

Gamma-tocopherol-*N,N*-dimethylglycine ester as a potent post-irradiation mitigator against whole body X-irradiation-induced bone marrow death in mice

Kazunori ANZAI^{1,2,*}, Megumi UENO², Ken-ichiro MATSUMOTO²,
Nobuo IKOTA³ and Jiro TAKATA⁴

¹Nihon Pharmaceutical University

²National Institute of Radiological Sciences

³School of Pharmacy, Shujitsu University

⁴Faculty of Pharmaceutical Sciences, Fukuoka University

*Corresponding author. Nihon Pharmaceutical University, 10281 Komuro, Inamachi, Kitaadachi-gun, Saitama, 362-0806, Japan. Tel: +81-48-721-6374; Fax: +81-48-721-7056; Email: anzai@nichiyaku.ac.jp

(Received 25 February 2013; revised 5 June 2013; accepted 24 June 2013)

We examined the radioprotective and mitigative effects of gamma-tocopherol-*N,N*-dimethylglycine ester (GTDMG), a novel water-soluble gamma-tocopherol derivative, against X-irradiation-induced bone marrow death in mice. Mice (C3H, 10 weeks, male) were injected intraperitoneally with GTDMG suspended in a 0.5% methyl cellulose solution before or after receiving of 7.5-Gy whole body X-irradiation. GTDMG significantly enhanced the 30-day survival rate when given 30 min before or immediately after the irradiation. Its mitigative activity (administered after exposure) was examined further in detail. The optimal concentration of GTDMG given immediately after irradiation was around 100 mg/kg body weight (bw) and the 30-day survival rate was $97.6 \pm 2.4\%$. When GTDMG was administered 1, 10 and 24 h post-irradiation, the survival rate was 85.7 ± 7.6 , 75.0 ± 9.7 and $36.7 \pm 8.8\%$, respectively, showing significant mitigation even at 24 h after irradiation ($P < 0.05$). The value of the dose reduction factor (100 mg/kg bw, given intraperitoneally (i.p.) immediately after irradiation) was 1.25. GTDMG enhanced the recovery of red blood cell-, white blood cell-, and platelet-counts after irradiation and significantly increased the number of endogenous spleen colonies ($P < 0.05$). Subcutaneous (s.c.) administration also had mitigative effects. In conclusion, GTDMG is a potent radiation mitigator.

Keywords: vitamin E analog; radiation mitigator; bone marrow death; X-ray; whole body irradiation

INTRODUCTION

Research into radiation modifiers has been revived for two reasons. The first is a drastic improvement in radiation therapy for cancer. For treatments to be effective, the target tissue must be irradiated with a sufficient dose, which increases the risk of damage to surrounding normal tissues. Finding ways to reduce this risk is fundamental to improving the outcome of radiation therapy. Using chemicals that modify the effects of radiation is one way to lessen damage to normal tissues, and various radioprotective compounds have been reported [1–4]. Such compounds are called ‘radiation protectors’, and are administered pre-irradiation. Although many compounds

have been reported, amifostine (WR-2721) is the only drug approved for clinical use by FDA in the USA. It is used for patients of head and neck cancer to reduce xerostomia when receiving radiotherapy. The dose reduction factor (DRF) of amifostine [500 mg/kg, intraperitoneal administration] has been reported to be 2.7 [5]. The second reason is a recent increase in the risk of accidental overexposure [6, 7]. Agents which reduce radiation damage in individuals unintentionally exposed through accidents can obviously only be given after the exposure has occurred. Such agents are called ‘radiation mitigators’. However, relatively few of these agents have been reported to date [8–20], although the number of such agents is increasing. We have reported a

zinc-containing heat-treated yeast powder [21] and a water-soluble alpha-tocopherol analog (alpha-tocopherol-mono-glucoside) [22] as radiation mitigators.

Naturally occurring vitamin E is a family of eight molecules, which are characterized by a chromanol ring structure and a side chain containing two methyl groups in the middle and two more methyl groups at the end [23]. Alpha-tocopherol is one of the isomers and its radioprotective action has been reported [24–27], although some authors have not been able to demonstrate its radiation protective activity [28, 29]. Post-irradiation administration of alpha-tocopherol is reportedly consistent in effectiveness against radiation-induced death of mice [19, 20, 30, 31]. Gamma-tocotrienol and delta-tocotrienol, other type of isomers, have also been reported as a radiation protector and radiation mitigator, respectively [32–37].

A unique compound, gamma-tocopherol-*N,N*-dimethylglycine ester (GTDMG, Fig. 1), was developed as a novel water-soluble vitamin E derivative [38]. GTDMG acts as a pro-drug of gamma-tocopherol. GTDMG and its major metabolite, 2,7,8-trimethyl-2*S*-(beta-carboxyethyl)-6-hydroxylchroman, have received attention concerning their unique pharmacological activities. The present study aimed to evaluate the preventive effects of GTDMG against bone marrow death of mice when administered post-irradiation. GTDMG showed a remarkable enhancement of survival when administered after X-irradiation, suggesting that it may be useful for ameliorating the effects of radiation following accidental overexposure.

MATERIALS AND METHODS

Materials

GTDMG was synthesized as described previously [38]. Other reagents were of analytical grade and used without further purification. The white powder of GTDMG was pulverized finely with a mortar and pestle. A 0.5% methylcellulose solution (MC, methylcellulose 50, Wako Ltd., Osaka, Japan) was added to the powder of GTDMG, and the suspension was mixed well. The turbid suspension was directly administered i.p. to mice.

Animals

The mice used in the present study were treated and handled according to the Recommendations for the Handling of Laboratory Animals for Biomedical Research compiled by

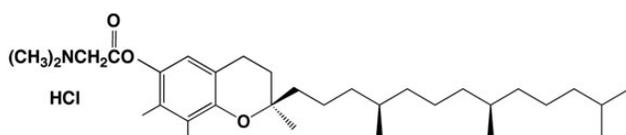


Fig. 1. Structure of gamma-tocopherol-*N,N*-dimethylglycine ester (GTDMG).

the Committee for Safety and Handling Regulations for Laboratory Animal Experiments in the National Institute of Radiological Sciences (NIRS). We used male C3H mice for the experiments, because the NIRS has accumulated data on the effect of radiation on C3H mice, and we have used male C3H mice for our earlier experiments on radioprotective agents. The mice were obtained from Japan SLC Co. (Hamamatsu, Japan) at 8 weeks of age. They were housed five per cage and allowed free access to a commercial diet (MB-1, Funabashi Farm Co., Funabashi, Japan) and acidified water (pH 3.0 ± 0.2) during the experimental period. The animal rooms were maintained on a 12-h light–dark cycle, at an air temperature of $23 \pm 1^\circ\text{C}$, and a humidity of $55 \pm 5\%$. The mice were 65–75 d old at the time of irradiation and weighed 24–29 g.

X-irradiation of mice

Each mouse was weighed and an average weight was calculated for each injection group. Usually, a 0.3-ml volume of the GTDMG suspension in 0.5% MC was administered intraperitoneally (i.p.) or subcutaneously (s.c.) before or after X-irradiation. A group of 10 mice were transferred to a round Lucite container (12 rooms, 23 cm in diameter, 4 cm high). The container was placed on the stand of an irradiator (Pantak HF-320, Shimadzu, Kyoto, Japan), and the mice were irradiated with X-rays at 200 kV and 20 mA with a filter (0.5 mm Cu/0.5 mm Al). The radiation dose was determined with a dose meter placed in a compartment of the container. The dose rate used was ~ 0.55 Gy/min. Throughout the experiments, the radiation dose was set at 7.5 Gy except where otherwise stated in the figure legends. After irradiation, the mice were separated into groups of 5 and assessed daily for survival for 30 d.

Administration of GTDMG

The turbid suspension of GTDMG was directly administered to mice i.p. or s.c. The dose of GTDMG was usually 100 mg/kg unless otherwise stated. Based on the experimental changing of the dose of GTDMG, no acute toxicity was observed, even at the dose of 1000 mg/kg body weight (bw) (data not shown).

Hematological examination of peripheral blood

A sublethal radiation dose (6.0 Gy) was used in the hematological examination to ensure that mice survived for the duration of the experiment. Five groups (total 83 mice) were used, as shown in Table 1. Group A: sham irradiation with no injection. Group B: sham irradiation and i.p. injection with the vehicle (0.5% MC). Group C: sham irradiation and i.p. injection with GTDMG (100 mg/kg bw). Group D: X-irradiation and i.p. injection with the vehicle. Group E: X-irradiation and i.p. injection with GTDMG. The GTDMG suspension or vehicle solution was i.p. injected immediately after the irradiation. The initial samples of blood corresponding

Table 1. Schedule for the hematological examination

Sampling Schedule						
(Days after irradiation)	0	3	7	14	21	28
Group A	5					
Group B				5		5
Group C				5		5
Group D		5	6	6	6	6
Group E		5	6	6	6	6

The values are numbers of mice used. Group A: Sham irradiation + no injection, Group B: Sham irradiation + vehicle, Group C: Sham irradiation + γ TDMG, Group D: 6 Gy + vehicle, Group E: 6 Gy + γ TDMG.

to time 0 were collected only for group A ($n=5$). Other samples were collected at Days 14 and 28 for groups B and C, and at Days 3, 7, 14, 21 and 28 for groups D and E. Mice were anesthetized with diethyl ether and the blood was collected from the drain of venous blood from a cut in the armpit. The blood was transferred immediately into EDTA-treated tubes (Sysmex Co., Kobe, Japan). Total red blood cells (RBCs), white blood cells (WBCs), platelets (PLTs), neutrophils, and lymphocytes were counted with an automated hematology analyzer (SF-3000, Sysmex Co., Kobe, Japan) attached to an analyzing unit for animals (SFVU-1, Sysmex Co., Kobe, Japan).

Endogenous spleen colony assay

The number of endogenous spleen colonies formed after X-irradiation was measured as reported previously [39]. Mice (five per group) were administered i.p. with the GTDMG suspension or control solution (methylcellulose or saline) immediately after receiving 7.5 Gy of whole body X-irradiation. The mice were sacrificed at Day 11 post-irradiation, and the spleens were removed. They were weighed and fixed in Bouin's solution. The number of colonies on the surface of the spleens was counted under a stereomicroscope (SZ-STU1, Olympus, Tokyo, Japan).

Statistical analysis

Data were statistically analyzed using the software GraphPad Prism (GraphPad Software Inc., La Jolla, CA, USA). For the survival data, Kaplan–Meier plots were analyzed with a log-rank test. For the spleen colony assay data, an unpaired t -test was used. Differences were considered statistically significant at $P < 0.05$.

RESULTS

As shown in Fig. 2, GTDMG (100 mg/kg bw) administered i.p. 30 min before whole body X-irradiation at a near lethal

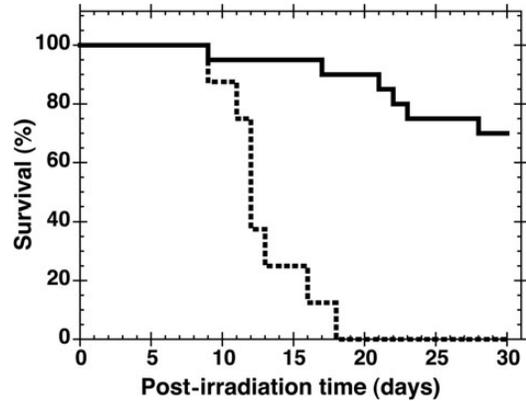


Fig. 2. Survival curves of mice after 7.5 Gy whole body X-irradiation. Vehicle (0.3 ml of 0.5% methylcellulose solution, broken line, $n=8$) or 100 mg/kg bw GTDMG (solid line, $n=20$) was i.p. injected 30 min before the irradiation. The survival rate of GTDMG-administered mice was $70 \pm 10\%$ ($n=20$).

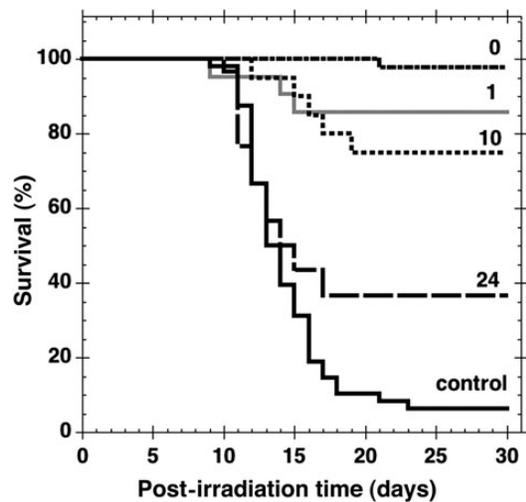


Fig. 3. Mitigative activity of GTDMG (100 mg/kg bw) given i.p. to mice after 7.5 Gy whole body X-irradiation. The mice were administered i.p. with GTDMG at different times after the exposure. The numbers 0, 1, 10 and 24 indicate the administration immediately, 1 h, 10 h and 24 h after irradiation, respectively. The survival rate was $98 \pm 2.4\%$ ($n=42$), $86 \pm 7.6\%$ ($n=21$), $75 \pm 9.7\%$ ($n=20$), and $37 \pm 8.8\%$ ($n=30$), respectively. Vehicle solution (MC) was administered i.p. for the control group immediately after irradiation ($n=48$).

dose (7.5 Gy) significantly protected mice from bone marrow death ($P < 0.05$). The survival rate of mice administered GTDMG was $70 \pm 10\%$ ($n=20$).

When GTDMG (100 mg/kg bw) was administered immediately after the irradiation, the survival curve indicated a potent reduction in bone marrow death with a 30-d survival rate of $97.6 \pm 2.4\%$ ($n=42$) (Fig. 3, the curve indicated as 0). Since the results suggested that GTDMG is a radiation

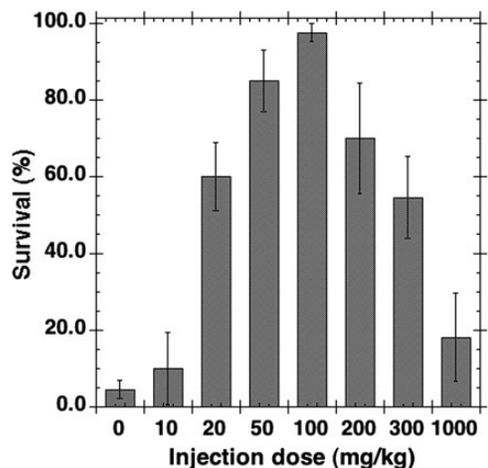


Fig. 4. The 30-d survival rate of mice injected with various doses of GTDMG immediately after exposure to 7.5 Gy of X-irradiation. The number of mice used was 66, 10, 30, 20, 42, 10, 21 and 11 for the injection dose of 0 (= control), 10, 20, 50, 100, 200, 300 and 1000 mg/kg bw, respectively.

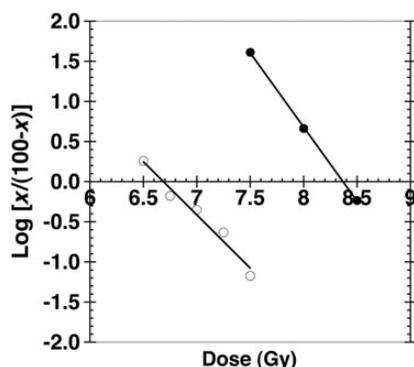


Fig. 5. Probit plot of the survival rate at Day 30. Closed circles are the data for the mice administered GTDMG (100 mg/kg bw) immediately after whole body X-irradiation. The number of mice used was 42, 28 and 19 for the irradiation with 7.5, 8.0 and 8.5 Gy, respectively. Open circles are the data for the control mice (administered the vehicle, a 0.5% methyl cellulose solution). The number of mice used was 17, 30, 81, 20 and 334 for 6.5, 6.75, 7.0, 7.25 and 7.5 Gy, respectively. The ordinate values were calculated using the equation $\log[x/(100-x)]$, where x is the survival rate expressed as a percentage. The line at 0 shows 50% survival and the crossing points of the 0-line and each linear regression line show the $LD_{50/30}$. The $LD_{50/30}$ of mice injected with 100 mg/kg bw GTDMG was 8.37 Gy, whereas that of control mice (given vehicle only) was 6.69 Gy.

mitigator, the timing of its administration was varied and the survival rate of mice was measured. As shown in Fig. 3, the survival rate was highest when GTDMG was given i.p. immediately after irradiation. The survival rate decreased gradually as the duration between the irradiation and administration increased. When GTDMG was administered at 1 h,

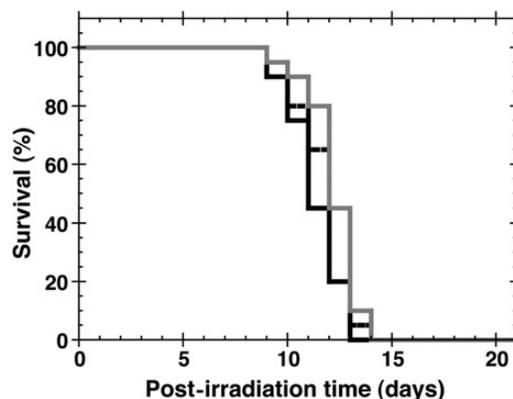


Fig. 6. Effect of dimethylglycine or gamma-tocopherol on the survival of mice administered after exposure to X-rays. Mice were whole body irradiated with 7.5 Gy X-rays and dimethylglycine in MC (25.9 mg/kg bw) or gamma-tocopherol in MC (77.4 mg/kg bw) was administered i.p. immediately after irradiation. Vehicle solution (MC) was used for the control. Black solid line, gray solid line, and black dotted line show the data for the group of mice injected with vehicle (MC), gamma-tocopherol, and dimethylglycine, respectively. The black dotted line almost overlaps with the other two lines. Number of mice used was 20 for each group.

10 h and 24 h post-irradiation, the survival rate at Day 30 was $85.7 \pm 7.6\%$ ($n = 21$), $75.0 \pm 9.7\%$ ($n = 20$), and $36.7 \pm 8.8\%$ ($n = 30$), respectively. Even following the administration at 24 h after exposure, the log-rank test showed the survival curve to be significantly different to that of the control ($P < 0.05$).

Figure 4 shows the survival rate of mice 30 d post-irradiation obtained while changing the dose of GTDMG. GTDMG was administered immediately after whole body irradiation with 7.5-Gy X-rays. The survival rate shows a bell-shaped dependency on the dose of GTDMG. The survival rates of mice injected with 20, 50, 100, 200 and 300 mg/kg bw were significantly higher than that of the control. The maximum effect was observed at 100 mg/kg bw but this was not significantly different from the rate observed at 50 mg/kg bw. The administration of doses higher than 100 mg/kg bw resulted in a significantly reduced effect. The survival rates of mice injected with 10 and 1000 mg/kg bw were not significantly different from that of the control.

The activity of GTDMG in protecting bone marrow death caused by whole body irradiation was estimated as a DRF. GTDMG was given i.p. immediately after X-irradiation at various doses. As shown in Fig. 5, the $LD_{50/30}$ of mice injected with 100 mg/kg bw GTDMG was 8.37 Gy, whereas that of control mice (administration of vehicle only) was 6.69 Gy. From these two values, the DRF of GTDMG (100 mg/kg bw, i.p. administration immediately after irradiation) was calculated to be 1.25.

When dimethylglycine, the concentration of which corresponded to 100 mg/kg bw GTDMG, was i.p. injected immediately after 7.5-Gy whole body irradiation ($n = 20$), no

significant extension of survival compared with the MC-injected group ($n = 20$) was observed (hazard ratio = 0.4774, $P = 0.0915$), while the mice injected with gamma-tocopherol, the concentration of which corresponded to 100 mg/kg bw GTDMG suspended in 0.5% MC ($n = 20$), showed only a small mitigative effect compared to the vehicle-injected control group (hazard ratio = 0.3573, $P = 0.0205$) (Fig. 6).

Changes to red blood cell (RBC), white blood cell (WBC), and platelet (PLT) counts after irradiation and/or GTDMG injection until Day 28 post-irradiation are shown in Fig. 7A–C in addition to body-weight changes (Fig. 7D). The RBC count increased slightly at Day 3 and then decreased till Day 14, after which it increased for the GTDMG-treated group (group E), whereas it remained low for the vehicle-treated group (group D) (Fig. 7A). The WBC count of both the GTDMG- and vehicle-treated groups

decreased drastically to almost zero at Day 3 after irradiation and remained very low till Day 14 (Fig. 7B). After Day 14, the WBC count of the GTDMG-treated group increased markedly to reach that in the un-irradiated group (group B and C), while the WBC count of the vehicle-treated group increased slightly. The PLT-count of both the GTDMG- and vehicle-treated groups decreased extensively till Day 7 and remained very low till Day 14 (Fig. 7C). The PLT count of the GTDMG-treated group recovered to the one third of the original level, while that of the vehicle-treated group recovered only slightly. Without irradiation, GTDMG did not change the RBC, WBC, or PLT count. Following irradiation, the body weight was decreased continuously from Day 7 to Day 21 in the vehicle-treated group (group D), while body weight in the GTDMG-treated group (group E) stopped decreasing and gradually recovered. Body weight in

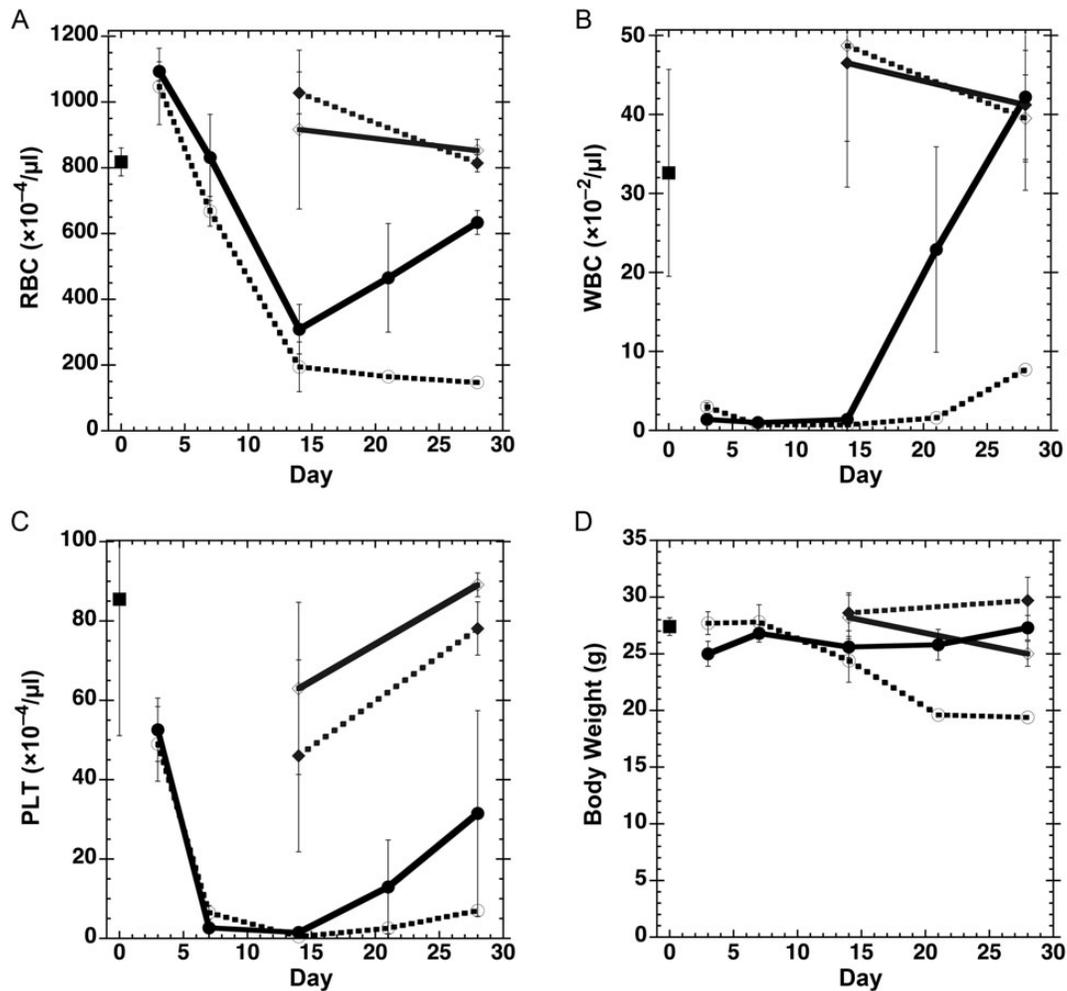


Fig. 7. Change in the number of (A) red blood cells (RBC), (B) white blood cells (WBC), and (C) platelets (PLT), and (D) the body weight of mice. Mice were whole body irradiated with X-rays at 6.0 Gy and each parameter was measured after the irradiation for 28 d. Group A (closed squares): sham irradiation with no treatment. Group B (closed diamonds and dotted gray line): sham irradiation and treatment with vehicle (MC). Group C (open diamonds and solid gray line): sham irradiation and treatment with GTDMG. Group D (closed circles and solid black line): irradiation and treatment with GTDMG. Group E (open circles and dotted black line): irradiation and treatment with vehicle (MC).

the GTDMG-treated group without irradiation (group C) was slightly lower than that in the vehicle-treated group (group B) at Day 28.

The effect of GTDMG on the formation of endogenous spleen colonies was examined. As shown in Fig. 8, the number of colonies was significantly larger in GTDMG-treated mice at Day 11 post-irradiation than in vehicle- or saline-treated mice ($P < 0.05$).

Finally, we examined routes of administration other than the i.p. route. As shown in Fig. 9, subcutaneous injection of

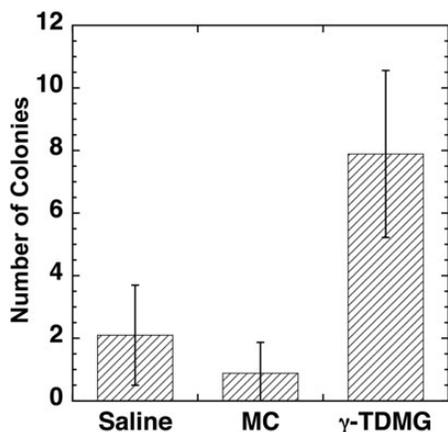


Fig. 8. Endogenous spleen colony formation in mice treated with GTDMG, vehicle (MC), or saline after receiving 7.5-Gy whole body irradiation. Each solution was injected i.p. immediately after the irradiation and the number of spleen colonies was measured at Day 11 post-irradiation.

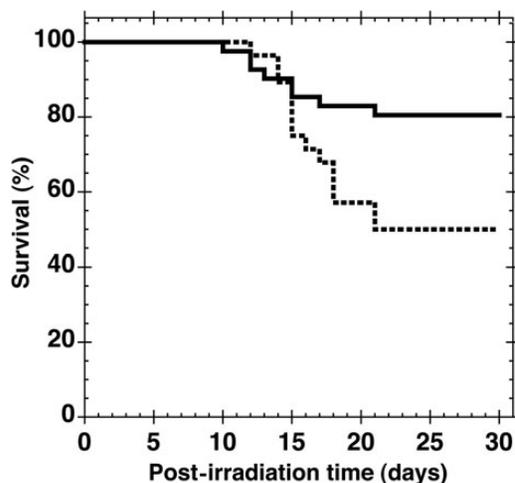


Fig. 9. Effect of subcutaneous injection of GTDMG on the survival curves of mice after 7.5-Gy whole body X-irradiation. Vehicle (0.3 ml of 0.5% methylcellulose solution, broken line) or 100 mg/kg bw GTDMG (solid line) was s.c. injected immediately after the irradiation. The survival rate of GTDMG-injected mice was $80.5 \pm 6.2\%$ ($n = 41$), whereas that of the irradiation control group was $50.0 \pm 9.4\%$ ($n = 28$).

GTDMG immediately after 7.5 Gy of whole body irradiation also significantly increased the 30-d survival rate ($P < 0.05$). The survival rate for the GTDMG-treated group was $80.5 \pm 6.2\%$ ($n = 41$), whereas that for the radiation control group was $50.0 \pm 9.4\%$ ($n = 28$). Oral administration of GTDMG was without effect (data not shown).

DISCUSSION

In the present study, we showed that GTDMG, a vitamin E analog, is a strong radiation protector (given before exposure) and also a strong radiation mitigator (given after exposure) acting to prevent bone marrow death in mice. Radiation mitigators should be useful in cases of accidental overexposure but relatively few compounds have been examined. Here we studied the mitigative activity of GTDMG in detail. Only a limited number of compounds have been reported to show radiation mitigation (radioprotection when administered after irradiation), for example, the vitamin E family (19, 20), glucan [4], heat-killed *Lactobacillus casei* [15, 16], tocopherol-monoglucoside [22, 40], heat-treated mineral yeast powder [21], CBLB502 [9], CBLB613 [13], ALXN4100TPO [10] and GR1977143 [8]. In the present study, we showed that the DRF value of GTDMG was 1.25 when given at 100 mg/kg bw by the i.p. route. This value is the highest for a mitigator among vitamin E derivatives reported so far. In addition, GTDMG had a significant mitigative activity, even at 24 h post-irradiation.

GTDMG is hydrolyzed to form gamma-tocopherol and dimethylglycine. We tried to determine whether the mitigative effect is due to GTDMG itself or due to hydrolyzed gamma-tocopherol and dimethylglycine. We observed that dimethylglycine (at a concentration corresponding to 100 mg/kg bw GTDMG) i.p. injected alone immediately after irradiation did not have a mitigative effect, and gamma-tocopherol injected alone had only a weak effect. Therefore, dimethylglycine is not responsible for the effect. Although this observation suggests that GTDMG itself is responsible for the mitigation, we cannot exclude the possibility that gamma-tocopherol is an active component, because it is not clear whether the concentration of gamma-tocopherol was high enough when injected alone in the MC solution. Alpha-tocopherol used in a properly solubilized form has been reported to act as a radioprotector [24–27]. Furthermore, gamma-tocotrienol and delta-tocotrienol have recently been reported as radioprotectors and mitigators [32, 33]. Experiments are in progress to measure GTDMG and gamma-tocopherol levels in plasma and bone marrow after i.p. administration. In addition to GTDMG, we observed that alpha-TDMG, alpha-tocotrienol-*N,N*-dimethylglycine ester, and gamma-tocotrienol-*N,N*-dimethylglycine ester had a significant mitigative effect (unpublished result).

GTDMG showed a bell-shaped dose dependency with maximum activity at around 100 mg/kg bw and less activity

at higher concentrations. A similar tendency, i.e. that a very high concentration is less effective than the optimum concentration, was reported for the prophylactic activity of alpha-tocopherol [30] and gamma-tocotrienol [32].

The hematological examination showed that whole body irradiation decreased peripheral blood counts, but the numbers recovered significantly after post-irradiation treatment with GTDMG. Similar changes were observed following LC9018 treatment [16], gamma-tocotrienol treatment [32], and delta-tocotrienol treatment [33] after exposure. The measurement of intrinsic colony formation also showed that GTDMG enhanced proliferation. These results suggest that GTDMG enhances the recovery of peripheral blood cells. It is possible that GTDMG induces radioprotective cytokines such as G-CSF and growth factors in the way that alpha-tocopherol succinate does [41], although as yet we do not have experimental data to support this. Several other mechanisms have been considered to explain the effect of tocopherols, tocotrienols and their derivatives in their action as radiation countermeasures [33, 35–37, 42, 43]. One such mechanism is based on the antioxidant property. Although GTDMG has antioxidant activity (unpublished result), the activity may not be responsible for the mitigative effect shown in this study (antioxidant activity is usually correlated with radioprotective effect). Other mechanisms, such as inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, modulation of the expression of antioxidative enzymes, inhibition of radiation-induced apoptosis, stimulation of extracellular signal-related kinase (Erk) activation-associated with the mammalian target of the rapamycin (mTOR) survival pathway, have not yet been examined with GTDMG.

Both i.p. and s.c. administration of GTDMG showed a significant mitigation effect. In contrast, oral administration of it had no significant effect on the survival of the mice (data not shown). Although we do not yet have the experimental results, it is plausible that GTDMG is hydrolyzed in the digestive tract and the resultant gamma-tocopherol (water-insoluble) is difficult to absorb in the intestine.

GTDMG has several advantages for possible practical use as a countermeasure, which are as follows: (i) a wide time window (significantly effective even at 24 h after exposure), (ii) a high DRF value compared with other known mitigators related to vitamin E, (iii) effective by s.c. injection, (iv) chemically stable and easy to handle because of its powdered form, (v) partially water-soluble, and (vi) low toxicity and immunogenicity (because it is a vitamin E analog). These features make GTDMG a good candidate for a radiation mitigator for human use.

FUNDING

This work was supported by a Grant-in-Aid for Scientific Research (C) from the Japan Society for the Promotion of

Science (22510066) and by funds from the Central Research Institute of Fukuoka University (091001).

ACKNOWLEDGEMENTS

We thank the members of the Laboratory Animal Science Section of the NIRS for help in performing mouse experiments.

REFERENCES

- Weiss JF, Landauer MR. History and development of radiation-protective agents. *Int J Radiat Biol* 2009;**85**:539–73.
- Poggi MM, Coleman CN, Mitchell JB. Sensitizers and protectors of radiation and chemotherapy. *Curr Probl Cancer* 2001;**25**:334–411.
- Nair CKK, Parida DK, Nomura T. Radioprotectors in radiotherapy. *J Radiat Res* 2001;**42**:21–37.
- Patchen ML. Immunomodulators and cytokines: their use in the mitigation of radiation-induced hemopoietic injury. In: Bump EA, Malaker K (eds). *Radioprotectors: Chemical, Biological, and Clinical Perspectives*. Boca Raton: CRC Press, 1997, 213–6.
- Weiss JF, Landauer MR. History and development of radiation-protective agents. *Int J Radiat Biol* 2009;**85**:539–73.
- Coleman CN, Stone HB, Moulder JE *et al.* Modulation of radiation injury. *Science* 2004;**304**:693–4.
- Anzai K, Ban N, Ozawa T *et al.* Fukushima Daiichi Nuclear Power Plant accident: facts, environmental contamination, possible biological effects, and countermeasures. *J Clin Biochem Nutr* 2012;**50**:2–8.
- Kiss GN, Lee SC, Fells JI *et al.* Mitigation of radiation injury by selective stimulation of the LPA(2) receptor. *Biochim Biophys Acta* 2013;**1831**:117–25.
- Burdelya LG, Krivokrysenko VI, Tallant TC *et al.* An agonist of toll-like receptor 5 has radioprotective activity in mouse and primate models. *Science* 2008;**320**:226–30.
- Satyamitra M, Lombardini E, Graves J, III *et al.* A TPO receptor agonist, ALXN4100TPO, mitigates radiation-induced lethality and stimulates hematopoiesis in CD2F1 mice. *Radiat Res* 2011;**175**:746–58.
- Fu Q, Berbee M, Wang W *et al.* Preclinical evaluation of Som230 as a radiation mitigator in a mouse model: postexposure time window and mechanisms of action. *Radiat Res* 2011;**175**:728–35.
- Kim H, Bernard ME, Flickinger J *et al.* The autophagy-inducing drug carbamazepine is a radiation protector and mitigator. *Int J Radiat Biol* 2011;**87**:1052–60.
- Singh VK, Ducey EJ, Fatanmi OO *et al.* CBLB613: a TLR 2/6 agonist, natural lipopeptide of *Mycoplasma arginini*, as a novel radiation countermeasure. *Radiat Res* 2012;**177**:628–42.
- Fu Q, Berbee M, Boerma M *et al.* The somatostatin analog SOM230 (pasireotide) ameliorates injury of the intestinal mucosa and increases survival after total-body irradiation by inhibiting exocrine pancreatic secretion. *Radiat Res* 2009;**171**:698–707.

15. Tsuneoka K, Ishihara H, Dimchev AB *et al.* Timing in administration of a heat-killed *Lactobacillus casei* preparation for radioprotection in mice. *J Radiat Res* 1994;**35**:147–56.
16. Nomoto K, Yokokura T, Tsuneoka K *et al.* Radioprotection of mice by a single subcutaneous injection of heat-killed *Lactobacillus casei* after irradiation. *Radiat Res* 1991;**125**:293–7.
17. Aoki Y. Protective action of OK-432 (Picibanil) on radiation-induced myelosuppression—examination of its action in whole body irradiated mice. *Nippon Igaku Hoshasen Gakkai Zasshi* 1982;**46**:1324–30.
18. Hiraoka A, Yamagishi M, Ohkubo T *et al.* Effect of a streptococcal preparation, OK-432, on hematopoietic spleen colony formation in irradiated mice. *Cancer Res* 1981;**41**:2954–8.
19. Bichay THE, Roy RM. Modification of survival and hematopoiesis in mice by tocopherol injection following irradiation. *Strahlentherapie und Onkologie* 1986;**162**:391–9.
20. Malick MA, Roy RM, Sternberg J. Effect of vitamin E on post irradiation death in mice. *Experientia* 1978;**34**:1216–7.
21. Anzai K, Ikota N, Ueno M *et al.* Heat-treated mineral-yeast as a potent post-irradiation radioprotector. *J Radiat Res* 2008;**49**:425–30.
22. Ueno M, Inano H, Onoda M *et al.* Modification of mortality and tumorigenesis by tocopherol-mono-glucoside (TMG) administered after X irradiation in mice and rats. *Radiat Res* 2009;**172**:519–24.
23. Kijima S. Chemistry of Vitamin E. In: Mino M, Nakamura H, Diplock AD *et al.* (eds). *Vitamin E – Its Usefulness in Health and in Curing Diseases*. Tokyo: Japan Scientific Societies Press, 1993, 3–12.
24. Sakamoto K, Sakka M. Reduced effect of irradiation on normal and malignant cells irradiated *in vivo* in mice pretreated with vitamin E. *British J Radiol* 1973;**46**:538–40.
25. Mutlu-Turkoglu U, Erbil Y, Oztezcan S *et al.* The effect of selenium and/or vitamin E treatments on radiation-induced intestinal injury in rats. *Life Sci* 2000;**66**:1905–13.
26. Sarma L, Kesavan PC. Protective effects of vitamins C and E against gamma-ray-induced chromosomal damage in mouse. *Int J Radiat Biol* 1993;**63**:759–64.
27. Felemovicus I, Bonsack ME, Baptista ML *et al.* Intestinal radioprotection by vitamin E (alpha-tocopherol). *Ann Surg* 1995;**222**:504–8; discussion 508–10.
28. el-Nahas SM, Mattar FE, Mohamed AA. Radioprotective effect of vitamins C and E. *Mutat Res* 1993;**301**:143–7.
29. Rostock RA, Stryker JA, Abt AB. Evaluation of high-dose vitamin E as a radioprotective agent. *Radiology* 1980;**136**:763–6.
30. Srinivasan V, Weiss JF. Radioprotection by vitamin E: injectable vitamin E administered alone or with WR-3689 enhances survival of irradiated mice. *Int J Radiat Oncol Biol Phys* 1992;**23**:841–5.
31. Roy RM, Petrella M, Shateri H. Effects of administering tocopherol after irradiation on survival and proliferation of murine lymphocytes. *Pharmac Ther* 1988;**39**:393–5.
32. Ghosh SP, Kulkarni S, Hieber K *et al.* Gamma-tocotrienol, a tocol antioxidant as a potent radioprotector. *Int J Radiat Biol* 2009;**85**:598–606.
33. Satyamitra MM, Kulkarni S, Ghosh SP *et al.* Hematopoietic recovery and amelioration of radiation-induced lethality by the vitamin E isoform delta-tocotrienol. *Radiat Res* 2011;**175**:736–45.
34. Berbee M, Fu Q, Boerma M *et al.* Reduction of radiation-induced vascular nitrosative stress by the vitamin E analog gamma-tocotrienol: evidence of a role for tetrahydrobiopterin. *Int J Radiat Oncol Biol Phys* 2011;**79**:884–91.
35. Berbee M, Fu Q, Garg S *et al.* Pentoxifylline enhances the radioprotective properties of gamma-tocotrienol: differential effects on the hematopoietic, gastrointestinal and vascular systems. *Radat Res* 2011;**175**:297–306.
36. Kulkarni SS, Cary LH, Gambles K *et al.* Gamma-tocotrienol, a radiation prophylaxis agent, induces high levels of granulocyte colony-stimulating factor. *Int Immunopharmacol* 2012;**14**:495–503.
37. Satyamitra M, Ney P, Graves J, III *et al.* Mechanism of radioprotection by delta-tocotrienol: pharmacokinetics, pharmacodynamics and modulation of signaling pathways. *Br J Radiol* 2012;**85**:e1093–103.
38. Takata J, Hidaka R, Yamasaki A *et al.* Novel d-gamma-tocopherol derivative as a prodrug for d-gamma-tocopherol and a two-step prodrug for S-gamma-CEHC. *J Lipid Res* 2002;**43**:2196–204.
39. Yonezawa M, Horie K, Kondo H *et al.* Increase in endogenous spleen colonies without recovery of blood cell counts in radioadaptive survival response in C57BL/6 mice. *Radiat Res* 2004;**161**:161–7.
40. Satyamitra M, Uma Devi P, Murase H *et al.* *In vivo* postirradiation protection by a vitamin E analog, alpha-TMG. *Radiat Res* 2003;**160**:655–61.
41. Singh VK, Shafran RL, Jackson WE, III *et al.* Induction of cytokines by radioprotective tocopherol analogs. *Exp Mol Pathol* 2006;**81**:55–61.
42. Roy RM, Petrella M, Shateri H. Effects of administering tocopherol after irradiation on survival and proliferation of murine lymphocytes. *Pharmacol Ther* 1988;**39**:759–64.
43. Singh VK, Singh PK, Wise SY *et al.* Radioprotective properties of tocopherol succinate against ionizing radiation in mice. *J Radiat Res* 2013;**54**:210–20.