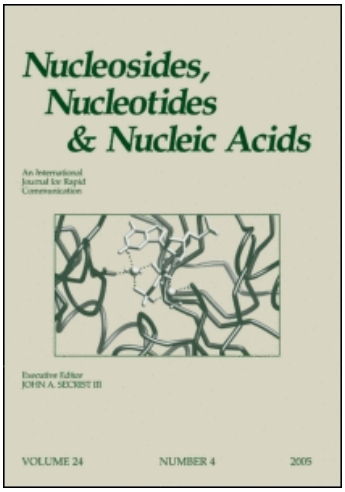

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ANTIOXIDATIVE AND RADIATION MODULATING PROPERTIES OF GUANOSINE-5′-MONOPHOSPHATE

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□ *Employing enhanced chemiluminescence in luminol-p-iodophenol peroxidase system and coumarine-3-carboxylic acid, it was shown that guanosine-5′-monophosphate (GMP) appreciably reduces formation of H_2O_2 and hydroxyl radicals induced by x-ray irradiation. Using immunoenzyme assay, we revealed that GMP lowered 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo) formation in DNA in vitro after irradiation. The results of survival test have shown that mice being injected intraperitoneally with GMP after irradiation with a dose of 7 Gy had better survival rate than the control mice. GMP reduced leucopoenia and thrombocytopenia in irradiated mice. Obtained results give premises that GMP may be promising therapeutic agent for treatment of radiation injuries.*

Keywords guanosine-5′-monophosphate; antioxidant; reactive oxygen species; 8-oxoguanine; x-ray irradiation; long-lived radicals

INTRODUCTION

Reactive oxygen species (ROS) are generated by the exogenous factors, such as ionizing radiation, ultraviolet (UV) radiation, chemical mutagens, carcinogenic substances, etc. Also, ROS are formed continuously in living cells of aerobic organisms as part of physiological processes, metabolic, and other biochemical reactions, but because of their reactive nature ROS can cause oxidative damage to lipids of cellular membranes, proteins, and DNA and other biomolecules.[1] Recent investigations have established that guanine is the main target for in DNA ROS.[2] Irradiation of DNA leads to appearance of a large number of oxidized derivatives of guanine in its structure.[3] In the middle of twentieth century, it was found that RNA[4] and DNA[5]

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protect plants and animals from radiation damage and reduce a chance of a fatal outcome. RNA hydrolyzed to mononucleotides was as effective as native RNA.[4] The component of nucleic acids, which is responsible for the protective effect, remained unknown for a long time. We have recently found that, among of five natural nucleosides composing mononucleotides of RNA and DNA, only guanosine has protective properties to some extent.[6] Based on this fact, we suppose that the guanosine-5′-monophosphate (GMP) is likely responsible for the protective and therapeutic effects of RNA and DNA.

Therefore, this investigation studied antioxidant and radioprotective (mitigation) properties of GMP. We have shown that GMP decreases radiation-induced generation of ROS, the amount of 8-oxodGuo formed in DNA upon x-ray irradiation and promotes elimination of bovine serum albumin radicals. GMP increases the survival rate of mice exposed to a lethal dose of ionizing radiation. This effect is most pronounced when this compound is administered shortly after irradiation. GMP facilitates the restoration of peripheral blood cell counts and protects bone marrow cells from damage.

MATERIALS AND METHODS

Irradiation

Irradiation was carried out on a RUT-15 therapeutical x-ray device (Mosrentgen, Moscow, Russia) at a dose rate of 1 Gy/min (focal distance 37.5 cm, current 20 mA, voltage 200 kV) or 4.5 Gy/min (immunoenzyme analysis) (focal length 19.5 cm, current 20 mA, voltage 200 kV). Mice were whole-body irradiated at room temperature with doses 7 or 1.5 Gy (1 Gy min^{-1}).

Determination of Hydroxyl Radicals

Hydroxyl radicals were detected using a highly specific fluorescent probe coumarin-3-carboxylic acid (CCA). Experimental conditions have been described in details elsewhere.[7] GMP solutions (0.02–1.0 mM) in 20 mM phosphate buffer, pH 7.4, were irradiated in glass flasks for liquid scintillation counter (Beckman, USA). Fluorescence intensity was measured on a SFM 25A spectrofluorometer (Kontron Instruments, Italy) at $\lambda_{ex} = 400$ nm and $\lambda_{em} = 450$ nm in a mirror quartz cell at room temperature. Standard calibration solution of 7-OH-coumarin-3-carboxylic acid (7-OH-CCA) were used for result evaluation.

Determination of Hydrogen Peroxide

The highly sensitive method of enhanced chemiluminescence in the luminol–*p*_iodophenol–horseradish peroxidase system was used.[8] GMP at

a concentration of 0.02–1.0 mM in 1 mM phosphate buffer, pH 7.4 was irradiated in liquid scintillation counter glass flasks (Beckman, USA). The chemiluminescence intensity was measured by a Beta 1 liquid scintillation counter (Medapparatura, Ukraine) under single photon counting conditions. The H_2O_2 solutions of known concentration were used for calibration of the measurement.

Immunoenzyme Analysis

The method of immunoenzyme analysis was described in detail previously.[9] Freshly made GMP solution in concentration 0.02–1.0 mM was always used for every experiment. The amount of 8-oxodGuo in DNA was determined on the basis of known value of radiation–chemical yield.

Determination of Protein Radicals

Effective and sensitive method to detect free-radical reactions is luminescence, when interaction of radicals yields energy emitted in the form of light quantum. We detected and studied protein radicals by measuring the intrinsic luminescence of protein solutions induced by x-ray radiation using a high-sensitivity Biotoks-7A chemiluminometer (ICE, Russia). Measurements were performed in 20-ml plastic polypropylene vials for a liquid scintillation counter. The use of larger volumes for measuring chemiluminescence compared to the conventionally used volumes (at most 0.1 ml) allowed us to increase significantly (more than 200 times) sensitivity of this method and to detecte formation of protein radicals at low doses of several Gy. The method and chemiluminometer characteristics were described in detail previously.[10] We use the concentration of bovine serum albumin is less physiological approximately 40 times. Protein concentration was 1 g/l, as soon as we found in previous experiments that maximal sensitivity of used method was reached at this concentration. At one molecule of BSA in solution contains about 15 molecules of GMP.

Animals

Male white outbred mice (Kv:SHK) were obtained from the central nursery of the Russian Academy of Medical Sciences, Kryukovo, Russia. SHK mice originate from the parent strain of Swiss mice. The animals were housed in polypropylene cages with sawdust as a bedding material. They were kept under controlled conditions of temperature ($22 \pm 3^{\circ}C$) and were given standard commercial mouse feed (Arno, Russia) and drinking water ad libitum. Five-week-old male mice weighing 17–20 g were used for all experiments. Animal handling was done according to institutional guidelines for animal care. All the experiments with mice were approved by the institutional bioethics committee.

Survival of Mice

Animals received a single whole-body exposure to x-rays. GMP was dissolved at 37°C in 0.14 M NaCl to a concentration of 5 mM just prior to the experiment. Mice were injected intraperitoneally (0.5 ml per mouse) with the GMP solution 15 minutes before or after the irradiation. Control mice received injections of 0.14 M NaCl solution. Animals were observed for 30 days after the irradiation, and the number of surviving mice, as well as their weight changes, were checked at the same time every day. Mice were weighted individually. The amount of food and water consumed by the animals was measured per cage, and the obtained values were divided into the number of caged mice

Blood Cell Count

Each group included initially 10 mice, 5 were randomly taken for blood cell count, and then the results were averaged for five. At the end of the experiment, if the number of survived mice in a group was less than five, all of the mice were taken for blood draws. Samples of peripheral blood were taken from the tail vein of mice. All experimental procedures were described in detail previously.[11]

Micronucleus (MN) Test

Isotonic GMP solutions (0.5 ml (5 mM in 0.14 M NaCl, 37°C)) were injected to mice i.p. 15 minutes prior or after the irradiation with a dose of 1.5 Gy. This dose value was chosen since the method is very sensitive and not intended for use at the doses greater than 4 Gy. A linear dose–response relationship is seen in the dose range of 0–2 Gy.[12] Histological samples for MN-test were prepared 28 hours after irradiation by the standard method[13] with minor modifications. More than 2,000 polychromatophilic erythrocytes (PCE) per mouse were counted.

Flow Cytofluorometry

GMP was injected intraperitoneally 15 minutes after or before irradiation as well as 0.14 M NaCl as a control for irradiated and nonirradiated mice. Thymus cells extraction was performed as described in Ormerod[14] in 22 hours after irradiation. After extraction thymus cells were fixed in 70% ice cold ethanol for 30 minutes and after rinsing and resuspending in phosphate buffer, were treated with RNA'se A 125 mkg/ml (Sigma, USA) and stained with propidium iodide (50 mg/ml). Cells were measured on Partec PAS III cytofluorimeter (Germany).

Statistical Analysis

Mean and standard error of the mean (SEM) were calculated for most variables. The means from the different treatment groups were compared by the Mann-Whitney U test or Student's unpaired t test when appropriate. In the survival experiments, the survival curves of different groups were compared by Fisher's exact test. Statistical significance was assigned to $p < 0.05$.

RESULTS

The influence of GMP on •OH formation in phosphate buffer under x-ray irradiation was investigated by method of trapping these radicals with coumarin-3-carboxylic acid. Its hydroxylation product, 7-OH-coumarin-3-carboxylic acid, fluoresces intensely. Table 1 shows that GMP in concentration 0.02–1.00 mM reduced amount of hydroxyl radicals in 20 mM phosphate buffer, pH 7.4. Effect of GMP on the formation of hydroxyl radical depends on the concentration of GMP. The quantity of hydroxyl radicals formed in phosphate buffer upon irradiation (1–7 Gy) in the presence or absence of GMP depended almost linearly on dose (data not shown).

The influence of GMP on H_2O_2 generation in phosphate buffer, pH 7.4, upon x-ray radiation was determined. It is shown in Table 1 that GMP in concentration 0.02–1.0 mM proved to be equally efficient in reducing H_2O_2 concentration. The effect of GMP on hydrogen peroxide formation is concentration-dependent. The amount of hydrogen peroxide formed in phosphate buffer upon irradiation (1–7 Gy) in the presence or absence of GMP is almost linearly dependent on dose (data not shown).

The effect of GMP on formation of 8-oxodGuo in salmon sperm DNA exposed to x-rays at doses of 3, 7, or 10 Gy was studied by an enzymelinked immunosorbent assay (ELISA) with monoclonal antibodies against 8-oxoG.

TABLE 1 Influence of GMP at various concentration on the generation of hydrogen peroxide, hydroxyl radicals in phosphate buffer, pH 7.4, and on the formation of 8-oxodGuo in a DNA solution irradiated in vitro with 7 Gy of x-rays

Treatment	OH-radicals, μM	DRF	H_2O_2, μM	DRF	8-oxodGuo per 10^5 Guo	DRF
7 Gy	1.67 ± 0.07	—	0.60 ± 0.02	—	5.5 ± 0.2	—
7 Gy + GMP 0.02 mM	$1.57 \pm 0, 05$	1.1	0.44 ± 0.02*	1.4	4.1 ± 0.2*	1.3
7 Gy + GMP 0.05 mM	1.40 ± 0.03*	1.2	0.36 ± 0.01*	1.7	3.8 ± 0.2*	1.5
7 Gy + GMP 0.1 mM	1.24 ± 0.06*	1.3	0.28 ± 0.01*	2.1	3.0 ± 0.1*	1.8
7 Gy + GMP 1 mM	0.54 ± 0.04*	3.1	0.21 ± 0.01*	2.9	2.6 ± 0.1*	2.1

The background level of hydrogen peroxide, hydroxyl radical, and 8-oxodGuo were subtracted from the observed values.

Data points are means ± SEM of three independent experiments.

*Significantly different from control at $P < 0.05$.

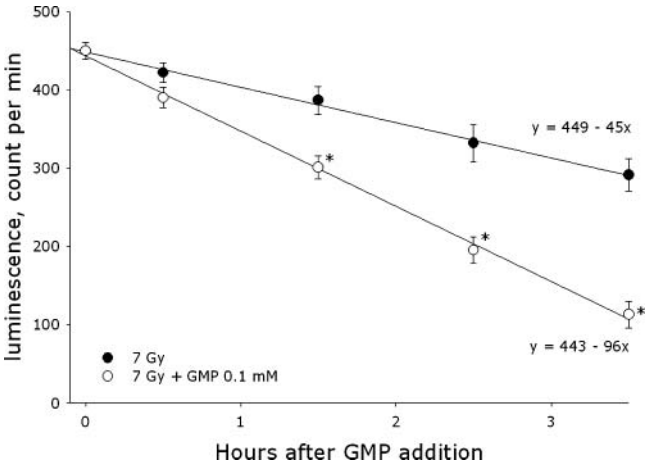

FIGURE 1 Time dependence of the effect of GMP on the elimination of bonive serume albumin radicals. The baseline values of the chemiluminescence intensity were subtracted. Bonive serume albumin concentration of 1 g/l. Data are represented as the means ± SEM of three independent experiments. Asterisk marks the values that significantly differ from the control at $p < 0.05$ (Student's unpaired t test).

As shown in Table 1, GMP in concentration 0.02–1.0 mM decreased the radiation-induced formation of 8-oxodGuo in DNA. The amount of 8-oxodGuo formed in DNA upon x-ray irradiation in the presence or absence of GMP depended linearly on dose (data not shown). GMP added to DNA after irradiation has no effect on the level of 8-oxodGuo. This means that GMP at the concentrations used did not cross-react with the antibodies.

The time dependence of the effect of GMP on elimination of bonive serume albumin (BSA) radicals formed under exposure to x-ray radiation (dose, 7 Gy) is shown in Figure 1. GMP was added to the protein solution 1 hour after protein irradiation and the intrinsic luminescence intensity was measured after incubation for 0.5, 1.5, 2.5, and 3.5 hours at room temperature in the dark. The half-life of BSA radicals constituted approximately 5 hours. At a concentration of 0.1 mM GMP neutralizes approximately 60% of protein radicals for 3.5 hours. The rate of BSA radical neutralization in the presence of GMP is twice as high as the control value. The amount of BSA radicals formed upon x-ray irradiation is linearly depends on dose (data not shown).

The survival of mice injected intraperitoneally with GMP 15 minutes before or 15 minutes after irradiation with a dose of 7 Gy was investigated (Figure 2). The median survival time of control mice was about 8 days and the maximum was 14 days. When GMP was injected before irradiation, the mean survival times was approximately 10 days, while the maximum survival time increased up to 24 days. A pronounced effect was observed when GMP was injected right after irradiation. In this case, approximately 40% of animals were alive on the 30th day after irradiation.

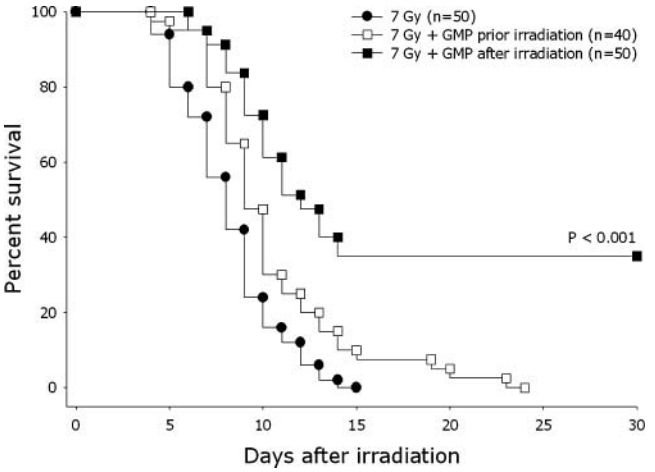

FIGURE 2 Kaplan-Meier estimate of 30-day survival of x-irradiated (7 Gy) mice injected i.p. with GMP (~45 μg g-1) intraperitoneally before or after irradiation. The data represent the means of 4 or 5 separate experiments with 10 mice per group in each experiment. Differences in survival between the irradiation control group and the GMP-treated groups were evaluated by Fisher's exact test.

For surviving animals, intake of water and food by the animals as well as changes in their body weight were also studied (Table 2). The amount of food and water consumed by intact mice was basically the same over the all experimental period. The same is true for un-irradiated GMP animals (data not shown). Mice irradiated with a dose of 7 Gy consumed food and water less by 20–40% and 40–50%, respectively. Intake of food and water was in average, similar in mice injected with GMP prior or after irradiation to

TABLE 2 Percentage changes in body weight (means ± SEM) and the amount of food and water intake of x-irradiated (7 Gy) mice injected i.p. with GMP (~45 μg g^{-1}) 15 minutes prior or after exposure

	Days after irradiation					
	0	3	5	10	20	30
Treatment	Change of body weight,% Food/Water intake, g					
0 Gy	100 ± 3	101 ± 3	102 ± 3	105 ± 3	108 ± 3	112 ± 4
	4.8/7.0	4.9/7.0	4.9/7.0	4.8/6.9	4.9/6.9	5.0/7.0
	(10)	(10)	(10)	(10)	(10)	(10)
7 Gy	100 ± 4	89 ± 3	80 ± 3	69 ± 5	—	—
	4,7/6.9	3,4/3.4	2,7/4.0	3,9/4.2	—	—
	(10)	(10)	(9)	(2)	(0)	(0)
7 Gy + GMP 15 minutes prior	100 ± 3	92 ± 3	80 ± 4	70 ± 7	75	—
	4.9/7.0	3.5/3.6	3.0/3.8	3.0/4.6	3.3/4.8	—
	(10)	(10)	(9)	(5)	(1)	(0)
7 Gy + GMP 15 minutes after	100 ± 4	91 ± 5	84 ± 4	65 ± 7	90 ± 5	102 ± 6
	4.7/6.9	3.8/4.1	3.4/4.1	3.1/4.9	4.5/5.9	4.9/6.7
	(10)	(10)	(10)	(7)	(2)	(2)

Data are means for n animals; *n* is given in parentheses.

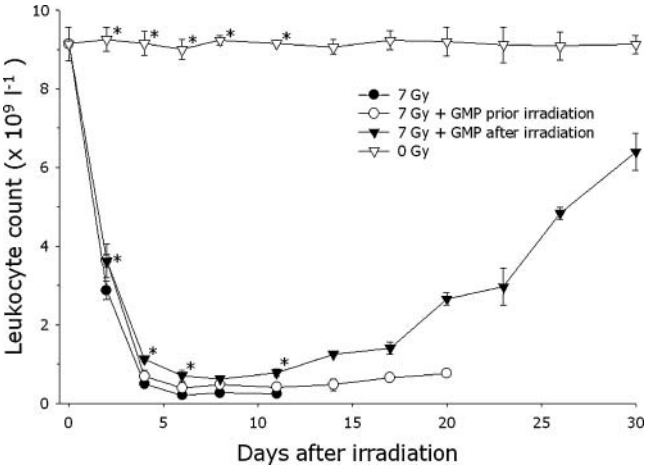

FIGURE 3 Circulating leukocyte counts of whole-body 7 Gy x-irradiated mice injected i.p. with GMP (~45 μg g-1) 15 minutes before or after exposure. Data points represent means ± SEM of 2–5 animals. Statistically significant differences between irradiation control group and the other groups (Mann-Whitney U test, $p < 0.05$) are marked by asterisks.

irradiated control mice. The consumption of food and water by GMP-treated irradiated mice significantly returned to norm by Day 30. The body weight in all groups of un-irradiated mice was increased, in the mean, by 10–14% over the course of experiment. The control mice lost 11% of their body weight within three days after irradiation and then up to 31% within 10 days after the irradiation. Body weight loss rate was similar in GMP-treated mice. No signs of diarrhea or obvious rectal bleeding were observed throughout the experiment in mice irradiated with 7 Gy of x-rays.

The effect of GMP administered i.p. to mice irradiated with 7 Gy of x-rays was studied on the leukocyte count of their peripheral blood (Figure 3). The amount of blood leukocytes in intact animals almost was not changed throughout the experiment. In un-irradiated mice receiving GMP the leukocyte count increased by 5% by Day 2, then decreased to its initial level by Day 4 and was not noticeably changed thereafter (data not shown). In irradiated mice, a sharp decrease of leukocyte count was observed by the second day, and the amount of leukocytes in irradiated control animals and in irradiated GMP-treated mice decreased by 98 and 93–96%, respectively, by Day 6, compared to the un-irradiated control. Hereafter, the leukocyte count in irradiated control mice was not changed substantially up to mice death. In fact, a similar pattern was observed in mice receiving GMP before irradiation. In animals receiving GMP after irradiation, the leukopenia was less severe; the leukocyte count began to rise gradually and the amount of leukocytes comprised 70% of its normal value by Day 30.

The effect of GMP injected to mice prior or after irradiation with 7 Gy on the granulocyte counts in peripheral blood was investigated (Figure 4). A change in the number of granulocytes after irradiation was largely parallel

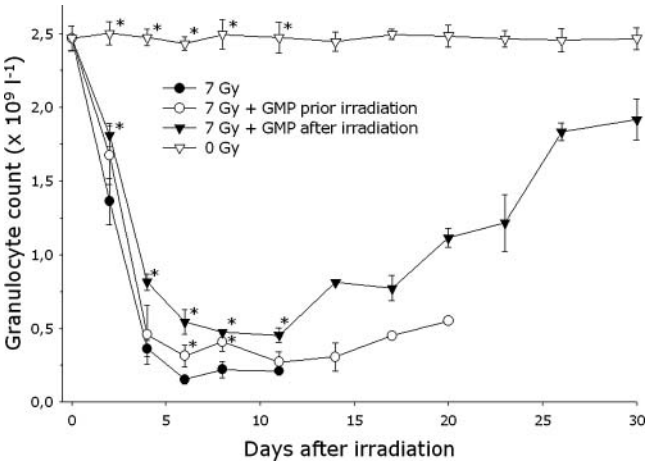

FIGURE 4 Peripheral granulocyte counts of x-irradiated (7 Gy) mice injected i.p. with GMP (~45 μg g-1) 15 minutes before or after exposure. Data points represent means + SEM of 2–5 animals. Statistically significant differences between irradiation control group and the other groups (Mann-Whitney U test, $p < 0.05$) are marked by asterisks.

to that of leukocytes. The amount of granulocytes in all irradiated animals decreased markedly by Day 2. Granulocyte counts in the groups of mice that received GMP after irradiation had a tendency to increase. They restored by Day 30 to 77% of their initial value.

The influence of GMP on the platelet counts in peripheral blood of mice exposed to x-rays at a dose of 7 Gy was studied (Figure 5). The thrombocyte

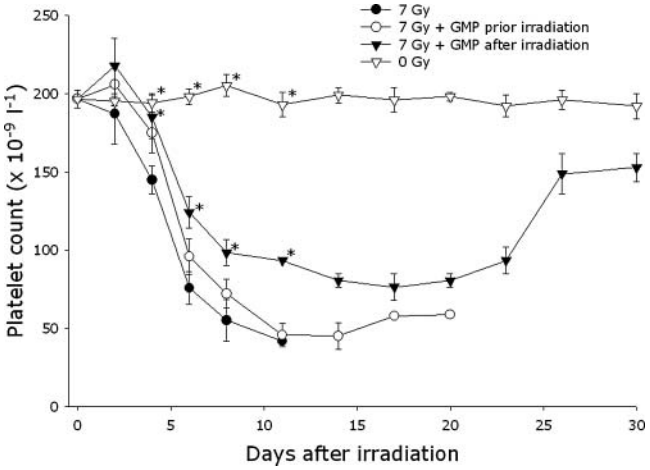

FIGURE 5 Peripheral platelet counts of x-irradiated (7 Gy) mice injected i.p. with GMP (~45 μg g-1) 15 minutes after exposure. Data points represent means + SEM of 2–5 animals. Statistically significant differences between irradiation control group and the other groups (Mann-Whitney U test, $p < 0.05$) are marked by asterisks.

TABLE 3 Effect of GMP (~45 mg g^{-1}) injected i.p. to mice 15 minutes after or before their irradiation with 1.5 Gy of x-rays on the formation of PCE with MN in the bone marrow of the animals

Treatment	Number of animals	Number of PCE	Number of PCE with MN	Percentage of PCE with MN
0 Gy	5	10260	50	0.49 ± 0.06*
1.5 Gy	5	10279	552	5.36 ± 0.26
1.5 Gy + GMP prior	5	10306	317	3.08 ± 0.25*
1.5 Gy + GMP after	5	10638	299	2.81 ± 0.28*

The data were obtained by the micronucleus test and are given as means±SEM for specified number of animals.

*Significant difference at 5% level (Student's unpaired *t* test) in comparison with the irradiation control.

count in non-irradiated mice (intact control and GMP-treated control) was not changed measurably over the experiment. In control irradiated mice and mice receiving GMP prior or after irradiation, the number of platelets fell sharply by Day 6 and comprised about 39, 48, and 63%, respectively, of its initial value. By Day 30, the thrombocyte count in blood of irradiated mice receiving GMP reached 77%, respectively, of its normal level.

The MN test, which is based on the registration of mouse bone marrow PCE containing MN, is a convenient tool for assessing the effectiveness of compounds protecting animals against the cytogenetic cell damage. We studied the effect of GMP injected to mice 15 minutes prior or after the irradiation with x-rays at a dose of 1.5 Gy on the formation of PCE with MN in the bone marrow (Table 3). As it is shown in the Table 3, the percentage of PCE with MN after irradiation increases about eleven-fold, from 0.49% in the un-irradiated control to 5.36% after irradiation with a dose of 1.5 Gy. The administration of GMP prior or after irradiation produced a marked protective effect against x-ray injury; the amount of PCE with MN decreased by 42 and 48%, respectively, compared with the irradiation control. Thus, GMP protected the bone marrow cells of mice against cytogenetic damage induced by x-rays.

During later stage of apoptosis extensive degradation of DNA occurs. Apoptosis is accompanied by fragmentation of DNA into oligonucleosomal fragments with length that are multiplies of 180–200 b.p. and nonspecific degradation of DNA. Lower molecular weight DNA leaches out during overnight incubation in phosphate citrate buffer. These cells with lower apoptotic DNA content can be observed as sub G1 peak on cytofluorimeter DNA histogram. Irradiation causes cells to be arrested in G1 phase. Thymus cells, extracted from 7 gray irradiated mice, show significally less amount of cells entering G2 phase comparative to control and accumulation of number of cells in G1 peak. Intraperitoneally injection of GMP in irradiated mice 15 minutes after irradiation does not prevent cells from G2 delay. However, sub-G1 peak is not clearly visible on DNA histogram of cells in

GMP treated mice as in irradiated without treatment, which could be explained as blocking of some apoptotic pathways and preventing cells from programmed death or more extensive degradation of DNA to smaller pieces, which can leak from cells, lowering DNA content. Gating area with sub G1 peak and extensive DNA degradation can give us comparative quantitative evaluation of DNA degradation for control, irradiated mice and irradiated mice treated with GMP. Thymus DNA degradation in nonirradiated mice after GMP peritoneal injection was in the same minimal value as in control mice. Irradiated with 7 gray mice were divided on three groups—first group received peritoneal injection of 5 mM GMP before irradiation, second group received injection of 5 mM GMP in 15 minutes after irradiation and third group—0.9% NaCl. All irradiated mice showed more than 5-time increase in thymus cell DNA degradation. Thymus cells degradation in irradiated mice treated with GMP 15 minutes after irradiation is non-significally less ($P > 0.05$) than in irradiated mice with NaCl control solution. Injection of GMP 15 minutes before irradiation has an effect similar to effect of GMP injection 15 minutes after irradiation.

DISCUSSION

Ionizing radiation produces shortlived reactive oxygen species during water radiolysis.[15] We found that GMP is scavenger of ROS such as hydroxyl radicals and hydrogen peroxide (Table 1). Our results agree with earlier published data. According toBiaglow et al.,[16] GMP decreases the concentration of hydroxyl radicals in ferric ion-linked Fenton reaction. Also we have shown that GMP exhibits the protective effect decreasing the yield of 8-oxodGuo in irradiated DNA in vitro (Table 1) and eliminating protein lesion (Figure 1). We suggest that these protective effects are mediated by reducing of generation of ROS due to the easy oxidation of GMP in water solutions. Guanine, the base of GMP, has the lowest redox potential among all natural nitrogen bases.[17] Moreover, in vivo GMP can be broken down to guanosine and uric acid, these compounds exert a wide range of biological effects, including significant antioxidative properties.[6,7,18] One can assume that it is guanosine and uric acid that bear a part of the antioxidant properties of GMP in vivo.

It was established recently that, after irradiation of animals, cells or protein solutions, long-lived protein radicals form.[19–22] Histone H1 as well as some other proteins and amino acids irradiated in the presence of oxygen produce long-lived radicals.[20] The formation of long-lived radicals with a half-life of about 20 hours by ionizing radiation was shown using electron paramagnetic resonance spectroscopy.[19] These radiation-induced long-lived protein radicals give rise to mutations and provoke cell transformation. These radicals trigger the formation of protein-DNA pyrimidine base crosslinks and oxidative base damage with the production of

8-oxodGuo.[10,21] It is suggested that protein radicals are not the final products but rather are the mediators of oxidative stress in biological systems.[20] Some low molecular-weight bioantioxidants (for example, guanosine, inosine, vitamin C) can effectively quench these long-lived radicals.[10,22] We have shown (Figure 1) that the GMP quenching the long-lived protein radicals. Furthermore, quenching effect of GMP is comparable and in some cases higher than that of the above compounds.

GMP administration has no influence on the weight of mice (Table 1). Probably GMP does not have a protective action on the gastrointestinal tract of mice. In our previous work it was shown that irradiated with a dose of 7 Gy SHK mice die from hematopoietic injury. The major factors contributing to death were tissue hemorrhages due to thrombocytopenia and opportunistic infections as a consequence of leukocytopenia.[11] Our results demonstrate that GMP administered early after lethal-dose irradiation facilitates hematopoietic recovery (Figures 3 and 4) and prevents full-blown thrombocytopenia (Figure 5). Also previous investigations have shown that GMP injected to rat before irradiation could decrease formation of skin hemorrhages.[23] Thus, we think that at the organism level, protective effect of GMP is implemented through prevention of hemorrhages formation and stimulation of hematopoiesis. Furthermore, some indirect data allow us to assume that a mechanism of the radioprotective action of GMP on cellular level is probably partially related to the DNA protection or stimulation of DNA repair (Table 3) and quenching long-lived protein radicals (Figure 1). Moreover, guanosine, as a part of methabolic breakdown of GMP, can affect regulatory signaling cell system. It was shown that guanosine interacts with A3 adenosine receptor subtypes, which are involved in signalic pathways of triggering of apoptose, inflamation and allergic reaction.[24]

According to Stone et al.,[25] all the agents able of correcting radiation injuries can be classified into protectors, mitigators, and treatments depending on the time an intervention is to be administered. GMP both protects against and mitigate radiation-induced damages and can be viewed as versatile radiation modulators. Although medical management of hematopoietic syndrome has improved in recent years, treatment options remain limited. Some hemopoietic factors, such as cytokines, demonstrated to be effective protectors when administered either prior or after irradiation.[26] Granulocyte-colony stimulating factor (G-CSF) increase survival of various animals and accelerating recovery from neutropenia and thrombocytopenia.[26] In clinic, G-CSF is used to hasten neutrophil recovery.[27] However, G-CSF therapy is not free from disadvantages. Cytokines may be toxic if given systematically and cause side effects (antigenicity, mild flu-like symptoms, bone pain, splenomegaly, etc.).[28] GMP is comparable with the cytokines ability to protect but is less expensive, more stable, readily available and well-tolerated (in mice, LD50 for GMP is 3.5–5.0 g/kg (intraperitoneal, subcutaneous, intravenous administration) or 15 g/kg (oral administration).[29]

All this makes it favorable as a protective agent. This compound introduced shortly after radiation exposure offers a promise as a therapeutic agent ameliorating radiation-induced injuries. However, several hours might be lost before radiation victim could obtain a medical help. For this reason, GMP, unlike the G-CSF, is not so convenient for use in radiological accident, but it is presumably convenient for clinical and scientific use.

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