*

1. Introduction

The history of investigations on pharmacological modulation of acute radiation disease is long but the reflection of these studies into the availability of the investigated agents for humans is generally unsatisfactory. Toxicity with accompanying undesirable side effects is the most important factor limiting the practical usefulness of substances with proven effectiveness to mitigate the radiation-induced damage to tissues and organs. In the past most of the efforts on optimizing pharmacological procedures aimed at alleviation of the consequences of radiation exposure by deterministic doses of ionizing radiation were concentrated on radioprotective agents, *i.e.* on substances administered before irradiation. Reduction of toxicity of an effective radioprotective agent, WR-2721, by its combined administration with other agents was the principal target of many studies [see, *e.g.*, 1].

Recent and current endeavours in the field of pharmacological modulation of acute radiation disease are concentrated on the investigation of therapeutical agents, *i.e.* of drugs administered after irradiation. These agents should help to treat patients and radiation victims exposed to high doses of ionizing radiation as a consequence of radiation accidents or radiological (nuclear) terrorist attacks [2-6]. The topic of "Therapeutic Agents (Postexposure Treatment)" has been given top priority in the Priority List of Research Areas for Radiological Nucelar Threat Countermeasures by Pellmar *et al.* [7].

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The laboratory of the authors has been engaged in studies on the topics sumarized above for many years. This minireview emphasizes two agents, meloxicam - a cyclooxygenase-2 inhibitor and IB-MECA - an adenosine A_3 receptor agonist, that may be promising given the possibility of their contingent use in clinical practice in the treatment of acute radiation disease.

2. Introduction to Radiobiological Studies on Cyclooxygenase Inhibitors

Inhibitors of cyclooxygenase, *i.e.* inhibitors of prostaglandin synthesis, also known as non-steroidal anti-inflammatory drugs (NSAIDs), are commonly used drugs. Cyclooxygenase exists in two subtypes, namely as cyclooxygenase-1 (COX-1), which is expressed constitutively in a variety of tissues including the gastrointestinal tract, and cyclooxygenase-2 (COX-2), which is inducible and responsible for production of prostaglandins during inflammatory states [8-10]. Prostaglandin E_2 was found to participate in the regulation of hematopoiesis and to play an important role in the negative hematopoietic feedback control [11,12]. Previous studies from our laboratory have shown that non-selective NSAIDs, like indomethacin, diclofenac, ibuprofen, or flurbiprofen, stimulate hematopoiesis in sublethally irradiated mice when administered before or after irradiation (reviewed in [13]). However, when non-selective NSAIDs were administered to lethally irradiated mice, their survival was observed to be significantly lower in comparison with control animals; this observation could be ascribed to the lack of COX-1 in the gastrointestinal tract where prostaglandins play a protective role [13]. An increased intestinal damage caused by the combination of lethal irradiation and administration of non-selective NSAIDs was also experimentally proved [14]. Modern NSAIDs, selective for COX-2, show lower incidence of undesirable effects on the gastrointestinal tract because synthesis of prostaglandins in these tissues is ensured by functional COX-1. Therefore, we have performed a series of experiments with one of the COX-2-selective NSAIDs, meloxicam, and we have shown that meloxicam retains the ability of non-selective NSAIDs to stimulate hematopoiesis in sublethally irradiated mice while increasing survival when given to lethally irradiated animals (summarized in [15] and recently confirmed by Hoggatt *et al.* [16]).

3. Introduction to Radiobiological Studies on Adenosine Receptor Agonists

Adenosine acts in a regulatory sense through cellular membrane receptors. Linden [17] stated that adenosine was a primordial signaling molecule which had evolved to modulate physiological responses in all mammalian tissues. Adenosine membrane receptors exist in four varieties, namely as A_1, A_{2a}, A_{2b}, and A_3 subtypes [18,19]. Studies from our laboratory have shown that non-selective activation of adenosine receptors, achieved by combined administration of adenosine monophosphate - an adenosine prodrug and dipyridamole, prevented adenosine uptake by cells thus increasing its extracellular concentration. This increase in extracellular adenosine stimulates hematopoiesis (also when administered in combination with G-CSF [20]) and increases survival of irradiated mice. Subsequent investigations have shown that selective stimulation of adenosine A_3 receptors induced by administration of a selective adenosine A_3 receptor agonist, such as IB-MECA, is responsible for the previously observed hematopoiesis-stimulating effects of non-selective activation of adenosine receptors [summarized in 21,22]. Thus, the field of study of effects of administration of IB-MECA to radiation-exposed mammals was opened.

4. Combined Administration of Meloxicam and G-CSF

Combined administration of meloxicam and G-CSF was tested in two therapeutical (post-irradiation) treatment regimens. The first one consisted of four doses of meloxicam, G-CSF, or meloxicam + G-CSF on days 3, 4, 5, and 6 after irradiation of mice with a dose of 4 Gy of γ-rays. The second regimen consisted of two doses of meloxicam on days 3 and 5 post-irradiation with the above radiation dose, two doses of G-CSF on days 4 and 6 after irradiation, or a combination of both previously mentioned administration schedules. In both the regimens mentioned, both meloxicam and G-CSF alone significantly increased the numbers of femoral bone marrow progenitor cells for granulocytes and macrophages (GM-CFC) in comparison with the vehicle-treated controls on day 7 after irradiation [23]. Moreover, the combinations of meloxicam + G-CSF were found to further significantly increase GM-CFC numbers when compared with those attained after administration of the aforementioned drugs alone [23]. Of interest is the fact that meloxicam itself was observed to elevate the serum levels of G-CSF [23,24]. Thus, a combination of

exogenous G-CSF with endogenously induced G-CSF elevation may prove useful in the treatment of the acute radiation disease.

5. Combined administration of IB-MECA and G-CSF

A post-irradiation treatment with IB-MECA and G-CSF administered as single agents or in a combination on days 1 and 2 after exposure of experimental mice to a dose of 4 Gy of γ-rays was followed by samples on days 3, 6, 12, 17, and 22 post-irradiation. In the compartment of bone marrow granulocyte/macrophage progenitor cells (GM-CFC), the highest values were found in the combination-treated group on days 3, 6, and 22 after irradiation. In these mice, the numbers of GM-CFC per femur were significantly higher not only in comparison with the controls, but also when compared with the groups of mice administered with IB-MECA or G-CSF alone [25]. The same findings were obtained for the marrow progenitor cells for erythrocytes (BFU-E) for the sampling intervals of days 3 and 6 [25]. With regards to peripheral blood neutrophils, a signficant elevation of their numbers in the combination-treated group when compared to those in the solvent-treated controls could be stated when their values were aggregated in the time interval of days 3 to 17 [25]. In our study [25] we did not confirm the previously reported ability of IB-MECA to induce G-CSF production [26]. It remains therefore to consider, by means of further studies, the role of IB-MECA-induced endogenous G-CSF under conditions of our radiobiological experiments.

6. Combined administration of meloxicam and IB-MECA

Following encouraging results with post-irradiation treatment with combinations of meloxicam or IB-MECA with G-CSF, a question remained whether meloxicam and IB-MECA are able to potentiate mutually their stimulatory action on radiation-suppressed hematopoiesis. In an experiment, in which meloxicam was administered in a single dose prior to irradiation (1 hour) and IB-MECA in two doses (24 and 28 hours) after irradiation with the dose of 4 Gy, an effectiveness of combined tretament with both the agents on day 3 after irradiation was observed when looking at femoral GM-CFC, BFU-E, and proliferative granulocytic cells (bone marrow granulocytic precursor cells) [27]. The combination of meloxicam and IB-MECA was also tested in a survival experiment. In this study, the mice were irradiated with the dose of 8.5 Gy of γ-rays, IB-MECA was administered in a single dose 0.5 hour after irradiation and meloxicam in a single dose 1 hour after administration. Thirty-day survival was 46.7%, 66.7%, 60.0%, and 86.7% in control mice, mice treated with IB-MECA alone, mice administered meloxicam alone, and mice administered the combination of IB-MECA + meloxicam, respectively. A significantly improved cumulative 30-day survival and of the mean survival time were observed in the combination-treated mice when compared with those found in vehicle-treated control animals [28].

7. Discussion

As stated by Waselenko *et al.* [4], the recommended therapy for the hematopoietic syndrome of acute radiation disease, which is clinically the most probable form of acute radiation syndrome, is using hematopoietic cytokines, blood transfusion, and/or stem cell transplantation, accompanied by supporting therapy like antimicrobial agents, antiemetics and analgesic agents. Hematopoietic cytokines are often administered in combinations, like megakaryocyte growth and development factor + G-CSF [29] or stem cell factor + erythropoietin + G-CSF [30]. “Nontraditional” means like meloxicam or IB-MECA could fittingly supplement the spectrum of drugs used or recommended for the treatment of acute radiation syndrome at present. Until now, combined therapies of acute radiation disease using COX-2 inhibitors and/or adenosine A_3 receptor agonists have not been studied by other investigators than the authors of this communication.

When considering pharmacological approaches to the therapy of acute radiation disease comprising of meloxicam and /or IB-MECA, it should be also taken into account that a part of these studies were done employing administration schedules using very early post-irradiation administration of the drugs tested. This holds true also for the combined administration of IB-MECA + meloxicam. Such an early approach to the treatment of irradiated humans would be in agreement with the findings and concepts of Hérodin *et al.* [31-33] which emphasize the necessity of ”The Sooner The Better” post-irradiation therapy of radiation victims with the aim to influence post-irradiation phenomena like cell apoptosis.

8. Conclusions

Our results on hematopoiesis-stimulating and survival-increasing effects of post-irradiation (therapeutically) administered drug combinations comprising

cyclooxygenase-2 inhibitor, meloxicam, and adenosine A_3 receptor agonist - IB-MECA, strongly suggest that these drugs could be useful in the treatment of the acute radiation disease in humans. A supporting factor for this conclusion comes also from the fact that meloxicam is already in clinical use for other indications. IB-MECA is under clinical studies (for other indications, too) in which it was found to be safe and well tolerated [34-36].

Conflict of interest

The authors stated that there are no conflicts of interest regarding the publication of this article. Research support played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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