

## Effect of Tocopherol-monoglucoside (TMG), a Water-soluble Glycosylated Derivate of Vitamin E, on Hematopoietic Recovery in Irradiated Mice

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### Tocopherol monoglucoside (TMG)/Sublethal dose of gamma irradiation/Hematopoiesis/Mice.

A preparation of alpha-tocopherol monoglucoside (TMG) administered i.p. at a dose of 600 mg/kg immediately after whole body gamma irradiation was examined for its radioprotective efficacy towards bone marrow and peripheral blood nucleated cells. When mice received X-rays at a dose of 5,6 Gy, a marked decrease in bone marrow karyocytes and a reduction of peripheral leukocytes within the early post-irradiated period were observed. However these changes were attenuated in TMG-treated mice. Significant protection of blood lymphocytes was found for the TMG group of mice. The return to normal value of the reduced blood leukocyte count starting from the 8th day was more rapid in TMG-treated mice than in untreated irradiated mice. TMG administration was found to enhance hematopoietic recovery, as measured by the exceeded nucleated bone marrow cell count due to elevated amount of both lymphoid and granulocytic elements in the TMG-group, in comparison with that of both control irradiated and non-irradiated animals. These findings indicate that the radioprotective effect of TMG is apparently realized through its influence on hematopoietic system.

### INTRODUCTION

Nowadays many synthetic as well as natural compounds have been investigated for their efficacy to protect against irradiation damage. They include sulfhydryl compounds, antioxidants, plant extracts, immunomodulators, and other agents.<sup>1,2)</sup> Many studies are focused on vitamins with antioxidant properties such as Vitamin A, Vitamin E (alpha-tocopherol) and Vitamin C (ascorbic acid). These vitamins are present in living cells and biological fluids and they have been shown to be inhibitors of free-radical processes in tissues. Among the others the effectiveness of alpha-tocopherol in the protection against irradiation-induced damage in bone marrow and survival in irradiated mice is well known.<sup>3)</sup> Since the mobility of Vitamin E in the biological membranes is restricted due to its long phytyl side chain, it seems to be unlikely to scavenge active oxygen, which might be gener-

ated in aqueous phase. Consequently water-soluble derivatives of Vitamin E tocopherol monoglucoside (TMG) have been synthesized by introduction of glucose into the molecule of vitamin E to scavenge reactive oxygen species in aqueous phase.<sup>4)</sup> TMG was demonstrated to exhibit its activity both in water and lipid phases due to the presence of chromanol ring and a short hydrogen tail in its molecule that allowed it to interact with free radicals in cell cytoplasm and cell membrane displaying a high antioxidant activity.<sup>5)</sup>

Using differential voltammetry we showed also a high TMG antioxidant activity that was nearly two-fold higher than that of ascorbic acid.<sup>6)</sup> Besides we have found that the more effective range for its concentration was 0,12-0,60 g/l<sup>-1</sup>. The higher antioxidant activity was observed for the pH from 6,9 to 9,1 with maximal effect for pH 7,3.<sup>6)</sup> These results are correlated to the effective concentration of TMG for protection against radiation damage in mice. TMG was revealed to show the radioprotective effect in mice irradiated at a dose of 6.6 Gy since administration of TMG at a dose of 600 mg/kg immediately after the whole body gamma-irradiation resulted in high rate of survival in mice (60%).<sup>7)</sup> Kapoor *et al* reported that TMG may act as an antioxidant to repair free radical damage to some biological compounds.<sup>8)</sup>

The survival after irradiation is really a result of recovery of several target system such as bone marrow, gastrointestinal tract and skin. Hematopoietic system is known to be one

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of the most radiosensitive and its damage may be critical for the survival due to hematopoietic syndrome death.<sup>9</sup> Satiyamitra *et al* showed that TMG given in a single dose of 400 or 600 mg/kg was effective in significantly reducing damage to bone marrow chromosomes in mice when administered within 10 min of radiation.<sup>10</sup> Nair *et al* found that the TMG administration immediately after exposure to gamma-irradiation can protect normal tissue in tumor-bearing mice.<sup>11</sup>

In our study we examined the effect of TMG on recovery of bone marrow and peripheral blood cell counts in mice that received whole-body gamma-irradiation.

## MATERIALS AND METHODS

### Animals

Male CBA mice, 32–48 weeks old (weighting 30–35 g) were obtained from Institute of Pharmacology (Tomsk, Russia). Animals were quarantined for a period of 2 weeks and were housed in rodent cages with 8–10 animals per cage at about 23°C. Research was conducted according to the principles set out in the “Guide for the Care and Use of Laboratory Animals” (Moscow Breeding Nursery, Russian Acad. Med. Sci.).<sup>12</sup>

### Irradiation of animals

Mice were placed in plexiglass containers and the whole-body exposed to 5.6 Gy of gamma rays. Gamma-radiation was performed with “RUM-17” unit (filter: copper 0.5 mm + 1.0 mm aluminum, 200 kV voltage on the tube, 5 mA anodic current) at the dose rate of 0.5 Gy per a minute.

### Administration of TMG

TMG was sent to our Institute by Dr. V. T. Kagiya (Health Research Foundation, Kyoto, Japan). TMG was dissolved in physiological saline for the administration of dose of 600mg/kg through intraperitoneal (i.p.) route in a maximum volume of 0,2 ml. Control animals were administered with 0,2 ml of normal saline. The effect of TMG on bone marrow and peripheral blood cell count was examined in healthy mice and gamma-irradiated mice.

### Analytical methods

Mice were anesthetized by ether before sacrificing them on days 3, 8, 14, 20, 30 after various treatments and blood and femur bone marrow cells were prepared as described by E.Goldberg *et al.*<sup>13</sup> Briefly, femoral bone was exposed under aseptic conditions, cells were washed with 199 medium, suspended by a syringe with a needle of various diameter, and washed again 2–3 times with 199 medium (centrifuged at 150 g, for 10 minutes). Smears of the cells were drawn on clean glass slides, fixed with methanol for 10 min and stained with May-Grunwald-Giemsa. At least 1000 cells were scored from each animal to determine differential elements (erythroid, lymphoid, myeloid cells). Total leukocyte

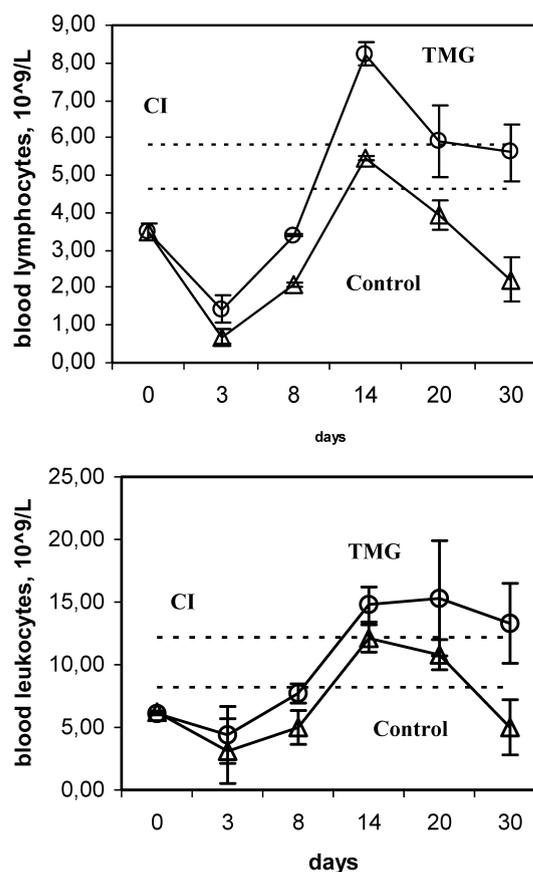
count as well as differential leukocytes (lymphocytes, neutrophils and monocytes) were studied in peripheral drawn from mice on the same days after irradiation. Splenic nucleated cell count was also examined at the same time.

### Statistical analysis

Data are presented in terms of the mean and SEM. Comparison between groups was made by nonparametric Mann-Whitney test. P values <0.05 was considered as significant.

## RESULTS

No evident changes in total number and different elements of both bone marrow and blood karyocytes were observed in nonirradiated mice treated with TMG at different post administration intervals (data not shown). Change in the blood differential leukocytes count following irradiation for various treatment groups is presented at Fig. 1. The total leukocyte count was nearly 3-fold decreased on day 3 after irradiation and no significant changes were found in mice treat-



**Fig. 1.** Time-dependent leukocyte and lymphocyte count in peripheral blood of mice given 5,6Gy of gamma-irradiation. Mice received injection i.p. with 600mg/kg of TMG (curve “TMG”) or saline (curve “control”) just after irradiation. Each value represents the mean  $\pm$  SD for 5 mice. CI - Confidence interval: mean value  $\pm$  SD for the index of nonirradiated mice within 30 days.

ed with TMG. The rate of neutrophil and monocyte level in mice of both group were similar to the changes in total leukocyte count. However significant protection of blood lymphocytes was apparent for the TMG group of mice (Fig. 1). Lymphocyte count and its proportion tended to be over in mice treated with TMG after irradiation than in the control group (Fig. 1,3)

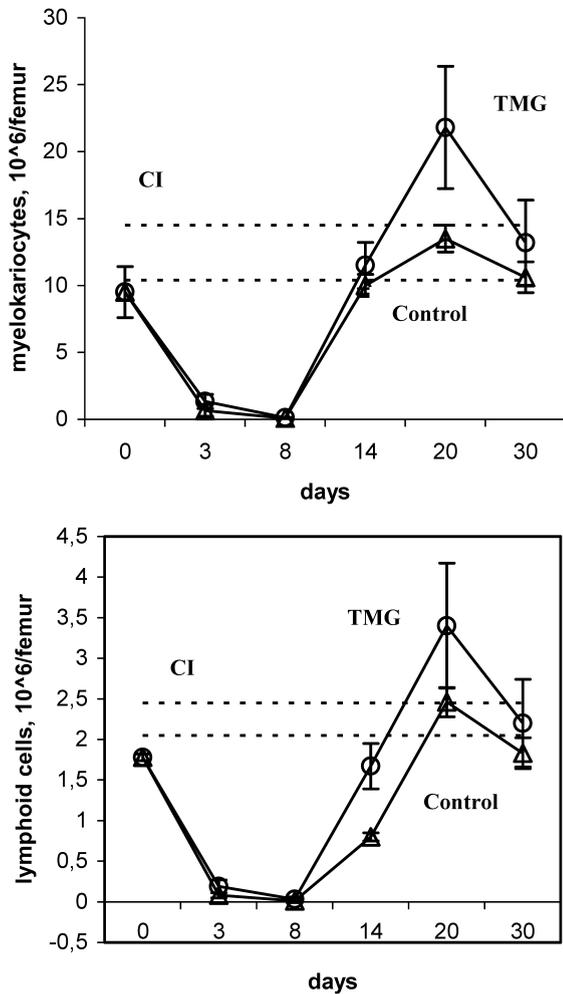
The recovery of leukocytes in peripheral blood following irradiation became evident from 8<sup>th</sup> day and the leukocyte level in TMG group was significantly higher within all days up to the 30<sup>th</sup> day compared with the irradiated control group (Fig. 1).

Figs 2 and 3 show the effect of TMG on bone marrow cell count in irradiated mice. The whole-body gamma- irradiation of mice at a dose of 5.6 Gy resulted in the decrease in

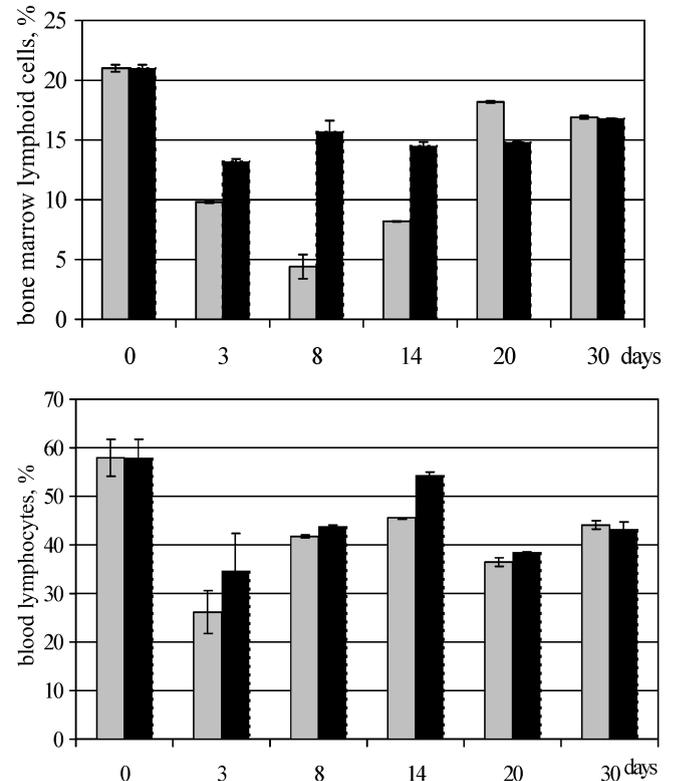
the number of bone marrow nucleated cell. Bone marrow cell count reduction was predominantly related to the dramatic decrease in the number of both lymphoid cells (Fig. 2), and neutrophilic granulocytes and erythroid cells (data not shown). Figure 2 shows that X-ray induced depletion of bone marrow cell reaching the minimum value on the 8<sup>th</sup> day was mitigated in the TMG group: total myelokaryocyte count was twice higher in the group of animals treated with TMG ( $0,13 \pm 0,03$  cells/femur ( $\times 10^6$ )) than that in the control group ( $0,07 \pm 0,02$  cells/femur ( $\times 10^6$ ))(p < 0,05). Treatment with TMG appeared to have a protective effect against irradiation-induced lymphoid cell death resulting in two-fold prevalence of lymphocyte count on day 3 compared with control ( $0,19 \pm 0,08$  cells/femur ( $\times 10^6$ ) versus  $0,08 \pm 0,03$  cells/femur ( $\times 10^6$ ) in control mice)(p < 0,05).

The prevention of lymphocyte proportion decrease by TMG was also revealed within early post-irradiated period (Fig. 3).

The render of number of bone marrow cells following irradiation was revealed from day 14. The lymphoid element count was returned to the normal level earlier in the TMG group than in the control group (Fig. 2). TMG administration was found to enhance hematopoietic recovery as mea-



**Fig. 2.** Time-dependent total myelocaryocyte and lymphocyte count in bone marrow of mice given 5,6Gy of gamma-irradiation. Mice received injection i.p. with 600mg/kg of TMG (curve "TMG") or saline (curve "control") just after irradiation. Each value represents the mean  $\pm$  SD for 5 mice. CI - Confidence interval: mean value  $\pm$  SD for the index of nonirradiated mice within 30 days.



**Fig. 3.** The rate of lymphocyte proportion in bone marrow (upper) and in peripheral blood (lower) of mice given 5,6Gy of gamma-irradiation. Mice received injection i.p. with 600mg/kg of TMG (black column) or saline (white column) just after irradiation. Each value represents the mean  $\pm$  SD for 5 mice.

sured by the exceeded nucleated bone marrow cell count on day 20 due to elevated amount both lymphoid and granulocytic elements in "TMG-group" in comparison with that of both control irradiated and non-irradiated animals (Fig. 2).

The early recovery of splenic nucleated cell count following the irradiation was also observed in TMG treated irradiated mice (data not shown).

## DISCUSSION

The whole-body high dose gamma-ray irradiation of mice is known to result in the depletion of hematopoietic organs owing to the intensive destruction of irradiated cells and the violation of their reproduction due to decreasing ability to proliferate.<sup>14,15</sup> The so-called hematopoietic syndrome death is often sufficient for the organism lethality as a result of infection due to the impairment of the immune system.<sup>12</sup> The whole-body X-ray irradiation for mice was found to produce DNA damage and a marked decrease in anti-oxidants in the bone marrow.<sup>16</sup> Various mechanisms such as prevention of damage through inhibition of free radical generation or their intensified scavenging, enhancement of DNA and membrane repair, replenishment of dead hematopoietic and other cells and stimulation of immune cell activity are considered important for radioprotection.

TMG was shown to give a significant protection when it was administered after irradiation with no evident toxicity at the effective dose of 600 mg/kg. Protective actions of TMG were displayed as the inhibition of gamma-radiation induced thymine glycol formation, protection of cells against radiation-induced loss of viability by stimulation of DNA repair and prevention of whole body irradiated mice lethality.<sup>10,7,17,18,3</sup> The antioxidant activity of TMG may contribute to the protective property, but the high efficacy when given post irradiation alludes to the involving of other mechanisms. One can expect that TMG can both prevent the destruction and promote faster recovery of bone marrow cells. Our experimental findings have confirmed this supposition.

Administration TMG after irradiation produced a significant increase in BM cell count that was reduced because of irradiation. We have found that TMG administered to irradiated mice resulted in attenuation in radiation-induced damage to lymphocytes, which are known to be less resistant to radiation than other differential leukocytes.<sup>15,19</sup>

Since within the whole post-irradiation period the peripheral blood pattern is mainly the reflection of processes occurring in hematopoietic organs,<sup>9</sup> significant protective effect of TMG against lymphocyte death in bone marrow can lead to their accelerated recovery in peripheral blood. In fact, the return to normal value of the reduced blood leukocyte count starting from 8th day was more rapid in TMG-treated mice than in untreated irradiated mice.

Since DNA damage in bone marrow cells is a critical

event for cell death the ability of TMG to protect DNA damage could be associated with the early recovery of peripheral leukocytes that originated from the bone marrow cells. It may be related to both the intensification of proliferative activity of survived stem cells and the migration of cells from peripheral blood and thymus to bone marrow.<sup>14</sup>

In previous study we demonstrated the ability of TMG at various doses to enhance both spontaneous and PHA-induced proliferation of splenic lymphocytes in mice. In addition we showed that TMG was able to stimulate the lymphocyte activity to inhibit tumor cell proliferation *in vitro*.<sup>20</sup> As the immune cells such as macrophages and lymphocytes are known to regulate hematopoiesis, the immunomodulatory activity of TMG is likely to also contribute to the hematopoietic recovery following irradiation.<sup>21</sup>

Evidences exist that alpha-tocopherol derivatives with short-cut side chain as well as unchanged alpha-tocopherol form are able to strongly inhibit the formation of lipid peroxidation reactive products and to modulate glutathioneperoxidase activity.<sup>22</sup> It can be expected that the direct antioxidant action of TMG may combine with its capacity to activate the antioxidant systems, that results in a reduction of the level of free-radical lipid peroxidation products and stabilization of cellular membrane structure.<sup>5,23</sup>

Thus, the direct antioxidant activity of TMG, its ability to protect against radiation-induced cytogenetic damage and enhance the post irradiation recovery of hematopoiesis, as well as immunomodulatory activity together could contribute to TMG radioprotective effect. All these findings suggest that TMG is a promising protector against unanticipated exposures in a case of accidental release of radioactivity and it may be exploited in the protection of individuals exposed to radiation accidents, dirty bombs, and nuclear terrorism. The high water solubility makes TMG as a superior protector than the parent vit. E since it overcomes complications arising from emulsifiers and other ingredients present in the solvent to deliver vit.E in the body.<sup>24</sup>

Since it is generally accepted that opportunistic infections due to immunosuppression following radiation exposure are the major complications of cancer radiation therapy the use of TMG in radio therapeutic applications may also be promising. The difficulty of employing radioprotectors in cancer therapy is the potential of affecting tumor response. However, according to data of Nair *et al*, TMG administration does not protect tumor cells from radiation damage in tumor-bearing mice with synchronous protective effect on normal hematopoietic cells.<sup>11</sup> This fact suggests that TMG application could be useful to decrease the side effect of radiation in the treatment of cancer patients. Further experiments are needed for discovering the required dose of TMG to use it in fractionated radiotherapy of cancer patient.

In conclusion, our findings show more favorable post-irradiation changes in the number of bone marrow karyocytes and peripheral blood leukocytes in animals treated with

TMG immediately after irradiation in comparison with those of control mice indicating that the radioprotective effect of TMG is apparently realized through its influence on hematopoietic system. These data allow one to elucidate the mechanism by which TMG can provide the whole body defense against high doses of gamma-irradiation.

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