Protective Effects of Inosine on Mice Subjected to Lethal Total-body Ionizing Irradiation

Bing HOU, Zhi-Wei XU, Chao-Wen YANG, Yan GAO, Shi-Fu ZHAO and Cheng-Gang ZHANG*

Inosine/Total-body irradiation/Spatial memory.

Mammals can barely survive total-body ionizing irradiation greater than 10 Gy. To date, there are few drugs available for radioprotective therapy under such circumstances. Inosine, a natural derivative of adenosine, has been known to provide powerful protection for many kinds of cells and tissues against various insults both in vitro and in vivo. In the present study, we examined whether inosine was also beneficial for mammals subjected to an absolutely lethal total-body ionizing irradiation. Immediately after adult Balb/c mice were exposed to 60Co γ-rays at a single dose of 12 Gy, a moiety of them were administered daily with inosine or adenosine, either at doses of 375 or 750 micromol/kg up to death, and their body weight and survival time were recorded. Some irradiated mice were administered inosine or adenosine daily at doses of 750 micromol/kg and assessed for spatial memory abilities using the Morris water maze. The results demonstrated that, although inosine could not prevent body weight loss in irradiated mice, it was able to significantly prolong their survival time at doses of 750 micromol/kg. Moreover, inosine but not adenosine could suppress spatial memory deficit in irradiated mice. The data suggested that inosine had protective effects on mammals suffering from total-body ionizing irradiation at a single lethal dose.

INTRODUCTION

The biological effect of ionizing radiation on the body is usually dose-dependent. It is believed that mammals can barely survive total-body ionizing irradiation greater than 10 Gy for 3–5 days, partially due to the gastrointestinal syndrome characterized by refractory diarrhea. 1) Unfortunately, ionizing radiation delivery may easily reach and exceed 10 Gy in the case of nuclear accidents and nuclear terrorism. To date, there are only a few drugs available for radioprotective therapy under such circumstances.

Inosine has been identified as protecting the myocardium 2,3) and the gut 4) from ischemia. In addition, it can provide a powerful protection for neurons damaged by respiratory inhibitors 5,6) and axotomy. 7) As a potent immunomodulator, inosine can also protect against endotoxin-induced shock, 8) pancreatitis-associated lung injury, 9) and the development of experimental diabetes. 10) The radioprotective action of inosine was demonstrated when it was introduced intraperitoneally to mice subjected to γ-irradiation at low dose rate, 11) but information is still lacking on its radioprotective effects in cases of lethal irradiation exposure at high doses. It is thus interesting to determine whether inosine is also beneficial for mammals exposed to ionizing radiation greater than 10 Gy. In the present study, the possible effects of inosine were studied in relation to weight loss and survival time of mice receiving a single dose of 12 Gy total-body ionizing irradiation. The radioprotective effect of a certain agent was usually assessed in relation to functions of the hematopoietic and digestive systems. However, the brain is also prone to radiation damage, both in adulthood 12) and in the developmental stages. 13) In order to provide a novel insight into radioprotection, we tried to evaluate the effect of inosine on brain dysfunction of irradiated mice, using behavioral tests.

MATERIALS AND METHODS

Experimental animals, grouping and drug administration

Seventy-six adult (two months old) male Balb/c mice weighing 21 ± 3 g were used in the present study. The study was approved by the Institute’s Committee of Experimental Animal Care and all the regulations were observed. All efforts were made to minimize the number of animals used as well as their suffering.

Forty-eight mice were used to explore the potential effect
of inosine on weight loss and survival. Forty mice received intraperitoneal injections of inosine or adenosine at doses of 375 or 750 micromol/kg respectively, or saline of the same volume, within 30 min after total-body irradiation (n = 8 for each group). After this, they received daily intraperitoneal injections till they died. The remaining 8 control mice were only exposed to total-body irradiation. Inosine and adenosine (for the chemical structures, see Fig. 1) were purchased from Sigma, St. Louis, USA.

The remainder of 28 mice were used to ascertain whether inosine on deficits in spatial memory induced by the irradiation. Twenty-one of these mice were irradiated and then given saline, inosine (750 micromol/kg) or adenosine (750 micromol/kg) (n = 7 for each group), following the protocols as mentioned earlier. Seven intact mice that did not undergo either irradiation or any other treatment served as controls.

**Total-body irradiation**

A $^{60}$Co irradiator was introduced for total-body ionizing irradiation. In detail: unanaesthetized mice were placed in well-ventilated plastic boxes and exposed to the $^{60}$Co irradiator at a distance of 3 m from the source. A single dose of 12 Gy γ-radiation was delivered at the dose rate of 2.44 Gy/min. The mice were then released from the plastic box and allowed free access to food and water.

**Assessments on weight loss and survival of irradiated animals**

Since weight loss is widely used as an indicator of the physical condition of irradiated experimental animals, the body weights of all mice were recorded immediately before irradiation (0 d), as well as 2 d and 3 d afterwards. To normalize the difference of body weight of mice before experiment, body weight loss rate (BWLR) was introduced to assess weight loss of irradiated mice. The parameter BWLR was calculated with the following formula:

$$\text{BWLR} = 100\% \times \frac{BW_0 - BW_1}{BW_0}$$

(BW0: body weight on 0 d; BW1: body weight on the day after irradiation)

Diarrhea was examined to determine severe gastrointestinal syndrome occurring in the given conditions. As a widely accepted standard to assess the effectiveness and efficiency of any putatively radioprotective agent, survival time (h) of each animal following irradiation was recorded for analysis.

**Assessments on spatial memory of mice**

One day after total-body ionizing irradiation, all mice were trained in a Morris water maze for two consecutive days (four trials per day). A platform of 6 cm in diameter was placed in the center of the northwest quadrant of a circular pool of 60 cm in height and 100 cm in diameter and 1 cm below the surface of the water. The water was rendered opaque by the addition of milk powder and maintained at 22–23°C throughout training and testing. During a given training trial, the mouse was introduced into the pool at one of four possible starting points (north, south, west, and east) and allowed a period of 60 s to find the platform. The order of starting points varied in a pseudorandom manner for each mouse every day. If the mouse had not found the platform within 60 s, it was directly placed on the platform by the experimenter. After staying on the platform for 30 s, the

![Chemical structures of adenosine and inosine](image-url)
mouse was then placed into a holding cage for a 30 s inter-
trial interval. Twenty-four hours after the final training trial, a probe test was conducted in which each mouse was allowed to swim for 60 s in the pool with the platform removed. The swimming behavior of the mice during the probe test was recorded with a computerized video tracking system (New Oriental, Beijing, China) to determine the percentage of time spent in the northwest (target) quadrant of the water maze.

Statistics
All data were shown in mean ± SD and analyzed with SPSS 13.0 for windows (SPSS Inc, USA), where P < 0.05 for significant difference.

RESULTS
After mice receiving a single dose of 12 Gy total-body ionizing irradiation at the dose rate of 2.44 Gy/min, the body weights of controls declined gradually with time. Like saline, neither inosine nor adenosine at any given dose, when administered daily, could prevent the loss of body weight. In each group, approximately one fifth of body weight was lost on the 3 d after irradiation. The least weight loss was found

<table>
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<tr>
<th>Tab. 1.</th>
<th>Changes in body weight of irradiated mice with different treatments (n = 8)</th>
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<tbody>
<tr>
<td>Groups</td>
<td>Body weight (g) / Body weight loss rate (%)</td>
</tr>
<tr>
<td></td>
<td>0 d</td>
</tr>
<tr>
<td>Control</td>
<td>21.3 ± 1.4</td>
</tr>
<tr>
<td>Saline</td>
<td>22.1 ± 1.1</td>
</tr>
<tr>
<td>Inosine</td>
<td>375 micromol/kg</td>
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<td></td>
<td>750 micromol/kg</td>
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<tr>
<td>Adenosine</td>
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The data were shown in mean ± SD and analyzed with one-way ANOVA followed by the Dunnett comparison test. Significant differences were only found among different time points. aa: p < 0.01, compared between 0 d and 2 d; aaa: p < 0.001, compared between 0 d and 2 d; bbb: p < 0.001, compared between 0 d and 3 d; c: p < 0.05, compared between 2 d and 3 d; ccc: p < 0.001, compared between 2 d and 3 d.

![Fig. 2. Survival times of irradiated mice after different treatments (n = 8). The difference among saline-, inosine- and adenosine-treated mice was analyzed using one-way ANOVA followed by the Dunnett comparison test. *: P < 0.05.](image-url)
in the control group. However, no significant difference in BWLR was found between any two groups, either on the 2 d or 3 d after irradiation (Table 1).

Within 2 days after irradiation, all the animals seemed to be normal in terms of regular activity and kept clean around their anus. Explosive membranate diarrhea happened on all mice on 3 d, while intake of food and water was hardly observed. There was no pronounced difference among the animals with different treatments as far as the time that diarrhea occurred was concerned.

In the control group, the mean survival time was 94.4 ± 5.9 h. There was no statistically significant difference in mean survival time between the controls and saline-treated animals (96.6 ± 6.2 h, P > 0.05). The mean survival time of animals treated with inosine at doses of 350 and 750 micro-mol/kg were 103.9 ± 6.5 h and 103.7 ± 4.5 h respectively, but only the latter survived significantly longer than the controls and the saline-treated animals (P < 0.05). Similarly, only doses of 750 micro-mol/kg of adenosine were able to significantly increase the mean survival time of irradiated mice (104.4 ± 3.8 h, P < 0.05) (Fig. 2).

To determine whether the BWLR could be used to predict the mean survival time of each group, we examined their relationship using regression analyses. However, we failed to find any correlation between them, either on 2 d or 3 d after irradiation (P > 0.05) (Fig. 3).

On the probe test day, the percentage of time spent in the northwest quadrant of the water maze was 22.3 ± 5.9% in the control group. Compared with controls, the irradiated mice that were administered saline and adenosine spent much less time in the target quadrant (11.9 ± 3.0%, P < 0.05; 11.1 ± 2.9%, P < 0.01, respectively). Compared with saline, inosine significantly increased the percentage of time of irradiated mice in the target quadrant (18.9 ± 3.8%, P < 0.05), while adenosine did not (P > 0.05) (Fig. 4).

**DISCUSSION**

Mammals receiving increasing doses of total-body irradiation (TBI) usually exhibit progressively severe radiation sickness. When the dose was higher than 6 Gy, the remarkable prodromal syndrome consisting of nausea and vomiting would be the first to be observed, followed by a subacute syndrome characterized by refractory diarrhea.1 In parallel to this, we found that mice deceased sharply their intake of food and water immediately after receiving total-body irradiation at a lethal dose and subsequently developed severe diarrhea after a recessive interval. Our results showed that without other supportive therapies, inosine and adenosine both at dose of 750 micro-mol/kg could increase survival time of irradiated mice with severe gastrointestinal syndrome, confirming that they have indications as protectors against radiation-induced tissue damage.11,16,17 Although in the present study the survival time was prolonged by only approximately 10 hrs, it is of potential importance to extend the therapy window for other effective interventions in the future. To our knowledge, this is the first report describing the radioprotective effect of inosine in the case of absolutely lethal γ-radiation at high doses.

It is believed that the radioprotective effect of adenosine and its precursor is mediated via activation of the receptors for adenosine.18 However, there are still controversies about...
how inosine functions. As an endogenous purine nucleoside, inosine is formed during the breakdown of adenosine by adenosine deaminase.\(^\text{19}\) Inosine was previously considered to be an inactive metabolite in most biological systems, but recent evidence indicates that it can exert powerful protection for many kinds of cells and tissues against various insults both \textit{in vivo} and \textit{in vitro}.\(^\text{2–10}\) It seems conceivable that inosine acts through a cell surface receptor, since nucleoside uptake inhibitors failed to reverse the effect of inosine.\(^\text{8}\) To date, the specific membrane receptor for inosine has not been identified yet, though inosine is able to bind and activate adenosine A3 receptors in mast cells.\(^\text{20}\) In contrast to this, the effect of inosine on the stimulation of axon outgrowth in neurons or on the protection of glucose-oxygen-deprived astrocytes could be prevented by dipyridamole.\(^\text{5,11,22}\) suggesting the involvement of an intracellular mechanism in these models. Thus, the unique mechanism underlying radioprotective effects of inosine remains to be clarified.

It is a known fact that ionizing radiation induces robust production of reactive oxygen species, leading to damage to cells and tissues. Previous investigations\(^\text{2,12,13}\) have shown that inosine could protect cells via purine nucleoside phosphorylase, a purine metabolic enzyme that can cleave inosine into hypoxanthine and ribose-1-phosphate. The final product of hypoxanthine is uric acid, a scavenger of peroxides,\(^\text{24}\) which may contribute to radioprotection of inosine. In addition, inosine could potently inhibit the production of many proinflammatory cytokines in a posttranscriptional manner.\(^\text{8}\) It is likely that inosine exerts its radioprotective effect via immunomodulation.

The radioprotective effect of a certain agent Researchers was usually assessed in view of functions of hematopoietic and digestive systems, though the brain is susceptible to radiation damage.\(^\text{12,13}\) As shown in the present study, the behavioral scores of irradiated mice merely reached to 50% of controls, demonstrating the dysfunction of spatial memory. In order to provide a novel insight into radioprotection of inosine, we further evaluated its effect on brain dysfunction of irradiated mice. Interestingly, in our study, inosine suppressed spatial memory deficits of irradiated mice assessed using the Morris water maze, a result that appeared to be different from those with adenosine. The difference did not seem to be due to physical conditions of irradiated mice, because: 1) either inosine or adenosine could not prevent weight loss of mice after irradiation; 2) the BWLR was not significantly different among groups with different treatments. This difference probably resulted from the fact that adenosine influenced the memory process via its receptors A1/A2. It has been demonstrated that A1 agonist impairs passive avoidance learning,\(^\text{25}\) while A1/A2 antagonist facilitates working memory.\(^\text{26}\) Notably, inosine does not have any of the apparent cardiovascular side effects that were reported for its precursor adenosine.\(^\text{4,25}\)

The present study demonstrated that inosine did not only facilitate the survival of the mice subjected to total-body irradiation at a lethal dose, but also suppressed radiation-induced spatial memory deficits. Taken together, inosine might be superior to adenosine with regard to the treatment of total-body irradiation at large doses.

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