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SHORT COMMUNICATION

IB-MECA, an Adenosine A_3 Receptor Agonist, Does Not Influence Survival of Lethally γ-Irradiated Mice

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Summary

In our previous studies, IB-MECA, an adenosine A_3 receptor agonist, was found to stimulate proliferation of hematopoietic progenitor and precursor cells in mice. This property of IB-MECA was considered to be responsible for its ability to support regeneration of suppressed hematopoiesis after irradiation with sublethal doses of γ-rays when the drug was given in a post-irradiation treatment regimen. This study was aimed at assessing the ability of IB-MECA to influence a 30-day survival of lethally irradiated mice. In a series of experiments, IB-MECA was administered following various lethal radiation doses in various numbers of drug doses and various administration routes. Though in some of these experiments a moderate increase in 30-day survival was observed in IB-MECA-treated mice, the differences in comparison with the controls were not significantly different. It can be inferred from these results and those of previous studies assessing the effects of IB-MECA after sublethal radiation doses that IB-MECA can probably influence only a substantially preserved hematopoiesis like that remaining after sublethal irradiation. Future studies should be aimed at evaluation of the abilities of IB-MECA to influence post-irradiation survival when administered as a part of combined treatment regimens.

Key words

Mouse • IB-MECA • Adenosine A_3 receptor agonist • Lethal γ-irradiation • Survival

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The topic "Therapeutic agents (postexposure treatment)" with the objective "To develop new therapeutic agents that can be used to treat people who have been exposed to ionizing radiation" was given a top priority among research areas for radiological nuclear threat countermeasures (Pellmar *et al.* 2005).

Adenosine, a naturally occurring nucleoside, was found to play a regulatory role in many organ systems in the mammals by acting on cell membrane receptors. It was stated that the regulatory activities of adenosine represented a universal intercellular communication system (Abbracchio 1996) and that adenosine was a primordial signaling molecule modulating physiological responses in all mammalian tissues (Linden 2001). Up to date four subtypes of adenosine membrane receptors were described, namely A_1, A_{2a}, A_{2b}, and A_3. Activation of adenosine receptors can be achieved either non-selectively, by adenosine, an endogenous agonist, or selectively by the use of various adenosine analogs which exhibit different degrees of receptor specificity (Abbracchio and Burnstock 1998, Klotz 2000).

In experimental studies on hematopoiesis, a non-selective adenosine receptor activation, achieved by a combination of adenosine monophosphate, an adenosine prodrug, and dipyridamole, a drug inhibiting the cellular

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uptake of adenosine, was found to stimulate regeneration from radiation-induced myelosuppression (Pospíšil *et al.* 1992, 1993, 1995, 1998, Hofer *et al.* 1997, 1999, 2002). The non-selective activation of adenosine receptors was found to induce also an increased survival of lethally irradiated mice (Pospíšil *et al.* 1993, 1995).

Further hematological investigations were carried out using synthetic adenosine analogs, more or less specific for the individual receptor subtypes. N^6-(3-iodobenzyl)adenosine-5'-*N*-methyluronamide (IB-MECA), a selective agonist of the adenosine A_3 receptors, was observed to stimulate proliferation of hematopoietic progenitor cells for granulocytes/ macrophages and erythrocytes (Pospíšil *et al.* 2004). IB-MECA was reported to positively influence the recovery from myelosuppression evoked by anti-cancer chemotherapy (Fishman *et al.* 2000, 2001, Bar-Yehuda *et al.* 2002, Hofer *et al.* 2006) and to act, together with N^6-cyclopentyladenosine (CPA), an A_1-selective adenosine receptor agonist, homeostatically in hematopoietic tissue during the phases of cell depletion and regeneration (Hofer *et al.* 2007, 2008). Moreover, adenosine A_3 receptor has been localized on hematopoietic precursor cells (Štreitová *et al.* 2010) and has been found to be expressed in premyelocytic cells in dependence on the cell cycle phase (Hofer *et al.* 2011a).

Hematopoiesis-stimulating abilities of selective activation of adenosine A_3 receptors were confirmed also in studies in which suppression of hematopoiesis was experimentally induced by ionizing radiation. After exposing mice to a sublethal dose of 4 Gy of γ-rays, IB-MECA administered in a therapeutic regimen on days 1 and 2 after irradiation was found to significantly increase important hematopoietic parameters (Hofer *et al.* 2010, 2011b).

Until now no studies were performed concerning the ability of IB-MECA to influence survival of experimental animals following their exposure to lethal radiation doses. Such studies would supplement the existing knowledge about mechanisms of action of IB-MECA in an irradiated mammalian organism and would be important also from the practical point of view – in determination of the extent of radiation doses which would represent indication for contingent therapeutic approach using an adenosine A_3 receptor agonist. We have tried to fill in this gap by experiments whose results are presented in this communication.

$B10CBAF_1$ 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 *at 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 carried out with the approval of the Institute's Ethical Committee.

The mice were whole-body irradiated at the dose rate of 0.15 Gy/min using a γ-ray source (^{60}Co, Chisostat, Chirana, Praha, Czech Republic).

N^6-(3-iodobenzyl)adenosine-5'-*N*-methyluronamide (IB-MECA, Sigma, St. Louis, MO, USA) was dissolved initially in dimethyl sulfoxide, diluted in sterile saline, and administered i.p. or p.o. in various post-irradiation (i.e., therapeutic) treatment regimens. The final concentration of dimethyl sulfoxide per one dose was always 2 %. DMSO itself was shown to have radioprotective effects (e.g., Chapman *et al.* 1979). Therefore, pertinent solvents containing 2 % DMSO concentration were used for control interventions.

Survival was recorded daily up to day 30 after irradiation. Analysis of survival time was carried out by Kaplan-Meier methodology; estimates of mean survival time were derived from the Kaplan-Meier curve. Differences in mean survival time between IB-MECA-treated and control mice were tested by a log-rank test. Differences in 30-day survival between IB-MECA-treated and control mice were tested by Fisher's exact test or maximum likelihood chi-square test (in experiment 5). The significance level was set at $P<0.05$.

Five experiments employing various radiation doses and various IB-MECA treatment regimens have been performed in total (1-5).

1) *IB-MECA administered i.p. (105 μg/kg per dose) on days 1 and 2 after irradiation with the dose of 9 Gy*: In this experiment the mice were irradiated with a nearly absoluthely lethal γ-ray dose of 9 Gy. The dosing and timing of IB-MECA was identical with that previously found to positively influence the recovery of hematopoiesis after an exposure to a sublethal radiation dose (Hofer *et al.* 2010, 2011b).

2) *IB-MECA administered i.p. (105 μg/kg per dose) on days 1 and 2 after irradiation with the dose of 8 Gy*: This survival study comprised the same pharmacological treatment regimen with IB-MECA and exposure of the mice to an approximately mid-lethal radiation dose.

3) *IB-MECA administered i.p. (105 μg/kg per dose) on days 6, 7, 8, and 9 after irradiation with the dose of 8.5 Gy*: The timing of administration of IB-MECA in this experiment was shifted to later post-irradiation time intervals (day 6 to day 10) with the aim to affect the phase of recovery of the hematopoietic tissues.

4) *IB-MECA administered p.o. (105 μg/kg per dose) on days 1, 2, 3, 4, 5, 6, 7, 8, and 9 after irradiation with the dose of 9 Gy*: A prolonged 9-day post-irradiation treatment regimen with IB-MECA administered perorally was also the object of our attention.

5) *IB-MECA administered i.p. (500 μg/kg per dose) on days 1 and 2 after irradiation with the dose of 8.5 Gy*: In this experiment, effects of high doses of IB-MECA, which can be expected to evoke also a non-selective activation of adenosine receptors, were evaluated.

Table 1. Numbers of mice surviving by day 30 after irradiation.

	N	Numbers of mice		p-value*
		Surviving	Died	
Experiment 1				
radiation dose 9 Gy, drug administration route i.p.				
Control	15	N=3 (20.0 %)	N=12 (80.0 %)	0.999
IB-MECA	15	N=3 (20.0 %)	N=12 (80.0 %)	
Experiment 2				
radiation dose 8 Gy, drug administration route i.p.				
Control	10	N=6 (60.0 %)	N=4 (40.0 %)	0.628
IB-MECA	10	N=8 (80.0 %)	N=2 (20.0 %)	
Experiment 3				
radiation dose 8.5 Gy, drug administration route i.p.				
Control	30	N=5 (16.7 %)	N=25 (83.3 %)	0.999
IB-MECA	30	N=5 (16.7 %)	N=25 (83.3 %)	
Experiment 4				
radiation dose 9 Gy, drug administration route p.o.				
Control	20	N=2 (10.0 %)	N=18 (90.0 %)	0.999
IB-MECA	20	N=3 (15.0 %)	N=17 (85.0 %)	
Experiment 5				
radiation dose 8.5 Gy, drug administration route i.p.				
Control	18	N = 6 (33.3 %)	N = 12 (66.7 %)	0.807
IB-MECA 150 μg /kg	21	N = 8 (38.1 %)	N = 13 (61.9 %)	
IB-MECA 500 μg /kg	21	N = 6 (28.6 %)	N = 15 (71.4 %)	

* Fisher exact test or Maximum likelihood chi-square test (in experiment 5).

The results of the experiments are summarized in Table 1 (numbers of mice surviving by day 30 after irradiation) and Table 2 (mean survival times after irradiation). Different percentages of surviving and died mice after a radiation dose of 8.5 Gy were obtained in experiment 3 and 5. Since the described studies were done in the course of the whole year, seasonal variations can be responsible for the observed differences. Another explanation of this finding may consist of differing actions of various numbers of injections and their timings.

Taken together, the results on survival of experimental mice administered the agonist of adenosine A_3 receptors IB-MECA in a variety of post-irradiation (therapeutical) treatment regimens following the exposure of the mice to various lethal doses of γ-rays show that

IB-MECA does not significantly modulate this parameter. Both scientific and practical importance of these findings consists in their joint evaluation with previously published results on the stimulatory action of IB-MECA on hematopoiesis in sublethally γ-irradiated mice (Hofer *et al.* 2010, 2011b). The reason for seemingly contradictory findings of radiation recovery-supporting effects of IB-MECA in sublethally irradiated mice and its ineffectiveness in lethally irradiated ones lies probably in the mechanisms by which IB-MECA influences the consequences of irradiation. If the positive action of IB-MECA in the irradiated mammalian organism is concentrated on the stimulation of hematopoietic progenitor and precursor cells, as follows not only from radiation experiments (Hofer *et al.* 2010, 2011b) but as supported also by the results of other hematological studies (Fishman *et al.* 2000, 2001, Bar-Yehuda *et al.* 2002, Pospíšil *et al.* 2004, Hofer *et al.* 2006, 2007, 2008), it can be assumed that there remain too few hematopoietic progenitor and precursor cells after a lethal irradiation to enable an effective employment of the hemopoiesis-stimulating properties of IB-MECA. Thus, when taking into account all results on the effects of IB-MECA in irradiated mice, IB-MECA can, in our opinion, be considered a promising drug for the treatment of the bone marrow radiation syndrome.

In all experimental groups of mice administered IB-MECA in the experiments reported here, survival of IB-MECA-treated mice was always the same or slightly better than that in the controls, with the only exception of mice repeatedly administered a high IB-MECA dose of 500 μg/kg. It can be deduced from this finding that administration of IB-MECA was not accompanied by undesirable side effects which can be clinically important in the conditions of a serious irradiation. Thus, even if IB-MECA was administered in association with higher radiation doses than those after which it could be expected to be most effective, its administration would highly probably be not accompanied with undesirable side effects. In human studies, CF-101, a commercial preparation of IB-MECA, was found to be safe and well tolerated (van Troostenburg *et al.* 2004, Bar-Yehuda *et al.* 2007). Therefore, IB-MECA can, in our opinion, be incorporated into the spectrum of agents suitable for treating radiation damage in humans.

Conflict of Interest

There is no conflict of interest.

Table 2. Mean survival times of mice after irradiation.

	N	Mean survival time (days)	p-value*
Experiment 1 radiation dose 9 Gy, drug administration route i.p.			
Control	15	17.9	0.760
IB-MECA	15	18.9	
Experiment 2 radiation dose 8 Gy, drug administration route i.p.			
Control	10	23.7	0.356
IB-MECA	10	26.7	
Experiment 3 radiation dose 8.5 Gy, drug administration route i.p.			
Control	30	15.8	0.994
IB-MECA	30	15.8	
Experiment 4 radiation dose 9 Gy, drug administration route p.o.			
Control	20	12.8	0.273
IB-MECA	20	14.3	
Experiment 5 radiation dose 8.5 Gy, drug administration route i.p.			
Control	18	19.1	
IB-MECA 150 μg /kg	21	20.3	0.819
IB-MECA 500 μg /kg	21	19.0	

* log rank test.

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