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Radioprotective Effect of Inosine and Its Enhancement by Magnesium and Global Hypoxia

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Summary

The slight radioprotective action of inosine, when injected intraperitoneally to mice shortly before gamma-irradiation, can be enhanced by the administration of magnesium aspartate. This effect can be explained by the additivity of the vasodilatory actions of both agents. Inosine increases the radioprotective effectiveness of hypobaric hypoxia (10 % O_2), probably due to the additivity of the hypoxic effects in radiosensitive tissues. Acute hypoxic toxicity, however, is decreased by inosine administration. The cumulation of radioprotective effects of inosine and of its antihypoxic action in vitally important organs can have a favourable influence in hypoxic radiotherapy.

Key words:
Radioprotection – Gamma-irradiation – Inosine – Magnesium – Hypoxia

Introduction

Experimental evidence suggests that mild deviations in the physiological state of the organism can modify its radiosensitivity (Pospíšil and Vácha 1983). For this reason current trends in radioprotection research have turned to the possibility of utilizing various metabolically active agents or their combinations, especially those available in human pharmacology (Weiss *et al.* 1990). Weissberg and Fischer (1981) observed radioprotective effects of inosine on the skin reactions and structural damage on the hind limbs of X-irradiated rats. Recently, inosine was found to reduce slightly 30-day lethality of mice when given shortly before irradiation (Vartanyan *et al.* 1989). Inosine, a tissue adenine nucleotide metabolite, exerts various physiological effects on blood flow and cell metabolism, and is used as a cardioprotective drug (Jones and Mayer 1980, Takeo *et al.* 1988). The possibility to utilize inosine in enhancing radioresistance led us to study the mechanism of its radioprotective action. As has been shown, the vasodilatory, hypotensive and thus hypoxic effects of inosine seem to be the most probable mechanisms of the radioprotection thus achieved. For this reason experiments were performed to enhance the protective effects of inosine by increasing the blood magnesium

concentration, which potentiates the vasodilation responses (Arnold and Tackett 1985, Charbon 1986, Nishio *et al.* 1988), and by the concomitant action of aerogenic hypoxia, which is known to increase radioresistance in radiosensitive tissues through the hypoxic mechanism (Jarmonenko *et al.* 1975, Neumeister and Révész 1987).

Material and Methods

Conventional male mice (CBA x C57BL)F_1, aged three months, with an average body weight of 30 g, were used. The mice were caged in groups of 20, under controlled lighting conditions (LD 12 : 12) and at a constant temperature of 22±1 °C. Pelleted sterilized standard diet (DOS-2ST Velaz) and HCl-treated tap water (pH 2–3) were given *ad libitum*. Control and experimental procedures were carried out concurrently in groups of mice from the same cage.

The mice were irradiated with single whole-body doses from a ^{60}Co gamma-ray source, at a dose rate of 0.37 Gy/min. During irradiation the mice were placed individually in chambers in a circular perspex container.

Inosine (Reanal, Hungary) was dissolved in saline and injected intraperitoneally 15 min before irradiation or hypoxia in doses of 9 or 15 mg per mouse and in volumes of 0.45 ml. LD_{50} of inosine, given i.p. to mice, is higher than 3000 mg/kg (Vartanyan *et al.* 1989). Monomagnesium D,L-asparagicum (Biotika) was dissolved in distilled water and injected subcutaneously 35 min before irradiation in doses of 13.3 mg per mouse and in volumes of 0.4 ml. Inosine or magnesium aspartate were given either alone or in combination (Mg-inosine). Saline was used for both s.c. and i.p. control injections.

Aerogenic hypoxia was induced by decreasing the barometric pressure of air in a hypobaric chamber as registered by an altimeter. When irradiating the animals in hypoxia, 3 min before the start of irradiation air pressure in the chamber was gradually decreased to the desired oxygen content (10 %) and, after termination of irradiation, adjusted again during 3 min to normal. In experiments where the toxic effects of hypoxia and their modification by the various drugs were evaluated, mice were exposed to 8 % oxygen in the air for 25 min. Deaths occurring during the exposure to hypoxia were recorded; no subsequent deaths were observed.

For measuring blood pressure and heart rate after magnesium aspartate, inosine alone and the Mg-inosine combination, the mice were anaesthetized (pentobarbital sodium, i.p. 50 mg/kg, 20 min before injecting the first drug). Arterial blood pressure was measured by an invasive method (catheterization of the right carotid artery) using a Statham transducer and an electronic apparatus LDP 102 Tesla; the heart rate was derived from blood pressure signal using a cardiomonitor LKM 205 Tesla.

The rectal temperature was measured in awake mice at various intervals after administration of the drugs, using a thermistor thermometer.

In lethally irradiated mice deaths were recorded up to the 30th day after exposure. Statistical significance of the results was evaluated using the distribution-free sequential test and the χ^2 test. The values given in the figures represent the means ± S.E.M.

Results

The effects of inosine (9 mg), magnesium aspartate and Mg-inosine combination on blood pressure and heart rate in anaesthetized mice are given in Fig. 1. The magnesium salt alone does not modify these functions significantly. Inosine alone and Mg-inosine combination markedly decreased the blood pressure ($P<0.05$ at all time intervals as compared to the controls) and there were no significant differences between inosine and Mg-inosine treatment. The heart rate was slightly, but nonsignificantly decreased after inosine treatment, while a higher decrease ($P<0.05$ at all intervals as compared to the controls) was observed after the Mg-inosine combination.

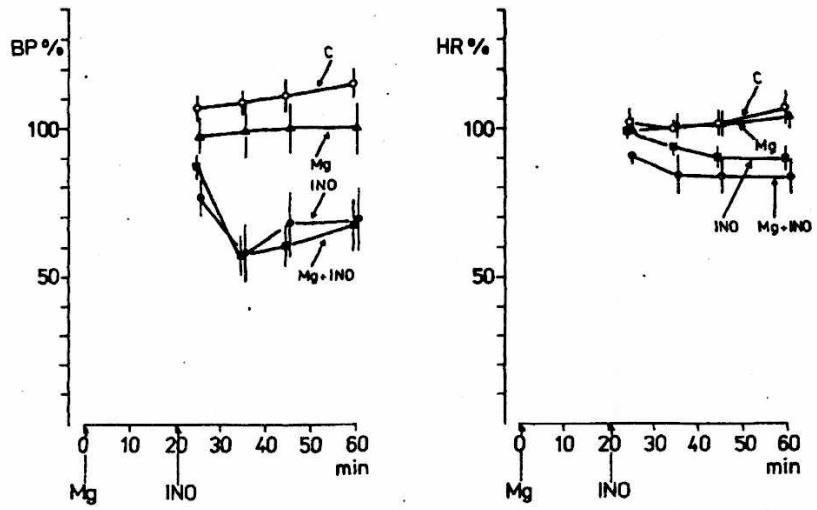

Fig. 1
Arterial blood pressure (BP) and heart rate (HR) changes induced by magnesium aspartate, inosine (9 mg) and Mg-inosine combination in anaesthetized mice, expressed as percentage of the initial values ascertained at time zero. Each value is the mean of 5–6 determinations.

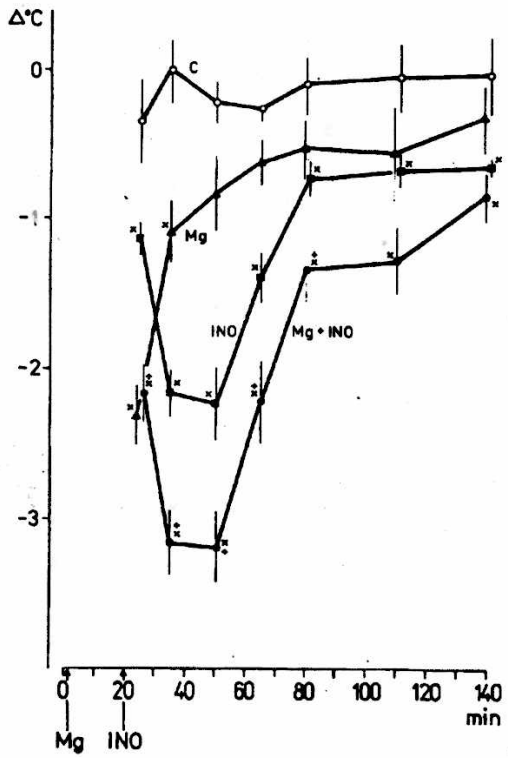

Fig. 2
Rectal temperature changes induced by magnesium aspartate, inosine (9 mg) and Mg-inosine combination in awake mice, expressed as a difference (Δ °C) from the initial values ascertained at time zero. Each value is the mean of 7–11 determinations. Statistical significance ($P<0.05$) as compared to controls (x) and to the group treated with inosine alone (+) is shown.

Rectal temperature in awake mice (Fig. 2) was slightly and transiently decreased after magnesium aspartate injection and more markedly decreased after inosine (9 mg) treatment. A still more distinct decrease was noted after administration of the Mg-inosine combination.

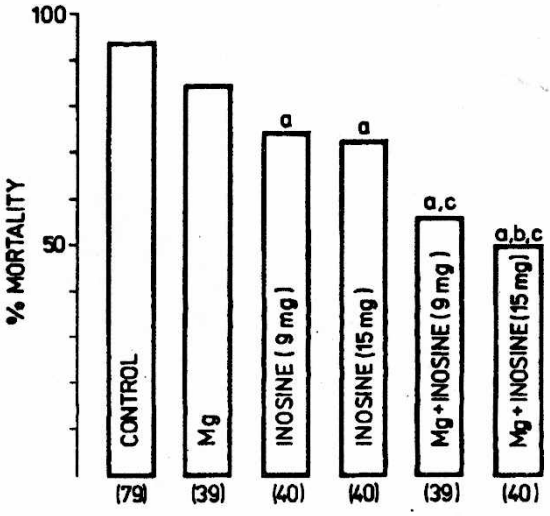

Fig. 3
Thirty-day mortality after 9 Gy in control mice, mice treated with magnesium aspartate, inosine (9 and 15 mg) and Mg-inosine combination. Numbers in parentheses refer to numbers of mice. a – statistical significance as compared to the controls ($P<0.01$), b – as compared to the inosine group ($P<0.05$), c – as compared to the magnesium aspartate group ($P<0.01$).

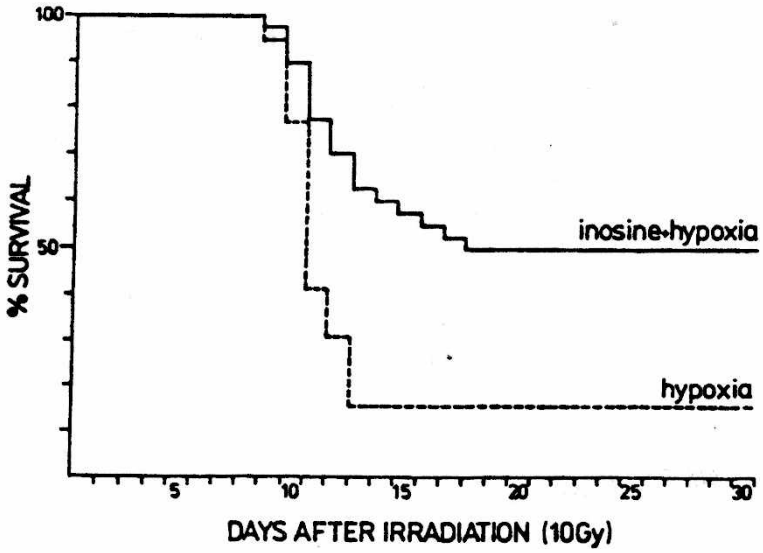

Fig. 4
Survival of mice irradiated with 10 Gy and protected with hypobaric hypoxia of 10 % O_2 (n=39) or inosine (9 mg) plus hypoxia combination (n=40). The difference in 30-day survival is statistically significant ($P<0.01$).

Fig. 3 illustrates the results on the effects of magnesium aspartate alone, inosine alone (9 and 15 mg) and the Mg-inosine combination on the 30-day mortality rate of mice irradiated with 9 Gy. A significant ($P<0.01$) decrease of mortality was observed in all the groups treated with inosine or Mg-inosine combination as compared to the controls. Magnesium aspartate *per se* does not provide significant protection. Groups of mice treated with the Mg-inosine combination exhibited highest survival and thus a potentiation of inosine protection with magnesium aspartate. An increase of the inosine dose from 9 to 15 mg per mouse did not have a further protective effect.

Fig. 4 shows the survival of mice irradiated with 10 Gy during hypobaric hypoxia (10 % O_2). As shown, inosine (9 mg) is able to increase the survival ($P<0.01$) of mice irradiated under hypoxic conditions.

In order to evaluate the effect of inosine on hypoxia toxicity, a 25 min exposure to hypobaric hypoxia, corresponding to 8 % O_2 in the inspired air, was used in control and inosine (9 mg, 15 min before the start of hypoxia) pretreated animals. In the control group ($n=20$) 90 % of the animals died during hypoxia exposure, as compared to 5 % in the group ($n=20$), pretreated with inosine ($P<0.01$). Thus inosine, in a time-dose schedule potentiating the hypoxia radioprotection, is able to decrease acute toxic effects of hypoxia.

Discussion

The observed decline of blood pressure following administration of either inosine alone or the Mg-inosine combination suggests that the radioprotective action of these agents can be explained by a physiological mechanism whereby hypoxia is induced. Our measurements were performed in anaesthetized mice and the depressive circulatory effects are probably higher than those elicited in awake mice, due to the lack of reflex sympathetic activation. Indirect evidence for the probable effect of hypoxia in awake mice is the lowering of body temperature. The hypothermic response to hypoxia reflects hypometabolic effects which are considered as compensatory reactions of the organism (Jílek 1966). From this point of view the degree of hypothermia, and thus probably hypoxia, induced by magnesium aspartate, inosine alone or the Mg-inosine combination at the critical time period of radiation exposure, is proportional to the radioprotective effect achieved. It should be mentioned that a decreased metabolism *per se* cannot be considered as radioprotective. Earlier radiobiological experience has shown that only hypothermia accompanied by hypoxia is radioprotective (Hope 1958, van den Brenk and Jamieson 1962). The most probable candidate for the induced radioprotective mechanism seems to be hypoxia elicited by hypotension and a reduction of blood flow in radiosensitive tissues which determine the survival probability of the organism, i.e. in the bone marrow and the intestine. As is known, direct oxygen dependence of radiation damage at all levels of biological organization is a universal phenomenon of radiobiology (Yarmonenko 1988).

The blood pressure decline after administration of various agents might be reasonably explained by vasodilatory effects. Even though inosine has generally been considered to have no vasoactive properties (Berne *et al.* 1983), more recent data indicate that inosine can indeed be vasoactive, especially at higher arterial concentrations. It was reported that inosine caused coronary vasodilation (Jones and

Mayer 1980) and vasodilation of the mesenteric bed (Granger *et al.* 1978). Some authors believe that the inosine effects may be attributed to inhibition of adenosine elimination from the extracellular space due to a reduction of its cellular uptake (Pfleger *et al.* 1969). Adenosine is a known vasodilator, acting *via* cell surface receptors, which stimulate adenylate cyclase activity (Collis 1989). Such a mechanism of adenosine action is probably responsible for the radioprotective effects of adenine nucleotides (Pospíšil *et al.* 1988); radioprotective effectiveness of adenosine monophosphate can be enhanced by the joint administration of dipyridamole, a drug inhibiting the cellular uptake of adenosine (Pospíšil *et al.* 1989).

With respect to the potentiating role of magnesium aspartate in radioprotection elicited by inosine, similar mechanisms of physiological action of both these drugs could be involved. In our recent experiments (Pospíšil *et al.* 1990), it was shown that the enhancing effect of magnesium aspartate on adenosine monophosphate radioprotection was induced by the elevation of magnesium in the serum, and that the possible metabolic effects of aspartic acid need not be taken into account. With the experimental doses used, magnesium aspartate induced transient hypermagnesaemia culminating (about 100 % of the norm) 20 min after aspartate administration. Arnold and Tackett (1985) have shown that the β-adrenoceptor-mediated vasodilation is enhanced in hypermagnesaemic states. Arteriolar constriction induced by epinephrine is attenuated by systemic i.v. infusion of magnesium salts (Nishio *et al.* 1988). Magnesium reduces the arterial tone, thus lowering arterial pressure, and mitigates excessive sympathetic activity (Charbon 1986). Magnesium is a positive allosteric effector of adenylate cyclase, which would lead to increased cyclic AMP levels (Wiemer *et al.* 1978) initiating vasodilation. In the light of this knowledge, the additivity of the inosine and magnesium effect on the circulatory mechanisms of tissue hypoxia and the consequent enhancement of radioprotection are comprehensible.

The additivity of inosine radioprotection and the protection achieved by aerogenic hypoxia indirectly favours the assumption of a hypoxic mechanism in the inosine effects. The radioprotection achieved by hypobaric hypoxia, corresponding to an exposure of 10 % of oxygen, is evident from the comparison of a similar mortality of control mice irradiated with 9 Gy and of mice irradiated under hypoxic conditions with a higher dose of 10 Gy (compare Fig. 3 and 4). As is shown, the protection attained by aerogenic hypoxia can be further enhanced by inosine administration. However, assuming that the hypoxia-induced mechanisms are additive, one could expect that near threshold or suprathreshold levels of hypoxia tolerance could become critical. The results showing the protective effect of inosine on acute hypoxia toxicity of mice do not support such apprehension. On the contrary, the mechanisms which protect vitally important organs against hypoxia seem to be stimulated. This effect can be explained by the following consideration. Vasodilation in the brain and heart ensures increased substrate and oxygen availability and protects these tissues under conditions such as hypoxia, ischaemia or an increased work-load (Berne *et al.* 1983, Newby 1984). It has been shown that inosine induces cerebral vasodilation in the presence of adenosine by augmenting the latter's vasodilatory action (Ngai *et al.* 1989). Exogenous metabolites of adenosine, including inosine, are utilized for the restoration of myocardial ATP during reoxygenation, which may lead to a beneficial recovery of the

hypoxia-induced loss of cardiac contractile force upon reoxygenation (Takeo *et al.* 1988). The protective effect of inosine on adrenaline-induced myocardial necrosis in rats has been demonstrated (Czarnecki and Hinek 1987). Inosine is thus not only a radioprotective agent, inducing hypoxia in radiosensitive tissues, but also an energy-preserving agent protecting vitally important organs against the toxic effects of hypoxia. The earlier concepts (Jílek 1966) stressed the role of the decreased energy metabolism in the brain. Indeed, the important protective mechanisms of this tissue to hypoxia and the lowering of body temperature after inosine observed in our experiments imply such a mechanism. However, the concept of regional blood flow regulation and of regulatory metabolites (Berne *et al.* 1983, Newby 1984) seems to us to be a more likely explanation of the results obtained.

The present study provides some practical conclusions. Inosine, as well as magnesium aspartate, available as clinical therapeutical agents, can induce, when used jointly and in appropriate dosage, tolerable hypoxia with a radioprotective effect. Furthermore, the use of inosine may be of importance in connection with hypoxic radiotherapy, which tries to improve tumour radiation treatment by protecting normal tissues *via* the induction of short-term global hypoxia and is presently being introduced into clinical practice (Jarmonenko *et al.* 1975, Neumeister and Révész 1987). When used in connection with this therapeutical regimen, inosine could increase the radioprotection thus achieved and concomitantly decrease the possible toxic effects of hypoxia on vitally important organs such as the brain and the heart.

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