Physiol. Res. 42: 333–340, 1993

Noradrenaline Reduces Cardiovascular Effects of the Combined Dipyridamole and AMP Administration but Preserves Radioprotective Effects of these Drugs on Hematopoiesis in Mice

M. POSPÍŠIL, M. HOFER, A. VACEK, J. NETÍKOVÁ, I. PIPALOVÁ, Š. VIKLICKÁ

Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno

Received April 20, 1993
Accepted June 8, 1993

Summary

Recent results of the authors have demonstrated 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), a soluble adenosine prodrug, mediates radioprotective effects in mice. Furthermore, it has been shown that this action is induced by at least two mechanisms: (1) protection by hypoxia as a result of the effects of treatment on the cardiovascular system (bradycardia, vasodilation), and (2) an enhanced regeneration of the radiation-perturbed hematopoiesis. Here, it was ascertained that the joint use of an optimal dose of noradrenaline given with dipyridamole and AMP combination eliminates the hypothermic and hypoxic effects of the treatment, but preserves the radioprotective action of dipyridamole and AMP combination in terms of hematopoietic recovery and partially also survival enhancing effects of the drugs in gamma-irradiated mice. These findings might be of importance for attempts to obtain available and tolerable radioprotective pharmacological prescriptions for clinical use.

Key words

Radioprotection – Hematopoiesis – Adenosine monophosphate – Dipyridamole – Noradrenaline

Introduction

Earlier, we observed 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), a soluble adenosine prodrug, mediates the radioprotective action in gamma-irradiated mice (Pospíšil *et al.* 1989). Later, it was shown that radioprotective effects of the extracellular adenosine elevation could be induced by at least two independent mechanisms: (1) protection by hypoxia as a result of the cardiovascular action of adenosine (bradycardia – vasodilation – hypotension), and (2) an enhanced regeneration of the hematopoietic cells due to either enhanced cell repair or increased proliferation of the hematopoietic stem cells induced probably by the direct, receptor-mediated action of adenosine on these cells (Pospíšil *et al.* 1992, Hošek *et al.* 1992, Pospíšil *et al.* 1993).

From a clinical standpoint, hypotension and the concomitant adverse effects of hypoxia may be unacceptable in many situations and could limit the potential use of this mode of radioprotection. We now investigated whether it is possible to separate the two proposed mechanisms of protection, to reduce the undesirable cardiovascular and hypoxic effects, and to preserve the proposed hematopoiesis stimulating action of extracellular adenosine. In order to counteract the cardiovascular effects of adenosine, the ability of noradrenaline (NA) to induce vasoconstriction and blood pressure elevation was utilized. The radioprotective effects of dipyridamole and AMP when combined with NA action were investigated. Monitoring of hypothermia as a measure

of hypoxic effects was used. Radioprotective effects of the drug combinations were evaluated in terms of the postirradiation hematopoietic recovery and survival of gamma-irradiated mice.

Material and Methods

Experimental animals

Conventional male (CBAxC57BL/10)F_1 mice, three months old and with an average body weight of 25 g, were used. Standardized pelleted diet and HCl-treated tap water (pH 2–3) were given *ad libitum*. The mice were kept under controlled lighting conditions (LD 12:12), temperature of 22 °C ± 1 °C being maintained.

Irradiation

Mice were irradiated with total-body doses of 7.5 Gy or 9.7 Gy from a ^{60}Co gamma-ray source, at a dose rate of 0.33 Gy/min. During irradiation the mice were placed in ventilated Plexiglas containers.

Drugs

Dipyridamole (Sigma, USA) was dissolved in 0.4 % tartaric acid and injected s.c. at a dose of 80 mg/kg, in a volume of 0.4 ml. Adenosine 5'-monophosphate sodium salt from yeast (Sigma, USA) was dissolved in distilled water and injected i.p. at a dose of 200 mg free acid/kg, in a volume of 0.2 ml. Both drugs were administered in combination, dipyridamole being given 20 min before AMP. Noradrenaline (Léčiva, Czech Republic) was injected s.c. at various doses (see Results), in a volume of 0.2 ml immediately before AMP administration. Solvents were used for control s.c. and i.p. injections. Radiation exposures were started 15 min after the last drug injection, i.e., AMP administration.

Measurements of rectal temperature and oxygen tension in the spleen

Rectal temperature was measured in conscious mice at a room temperature of 22 °C with a thermistor thermometer. The oxygen tension in the exteriorized spleen of anaesthetized mice (Pentobarbital sodium, i.p. 50 mg/kg, 20 min before injecting the first drug) was estimated polarographically by using the Pt electrode (Vacek and Ševčík 1963).

Hematological methods

Endogenous spleen colonies (indices of pluripotent stem cell survival) were determined after the irradiation of mice with 7.5 Gy. Twelve days after irradiation, mice were euthanized by cervical dislocation, their spleens were removed, fixed in Bouin's solution and the nodules greater than 0.4 mm were counted (Till and McCulloch 1963). At the same postirradiation day hematopoietic progenitor cells committed to granulocyte and/or macrophage development (GM-CFC) were assayed in the femoral marrow by a semi-solid plasma-clot technique (Vacek *et al.* 1990). Briefly, bone marrow suspensions were plated in quadruplicate using 10 % murine lung conditioned medium as a source of colony-stimulating factor. Colonies (> 50 cells) were counted after 7 days of incubation in a 37 °C humidified environment containing 5 % CO_2. The nucleated cells in the femur and peripheral blood cells were counted by a Coulter Counter. Bone marrow was washed out from the femoral diaphyses, blood was withdrawn from a fine incision in the tail vein. For differential counting of the main cell lines, bone marrow and blood smears were stained by the May-Grünwald and the Giemsa-Romanowski methods.

Survival studies

In lethally irradiated mice (9.7 Gy) deaths were recorded daily up to the 30th day after exposure.

Statistics

The values given in the figures and tables represent the means ± SEM. Statistical significance of the results was evaluated using the Man-Whitney rank sum test, a t-test, Dunnett's tables for multiple comparisons with a control (Dunnett 1964), and the χ^2-test. P value of < 0.05 was considered a basis for statistically significant difference.

Table 1

Oxygen tension in spleen of anaesthetized mice (percentage of initial value) determined at various time intervals after the administration of dipyridamole (DP) and adenosine-5-monophosphate (AMP) or DP+AMP with noradrenaline (NA)

	Time (min)			
	15	40	60	120
DP + AMP	3.9 ± 1.0	22.8 ± 7.7	43.4 ± 8.1	63.8 ± 3.6
DP + NA + AMP	82.4 ± 2.5	82.0 ± 2.9	81.8 ± 5.4	98.6 ± 5.7

A significant ($P < 0.01$) difference between both groups was found in all time intervals, 5 animals per group were used.

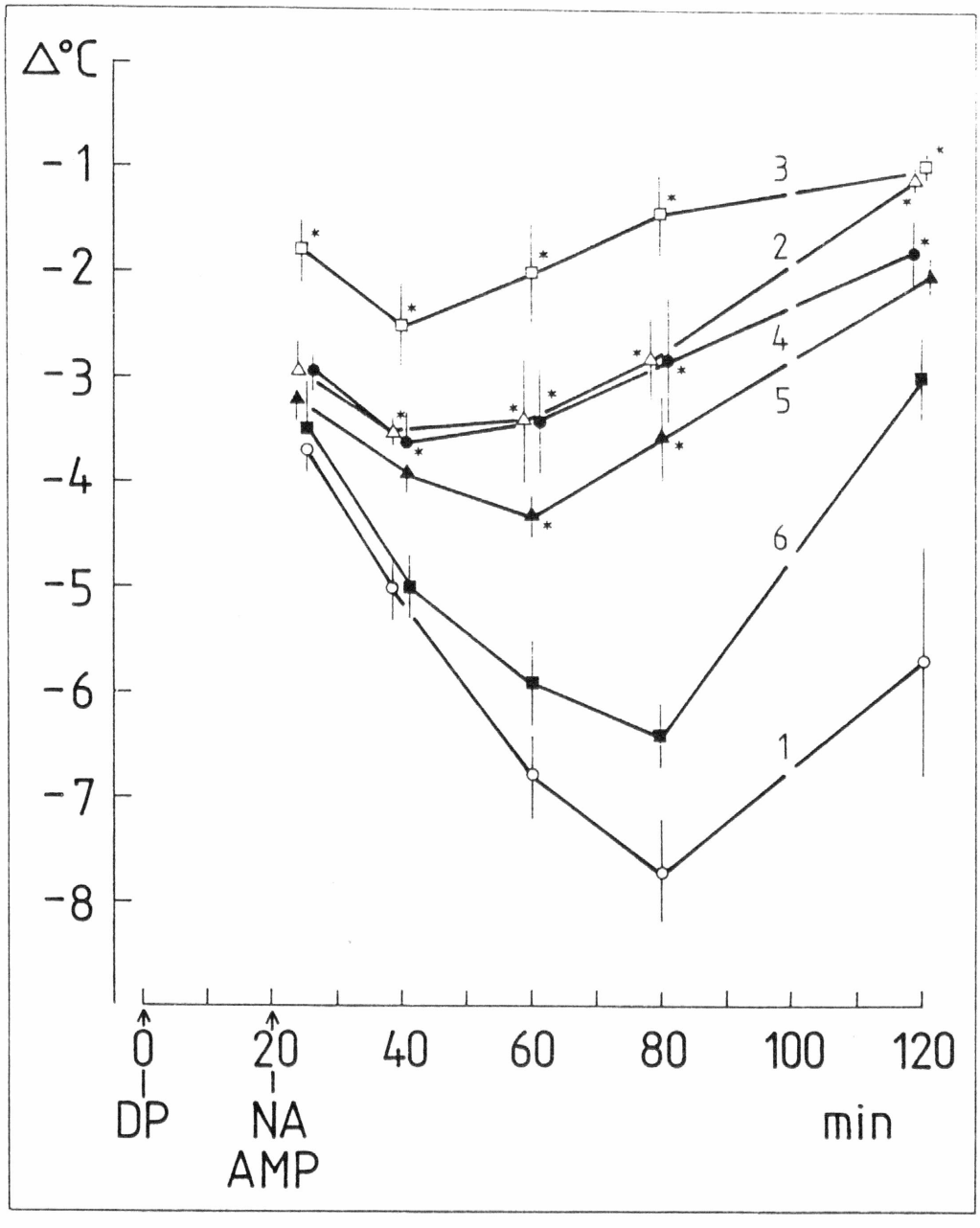

Fig. 1
Rectal temperature changes expressed as differences from the initial values ascertained at time "0" (37.3 ± 0.3 °C) and measured in mice treated with dipyridamole and AMP combination without NA (1), + NA 0.8 mg/kg (2), + NA 1.4 mg/kg (3), + NA 2.0 mg/kg (4), + NA 3.2 mg/kg (5), + NA 4.0 mg/kg (6). Asterisks denote statistical significance when compared to the group treated with DP + AMP without NA (Mann-Whitney rank sum test); 5 animals per group were used; for the sake of simplicity the significance at the 0.05 level was used for all comparisons.

Results

The results of experiments evaluating the ability of NA to influence the rectal temperature response to dipyridamole and AMP administration in normal (non-irradiated) conscious mice are given in Fig. 1. Rectal temperature of mice treated with dipyridamole and AMP (without NA) declined to its lowest level at 60 min after the administration of drugs. The most evident prevention of the temperature decline was obtained by NA dose of 1.4 mg/kg. Lower as well as higher doses of NA were less effective in this respect. NA given alone in a dose of 1.4 mg/kg induced only a moderate decline of rectal temperature attaining its maximum (−1 °C) at the interval of 60 min posttreatment (data not shown).

Table 2
Hematological indices determined on day 12 after 7.5 Gy irradiation in control mice and mice pretreated with noradrenaline (NA) alone, dipyridamole (DP) + AMP, DP + NA + AMP[a)]

	Control (1)	NA (2)	DP + AMP (3)	DP+NA+AMP (4)	Statistical significance[b)]
Spleen weight (mg)	23.4±0.9	26.3±1.4	31.1±2.1	31.6±3.0	1–3,1–4
Endogenous spleen colonies (No.)	3.0±0.5	3.1±1.3	12.8±1.2	9.3±1.4	1–3,2–3, 1–4,2–4
Nucleated cells per femur ($x10^7$)	0.88±0.06	0.93±0.05	1.21±0.11	1.29±0.12	1–3,1–4, 2–4
Myeloid cells	0.22±0.03	0.22±0.03	0.37±0.06	0.45±0.08	1–4
Erythroid cells	0.52±0.06	0.53±0.04	0.68±0.06	0.63±0.07	n.s.
Lymphoid cells	0.14±0.02	0.18±0.02	0.16±0.03	0.21±0.03	n.s.
GM-CFC per femur	269±18.0	204±11	701±65	838±78	1–3,2–3, 1–4,2–4
Granulocytes per μl of blood	119±29	155±17	416±42	294±54	1–3,2–3, 1–4
Lymphocytes per μl of blood	546±56	667±87	847±60	802±97	1–3
Erythrocytes per μl of blood ($x10^6$)	6.27±0.20	6.19±0.19	6.69±0.21	6.87±0.24	n.s.

[a)] Values found in intact nonirradiated mice: spleen weight 72.0±3.2 mg; femoral cellularity 2.51±0.20 x 10^7 (myeloid cells 1.57±0.16, erythroid cells 0.69±0.08, lymphoid cells 0.25±0.05); GM-CFC per femur 23600±1300; blood granulocytes 1018±90, lymphocytes 5429±407, erythrocytes 10.2±0.2 x 10^6 (per μl).
[b)] t-test and Dunnett's tables for multiple comparisons with a control were employed; for the sake of simplicity the significance at the 0.05 level was used for all comparisons; n.s. – no significant differences were found; two independent experiments were performed and the data pooled; 10–15 animals per group were used.

Table 1 summarizes results of splenic oxygen tension recordings performed in anaesthetized (non-irradiated) mice treated with dipyridamole and AMP combination given either without or with NA at a dose of 1.4 mg/kg. The results suggested the ability of NA to prevent the pronounced oxygen tension decrease induced by dipyridamole + AMP.

Irradiation was started 15 min after AMP or NA+AMP administration. Indices of hematopoietic functions were ascertained on day 12 after near-lethal irradiation (7.5 Gy) of mice, i.e., at the time interval when the recovery of hematopoiesis perturbed by radiation occurs. As shown in Table 2, protection of mice with dipyridamole and AMP combination given either without or with NA, enhanced with the approximately equal efficiency the spleen weight, endogenous spleen colony formation, femoral marrow cellularity, counts of bone marrow progenitor cells committed to granulocyte and/or macrophage development (GM-CFC), and counts of peripheral blood granulocytes. NA given alone did not influence significantly the evaluated indices of hematopoiesis. Investigations of hematopoietic functions on day 16 after the irradiation were also performed and similar effects were observed (data not shown).

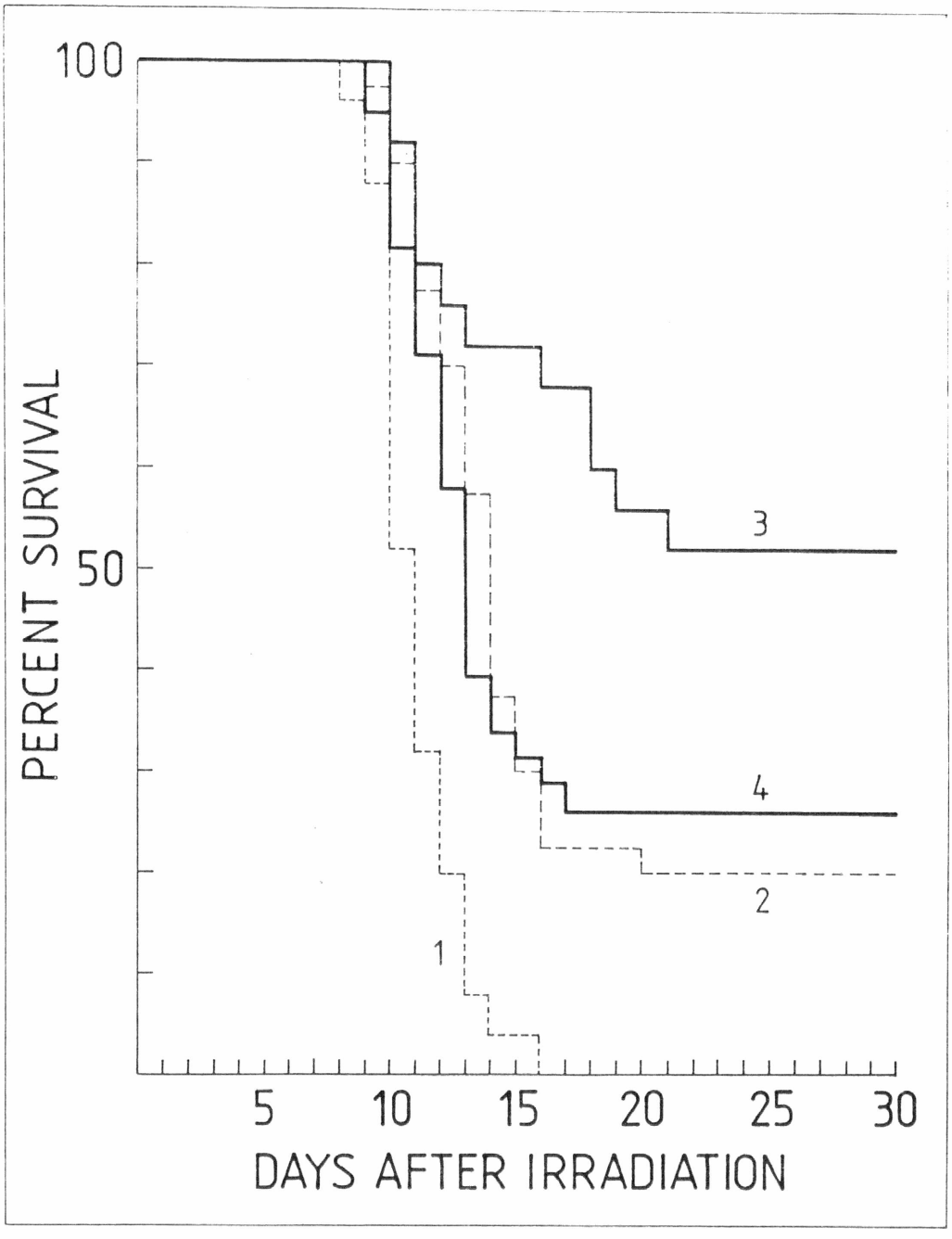

Fig. 2
Survival of mice irradiated with a dose of 9.7 Gy. The respective groups comprised 23–40 animals: 1 – control animals, 2 – NA alone, 3 – dipyridamole (DP) + AMP, 4 – DP + NA + AMP. Statistically significant differences between groups: 1–2 ($P<0.05$), 1–3 ($P<0.001$), 1–4 ($P<0.05$), 2–3 ($P<0.01$), 3–4 ($P<0.05$); χ^2-test with Yates correction was used.

The data on the survival of animals irradiated with the absolute-lethal dose of 9.7 Gy are shown in Fig. 2. The results indicate the highest radioprotective effectiveness of dipyridamole + AMP given without NA. Though the addition of NA (1.4 mg/kg) to this drug combination decreased this protective effect, a significant protection was still preserved. A moderate protection in terms of survival was induced also by NA given alone.

Discussion

The findings of moderate radioprotective action of adenosine nucleotides in mice (Langendorff and Langendorff 1972, Grant *et al.* 1976) have raised the question of the mechanism of these effects. Since the radioprotective effectiveness of these compounds is not dependent on the degree of their phosphorylation and endogenous nucleotides are rapidly converted to adenosine by ectonucleotidases (Gordon *et al.* 1989), adenosine seems to be the mediator of the radioprotective effect. The role of extracellular adenosine signalling in these effects was supported by findings suggesting the enhancement of the radioprotective action of AMP when given in a combination with dipyridamole, a drug which inhibits cellular uptake of adenosine and increases its extracellular concentration (Pospíšil *et al.* 1989). Extracellular adenosine is generally believed to exert its physiological effects by binding to specific membrane receptors regulating adenylate cyclase activity (Daly 1982). Through these receptor-mediated mechanisms adenosine is able to induce vasodilation in all vascular beds except the kidney (Collis 1989) and to exhibit negative chronotropic effects in the heart (Belardinelli *et al.* 1989). These cardiovascular effects can lead to hypotension and systemic hypoxia.

Because tissue hypoxia has been found to be the causative factor in the radioprotective action of various drugs (Livesey and Reed 1987), also adenosine-induced hypotension and subsequent hypoxia may contribute to protective efficiency of the dipyridamole and AMP combination. Our recent experiments (Pospíšil *et al.* 1993) investigating the cardiovascular effects of these drugs have really supported such a possibility and have shown a relationship between indices of cardiovascular response, tissue hypoxia and decreased intrinsic radiosensitivity of hematopoietic stem cells. However, in these experiments evidence has been presented that beside hypoxic mechanisms also other hypoxia-independent protective actions of extracellular adenosine have to be taken into account. This was evident especially in irradiated animals at the later post-treatment intervals when the hypoxic state wanes. In order to ascertain the role of these probable hypoxia-independent mechanisms in adenosine-induced radioprotection, experiments were performed investigating the radioprotective effects of dipyridamole and AMP combination under conditions of the concomitant NA action. The ability of NA to induce vasoconstriction and thus to counteract the adenosine-induced vasodilation and hypotension (Edlund *et al.* 1990) was employed.

For indirect investigation of the cardiovascular effects leading to hypoxia in conscious mice, measurements of rectal temperature were used with the assumption that hypothermic response reflects hypometabolic effects which are considered as compensatory reaction of the organism to hypoxia (Wood 1991). As shown, dipyridamole and AMP combination elicits a distinct and transient hypothermic response that can be influenced by NA administration. The dose of 1.4 mg NA/kg was found to be optimal for a significant inhibition of the hypothermic response. Higher doses of NA lose this ability probably due to the preponderance of vasoconstriction that again could lead to systemic hypoxia. Although the vasoconstrictory action of NA seems to be the most probable mechanism participating in the observed effects, the possibility that also calorigenic action of NA influences the hypothermic response cannot be excluded (Hošek and Novák 1968). The data of direct measurements of oxygen tension in the spleen of anaesthetized mice support the assumption that NA at a dose of 1.4 mg/kg reduces the hypoxic response to the dipyridamole and AMP administration.

The results demonstrating an equal enhancement of hematopoietic recovery in sublethally irradiated mice treated with dipyridamole and AMP combination given either without or with NA (at a dose attenuating most effectively the hypothermic response) suggest that hypoxia effects need not be the only causative factor in thus induced radioprotection of the hematopoietic system. Mechanisms of extracellular adenosine action linked to the cyclic AMP system and influencing cell proliferation might play a role. In our recent experiments the ability of dipyridamole and AMP combination to enhance cell proliferation in hematopoietic tissue of non-irradiated mice was observed (Pospíšil *et al.* 1992). Byron (1973) has shown that β-adrenergic stimulation of adenylate cyclase activity triggers *in vitro* hematopoietic stem cells to enter the cell cycle. Lehnert (1975) also postulated that a short burst of cyclic AMP accumulation precedes the initiation of DNA synthesis and proliferation of a wide variety of mammalian cells *in vivo*. Thus, if these proliferation enhancing mechanisms are activated by the elevation of extracellular adenosine under conditions of hypoxia absence, more effective repopulation of the radiation perturbed hematopoietic system could result.

The results of our survival studies have shown some peculiarities when compared with those obtained in the evaluation of hematopoietic recovery after near-lethal dose. Unlike in the case of hematopoietic recovery, the protection in terms of survival afforded by the dipyridamole and AMP combination was reduced when avoiding the hypoxic effects due to NA action, although in this situation the radioprotective effect was still significant. When considering these results, the complexity of pathophysiological events leading to radiation death has to be taken into account. The overall lethal response of the irradiated organism is dependent on the relative status of at least two sensitivities, i.e. the hematopoietic and gastrointestinal mode of damage. Moderate gastrointestinal damage

can result from radiation doses inducing the hematopoietic syndrome and could contribute to deaths of animals occurring during the second postirradiation week. It can be hypothesized that hypoxia and hypoxia-independent mechanisms of protection may operate differently in the hematopoietic and gastrointestinal tissues. Assuming that the hypoxia-independent mechanisms activated in animals by the dipyridamole and AMP combination can reduce only hematopoietic damage, while those of hypoxia influence both the hematopoietic and gastrointestinal injuries, a less pronounced protection in terms of survival could really occur in mice protected with the dipyridamole + NA+AMP combination. Interestingly, also NA given alone provides some moderate protection in terms of survival. Such an effect is compatible with the observations of other authors and could be attributed to the vasoconstriction-hypoxia action of NA in the radiosensitive tissues (Prewitt and Musacchia 1975).

More data are needed to understand the mechanism of the described radioprotective effects. Nevertheless, the results clearly indicate that in spite of the reduction of the cardiovascular effects of dipyridamole and AMP combination by the use of NA, the hypoxia-independent action of these drugs on hematopoiesis occurs and may protect hematopoietic functions against ionizing radiation.

Acknowledgements

This study was supported by grant No. 60402 of the Academy of Sciences of the Czech Republic.

References

BELARDINELLI L., LINDEN J., BERNE R.M.: The cardiac effects of adenosine. *Prog. Cardiovasc. Dis.* **32:** 73–97, 1989.

BYRON J.W.: Drug receptors and the haemopoietic stem cell. *Nature New Biol.* **241:** 152–154, 1973.

COLLIS M.G.: The vasodilator role of adenosine. *Pharmacol. Ther.* **41:** 143–162, 1989.

DALY J.W.: Adenosine receptors: targets for future drugs. *J. Med. Chem.* **25:** 197–207, 1982.

DUNNETT C.W.: New tables for multiple comparisons with a control. *Biometrics* **20:** 482–491, 1964.

EDLUND A., SOLLEVI A., LINDE B.: Haemodynamic and metabolic effects of infused adenosine in man. *Clin. Sci.* **79:** 131–138, 1990.

GORDON E.L., PEARSON J.D., DICKINSON E.S., MOREAU D., SLAKEY L.L.: The hydrolysis of extracellular adenine nucleotides by arterial smooth muscle cells. *J. Biol. Chem.* **264:** 18986–18992, 1989.

GRANT G.A., BARLOW J.A., LEACH K.E.: Modification of survival of gamma irradiated mice by adenosine nucleotides. *Strahlentherapie* **152:** 285–291, 1976.

HOŠEK B., NOVÁK L.: A contribution on the effect of noradrenaline on heat production in mice. *Experientia* **24:** 1214–1215, 1968.

HOŠEK B., BOHÁČEK J., ŠIKULOVÁ J., POSPÍŠIL M., VACEK A.: Protection of early cellular damage in 1 Gy-irradiated mice by the elevation of extracellular adenosine. *Radiat. Environ. Biophys.* **31:** 289–297, 1992.

LANGENDORFF H., LANGENDORFF M.: Adenosin-Nukleotide und Strahlenempfindlichkeit. *Strahlentherapie* **144:** 451–456, 1972.

LEHNERT S.: Modification of postirradiation survival of mammalian cells by intracellular cyclic AMP. *Radiat. Res.* **62:** 107–116, 1975.

LIVESEY J.C., REED D.J.: Chemical protection against ionizing radiation. In: *Advances in Radiation Biology,* Vol. 13. J.T. LETT, U.K. EHMANN, A.B. COX (eds), Academic Press, San Diego, 1987, pp. 285–353.

POSPÍŠIL M., NETÍKOVÁ J., PIPALOVÁ I., KOZUBÍK A.: Enhancement of radioprotective effectiveness of adenosine monophosphate by dipyridamole. *Stud. Biophys.* **132:** 203–208, 1989.

POSPÍŠIL M., HOFER M., NETÍKOVÁ J., VIKLICKÁ Š., PIPALOVÁ I., BARTONÍČKOVÁ A.: Effect of dipyridamole and adenosine monophosphate on cell proliferation in the hemopoietic tissue of normal and gamma-irradiated mice. *Experientia* **48:** 253–257, 1992.

POSPÍŠIL M., HOFER M., NETÍKOVÁ J., PIPALOVÁ I., VACEK A., BARTONÍČKOVÁ A., VOLENEC K.: Elevation of extracellular adenosine induces radioprotective effects in mice. *Radiat. Res.* 134: 323–330, 1993.

PREWITT R.L., MUSACCHIA X.J.: Mechanisms of radio-protection by catecholamines in the hamster (Mesocricetus auratus). *Int. J. Radiat. Biol.* **27:** 181–191, 1975.

TILL J.E., McCULLOCH E.A.: Early repair processes in marrow cells irradiated and proliferating in vivo. *Radiat. Res.* **18:** 96–105, 1963.

VACEK A., ŠEVČÍK F.: Electrochemical determination of the tension of oxygen in tissue. *Physiol. Bohemoslov.* **12:** 269–274, 1963.

VACEK A., ROTKOVSKÁ D., BARTONÍČKOVÁ A.: Radioprotection of hemopoiesis conferred by aqueous extract from chlorococcal algae (Ivastimul) administered to mice before irradiation. *Exp. Hematol.* **18**: 234–237, 1990.

WOOD S.C.: Interactions between hypoxia and hypothermia. *Annu. Rev. Physiol.* 53: 71–85, 1991.

Reprint Requests

Dr. M. Pospíšil, Institute of Biophysics, Academy of Sciences of the Czech Republic, 612 65 Brno, Královopolská 135, Czech Republic.