Effects of Zinc Compound on Body Weight and Recovery of Bone Marrow in Mice Treated with Total Body Irradiation

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This study aimed to investigate if zinc compound would have effects on body weight loss and bone marrow suppression induced by total body irradiation (TBI). ICR mice were divided randomly into two groups and treated with test or control compounds. The test compound contained zinc (amino acid chelated with bovine prostate extract), and the control was reverse osmosis pure water (RO water). One week after receiving the treatment, mice were unirradiated, or irradiated with 6 or 3 Gy by 6 MV photon beams to the total body. Body weight changes were examined at regular intervals. Three and 5 weeks after the radiation, animals were sacrificed to examine the histologic changes in the bone marrow. Lower body weight in the period of 1–5 weeks after radiation and poor survival rate were found after the 6 Gy TBI, as compared with the 3 Gy groups. The median survival time after 6 Gy and 3 Gy TBI for mice given the test compound were 26 and 76 days, respectively, and the corresponding figures were 14 and 70 days, respectively, for mice given the control compound ($p < 0.00001$). With zinc supplement, the mean body weight in mice which received the same dose of radiation was 7–8 g heavier than in the water-supplement groups during the second and third weeks ($p < 0.05$). Hence, there was no statistically significant difference in survival rate between zinc and water supplement in mice given the same dose of irradiation. Histopathologically, there was less recovery of bone marrow cells in the 6 Gy groups compared with the 3 Gy groups. In the 3 Gy water-supplement group, the nucleated cells and megakaryocytes were recovered in the fifth week when recovery was still not seen in the 6 Gy group. With zinc supplement, these cells were recovered in the third week. In this study, we found that zinc is beneficial to body weight in mice treated with TBI. Histologic examination of bone marrow showed better recovery of bone marrow cells in groups of mice fed with zinc. This study suggests that zinc can be used as supplements in cancer patients receiving radiotherapy to reduce radiation-induced complications.

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Zinc is one of the most important trace elements in the body; it is indispensable as a catalytic, structural and regulatory ion. Many enzymes which are associated with the synthesis of DNA and RNA contain zinc metalloenzyme [1]. Zinc ions are needed to induce the synthesis of metallothionein (MT), which has been shown

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to be a protective medium in oxidative stress during exposure to toxic metals, infections and immune responses [2–4]. Zinc plays a major biochemical role in maintaining the structure and function of membranes. It also plays a role in the metabolism of skin and connective tissue in wound healing [2,3,5,6].

The benefit of zinc supplementation has been demonstrated under certain circumstances [7,8]. In the case of diarrhea, zinc has been shown to have multiple functions, including helping to maintain the integrity of intestinal mucosa to reduce or prevent fluid loss [6]. Zinc was effective in preventing radiation-induced oropharyngeal mucositis in patients with head and neck cancers [9,10]. All these investigations indicated that zinc acts as an antioxidant, organelle stabilizer, and a stabilizer for the structure of DNA, RNA, and ribosome.

The side effects of total body irradiation include nausea, poor appetite, and bone marrow suppression. Several studies have shown that pretreatment of mice with zinc could protect against damage induced by ionizing radiation, and the LD50 could be increased from 8 Gy to 12.2 Gy by using zinc pretreatment [11]. In this study, we therefore investigated if zinc, administered in the form of biochemical compounds, could indeed have similar protective effects in radiation-induced bone marrow suppression and body weight loss.

Materials and Methods

One hundred and sixteen ICR male 5-week-old mice obtained from the National Laboratory Animal Breeding Research Center of the National Science Council in Taiwan were used in this experiment. After a week of acclimation, the mice were divided into six groups. Table 1 shows the dose of radiation and the kind of feeding supplements that were administered to each group of mice.

Table 1. Groupings of the mice in the experiment

	Group					
	1	2	3	4	5	6
Compound	W	W	W	P	P	P
Radiation dose (Gy)	6	3	0	6	3	0
Number of mice	20	20	10	23	23	10

W = reverse osmosis water; P = zinc amino acid chelated bovine prostate extract.

As shown in Table 1, zinc amino acid chelated bovine prostate extract (Banner Pharmacaps Inc., High Point, NC, USA) in capsule form (abbreviated as P) was employed as the chemical agent in this study. Reverse osmosis pure water (abbreviated as W) was given to mice in control Groups 1, 2 and 3. P contains 20 mg of zinc per capsule. Each mouse received either P or W. Tested drug P was dissolved in distilled water and 0.5 mL was administered daily by oral route, which contained 0.25 mg zinc. Use of zinc compounds and their dose in this study was pre-approved by both the animal study subcommittee and research and development committee of Kaohsiung Medical University.

Whole body irradiation to each mouse was performed by a linear accelerator which produced 6 MV photons at a dose rate of 2.4 Gy/min. Animals were secured by a holder in a supine position for irradiation. During irradiation, they were under constant observation through a monitoring system. Anterior and posterior fields were used for all irradiations to set a better radiation dose distribution for the whole mouse. The center of the field was placed on the midline of the animal's abdomen.

As can be seen from Table 1, there were three irradiation doses: 6 Gy, 3 Gy and 0 Gy. Each dose was irradiated to two out of six groups of mice. Mice in the two groups with 0 Gy received no irradiation. The first three groups were fed with RO water only. The second three groups of mice which were irradiated with the different doses of 0, 3 and 6 Gy were given zinc in P compound at 10 mg/kg body weight/day. Zinc and water were given to the animals for 7 days prior to irradiation and continued after irradiation until the time for necropsy by cervical dislocation at 3 and 5 weeks after irradiation. After a complete necropsy, the femoral bones were excised and bone marrow smears were stained with Liu's stain. The average number of nucleated cells and megakaryocytes per high power field were determined under light microscopy at 100× magnification.

Analysis of variance (ANOVA) test and repeated measures ANOVA test were used to analyze variances and to determine the p values for multiple test groups [12,13]. Survival curves were constructed using the Kaplan–Meier method, and comparisons between curves were made using the log-rank test. All p values were two-tailed and considered statistically significant if less than 0.05.

Results

The mean body weight of irradiated mice incrementally diminished in the period of 5 weeks in all groups with the exception of mice in Groups 3 and 6 in which the mice were not irradiated. Mean body weight increase was noted in Groups 2 and 5 after the sixth week following irradiation, but was not seen in Groups 1 and 4 where mice received the highest (6 Gy) dose. There was no difference in body weight between mice treated with zinc and water as shown in Figures 1 and 2.

In irradiated mice not given any zinc supplement, the mean body weight of the 6 Gy group was 2–6 g lighter than the 3 Gy group, which was statistically significant in the period of the 2nd to 54th days ($p<0.05$). With zinc supplement, the 6 Gy group was 1–6 g lighter than the 3 Gy group in the period of the 2nd to 25th days ($p<0.05$). More body weight decrease was found in mice treated with 6 Gy than in those treated with 3 Gy.

With zinc supplement, the mean body weight was increased up to 8 g over that of the water-supplement group during the 9th to 12th days after 6 Gy irradiation; the difference was 7 g during the 9th to 25th days after 3 Gy irradiation. In the period of the 2nd to 16th days in the 6 Gy group and 9th to 25th days in the 3 Gy group, analysis with ANOVA indicated a statistically significant difference among the zinc- and water-supplement groups ($p<0.05$) (Figures 1 and 2). Also, irradiated mice had less body weight when compared to unirradiated mice.

The survival times of mice in this study were 9–102 days after irradiation. Higher irradiation dose resulted in poorer survival rate. In water-supplement groups, the median survival was 14 days in the 6 Gy group and 70 days in the 3 Gy group ($p<0.0001$). In zinc-supplement groups, the median survival was 24 days in the 6 Gy group and 76 days in the 3 Gy group ($p<0.0001$). Analysis with log-rank test indicated a statistically significant difference among the radiation dose effect ($p<0.0001$). Poor survival was found in the higher dose (6 Gy) total body irradiation groups compared with that of the lower dose (3 Gy) groups, and such findings were similarly noted in the zinc and water supplement groups.

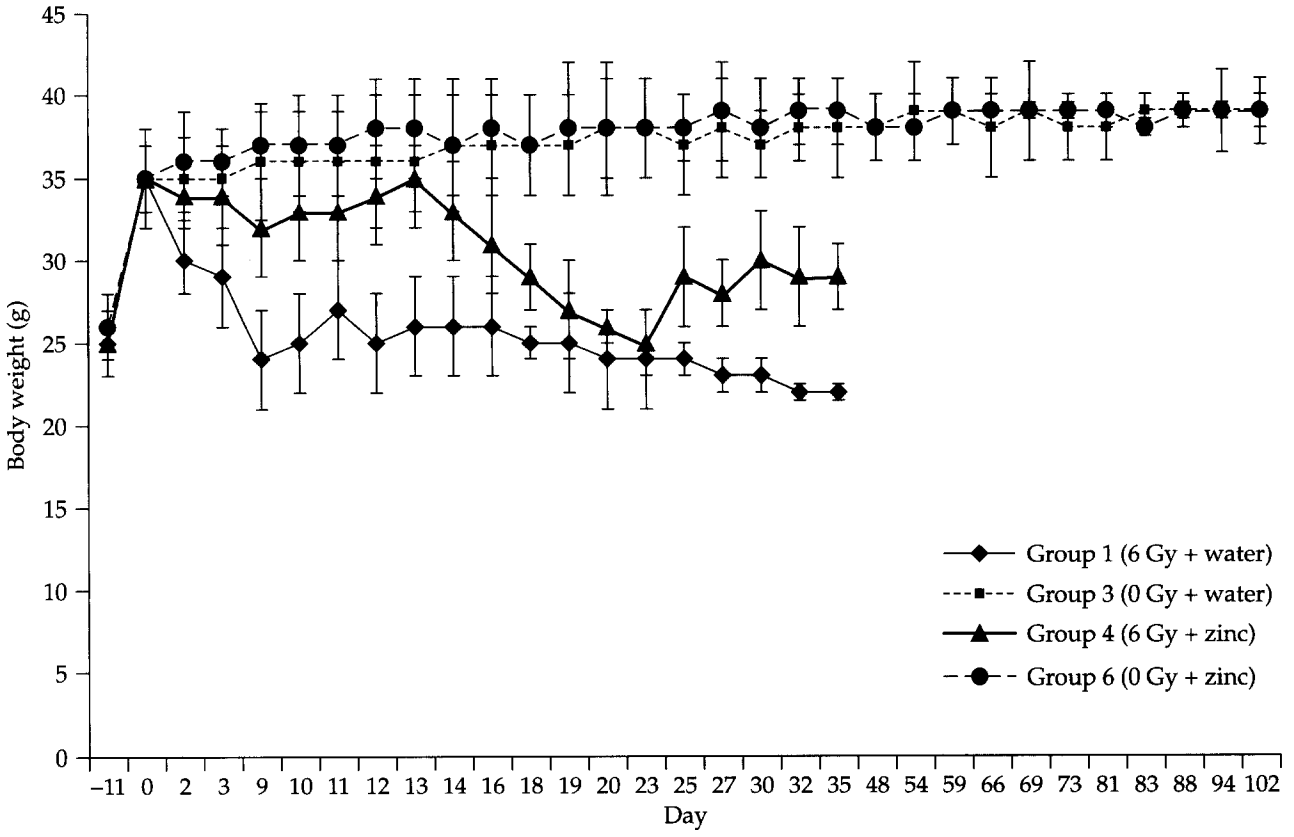


Figure 1. *Body weight change in the mice that received 6 Gy total body irradiation.*

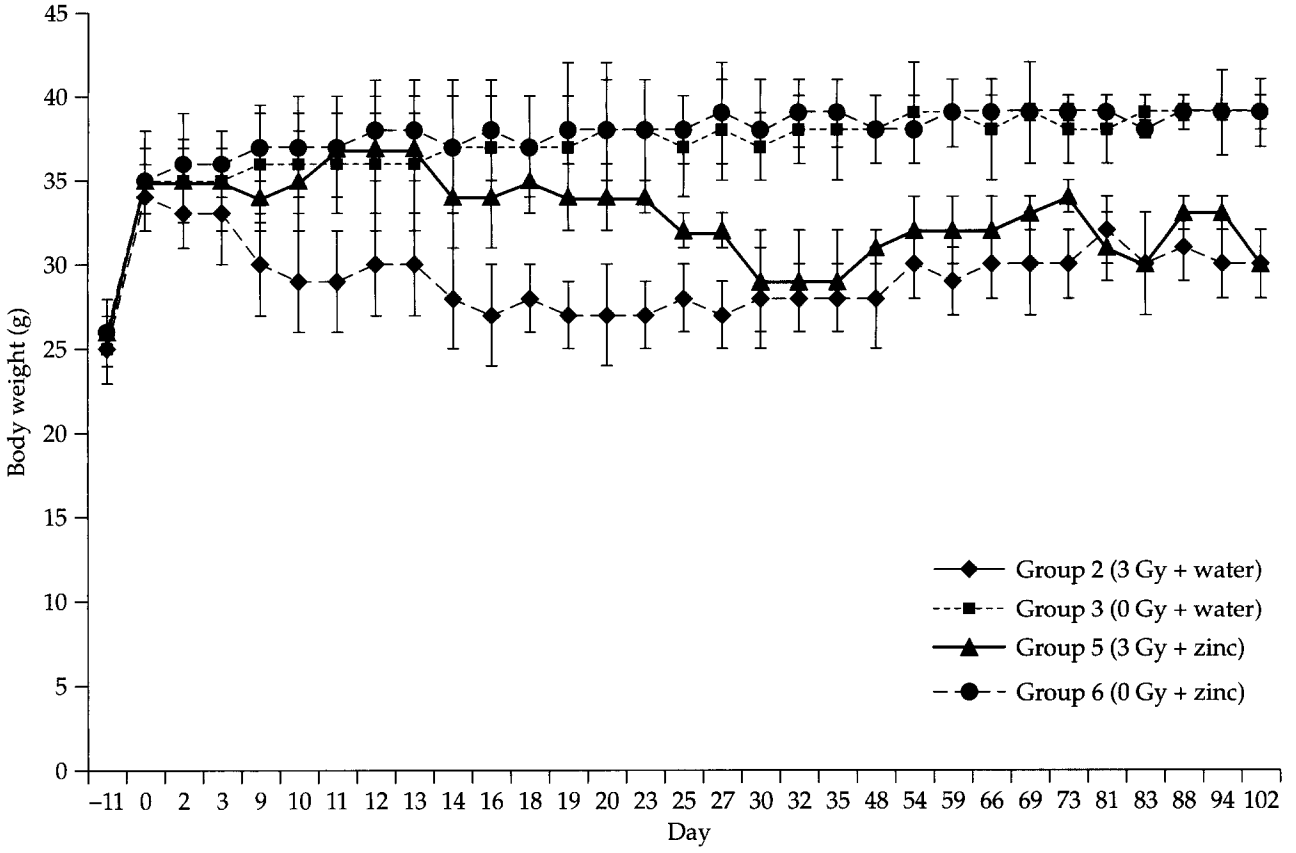


Figure 2. *Body weight change in the mice that received 3 Gy total body irradiation.*

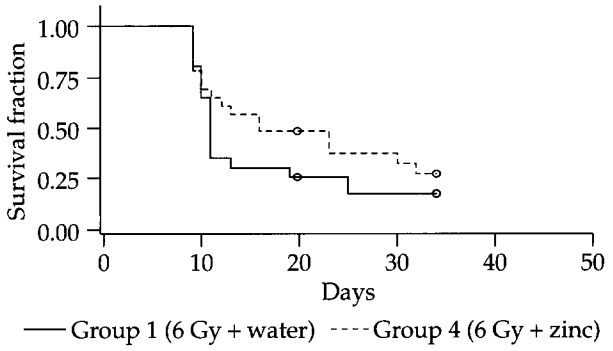


Figure 3. *Survival curves of the water and zinc supplement groups after 6 Gy total body irradiation.*

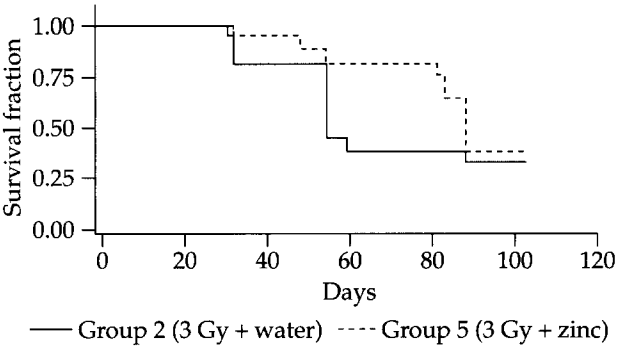


Figure 4. *Survival curves of the water and zinc supplement groups after 3 Gy total body irradiation.*

Even with zinc supplement, mice of Groups 1 and 4 which received 6 Gy total body irradiation died within 34 days after radiation ($p=0.2608$) (Figure 3). Between Groups 2 and 5, the observation time was extended up to 102 days after radiation and log-rank test indicated no statistically significant difference in survival among the zinc- and water-supplement groups ($p=0.6283$) (Figure 4). These results revealed that there is no statistical significance in the groups of mice treated with the same dose of total body irradiation but given zinc or water supplement. The results have been adjusted by the factors of early sacrifice of mice for histologic examination.

Histopathologically, we quantified bone marrow cells by Liu's stain. The results in Table 2 show that water-supplement mice in the groups receiving the higher dose have significantly less recovery of bone marrow cells in 3 and 5 weeks after radiation than

Table 2. Bone marrow cells for each group of mice that received different does of irradiation at different time intervals throughout the experiment

Group	Dose (Gy)	Supplement	Nucleated cells (100× microscopic examination)		Megakaryocytes (100× microscopic examination)	
			3 weeks	5 weeks	3 weeks	5 weeks
1	6	W	<500	500–2,000	<5	<5
2	3	W	500–2,000	>2,000	<5	5–10
3	0	W	>2,000	>2,000	5–10	5–10
4	6	P	>2,000	>2,000	5–10	5–10
5	3	P	>2,000	>2,000	5–10	5–10
6	0	P	>2,000	>2,000	5–10	5–10

W = reverse osmosis water; P = zinc amino acid chelated bovine prostate extract.

those with lower dose radiation (Figures 5 and 6). However, recovery of bone marrow was observed at 3 weeks after radiation in mice given zinc supplement (Figure 5).

With respect to the potency of zinc compounds, in contrast to RO water alone, zinc compound in general was able to guard against body weight loss, with significantly less body weight loss observed in the mice receiving irradiation and zinc. A similar tendency was observed in the improvement of bone marrow recovery.

Discussion

The practical significance of radioprotective compounds is undisputed [14]. The introduction of phosphorothioates as radioprotective drugs with WR-2721 (S-2-(3-aminopropylamino) ethylphosphorothioic acid) was thought to be the most promising agent, but it was compromised by toxic effects [15]. The importance of zinc as an antioxidant to prevent cellular damage has been demonstrated in various studies in *in vitro* cell systems, including protection against ionizing radiation-induced cellular damage [5,16].

The radioprotection by zinc and its synergism with thiols is explained by having the stabilization of thiols through the formation of zinc complexes [11]. Zinc used in this study was from amino acid chelated bovine prostate extract. It has been established that for male rats, LD50 for zinc gluconate is 1,900 mg/kg of body weight [17]. Zinc concentration of 0.25 mg per 0.5 mL is well below that lethal dose orally. Zinc compound appears to have obvious protection against the dose of radiation as seen in having a significant benefit to body weight in post-irradiation mice. Besides, zinc protects early body weight loss, but not in the late phase, especially in the low dose radiotherapy group. Our results showed that there was better recovery of bone marrow cells in the group of mice given 3 Gy radiation and zinc supplement.

We know that mammalian prostates contain high levels of zinc, prostaglandin, citric acid, and cyclo-hispro, all of which have been shown to enhance intestinal zinc absorption. Prostaglandin and arachidonic acid chelated zinc are capable of regulating intestinal zinc absorption and secretion in normal and diabetic rats [18,19]. When zinc is chelated to citric acid, it becomes available for intestinal absorption [20]. To absorb zinc, amino acids such as L-histidine are required [21]. Cyclo-hispro is a major thyrotropin-releasing hormone metabolite with a strong zinc chelating capacity [22]. For these facts, rate of zinc uptake through intestinal mucosa was improved in the mice which were orally given zinc amino acid chelated bovine prostate extract. Animals in these groups receiving irradiation were found to have benefits in bone marrow recovery and a slower rate of body weight loss.

Zinc participates in the regulation of cell proliferation in several ways; it is essential to enzyme systems that influence cell division and proliferation. Zinc also influences hormonal regulation of cell division. Specifically, the pituitary growth hormone–insulin-like growth factor-I (IGF-I) axis is responsive to zinc status; it appears to be essential for IGF-I induction of cell proliferation [1]. Ertekin et al summarized the evidence that zinc sulfate had significant protective effects on peripheral white blood cell count of rats against total body irradiation [23]. The effect of zinc in growth and

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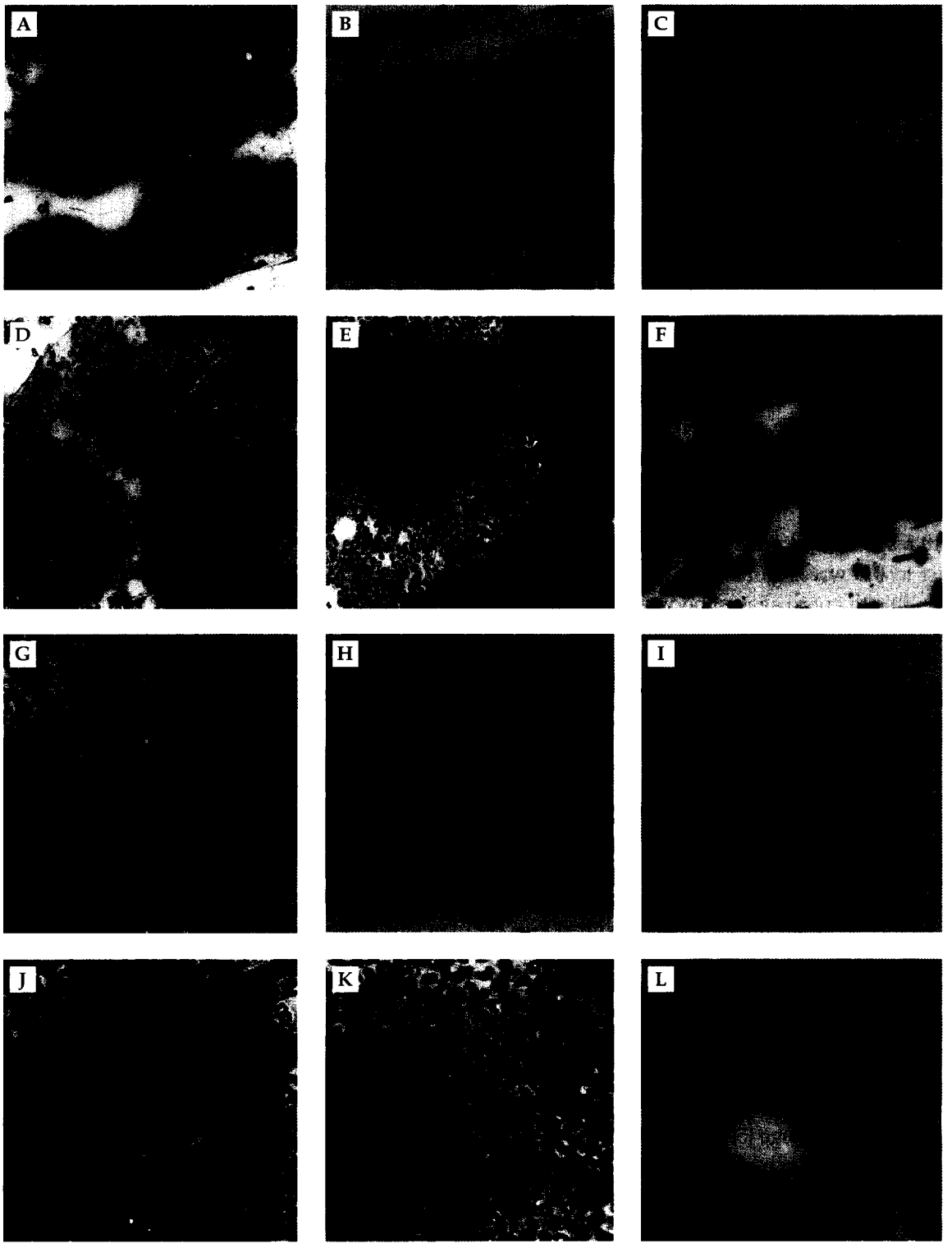


Figure 5. *Histologic examinations of the bone marrow of mice which received different doses of radiation (RT) and water supplement (W) or zinc compound supplement (P) 3 weeks after total body irradiation. Liu's stain, 40× microscopic examination: (A) no RT+W; (B) 3 Gy+W; (C) 6 Gy+W; (D) no RT+P; (E) 3 Gy+P; (F) 6 Gy+P. Liu's stain, 400× microscopic examination: (G) no RT+W; (H) 3 Gy+W; (I) 6 Gy+W; (J) no RT+P; (K) 3 Gy+P; (L) 6 Gy+P. Note that water-supplement mice in the groups receiving higher irradiation dose have less recovery of bone marrow cells than those with lower dose irradiation that already recovered in the zinc supplement groups.*

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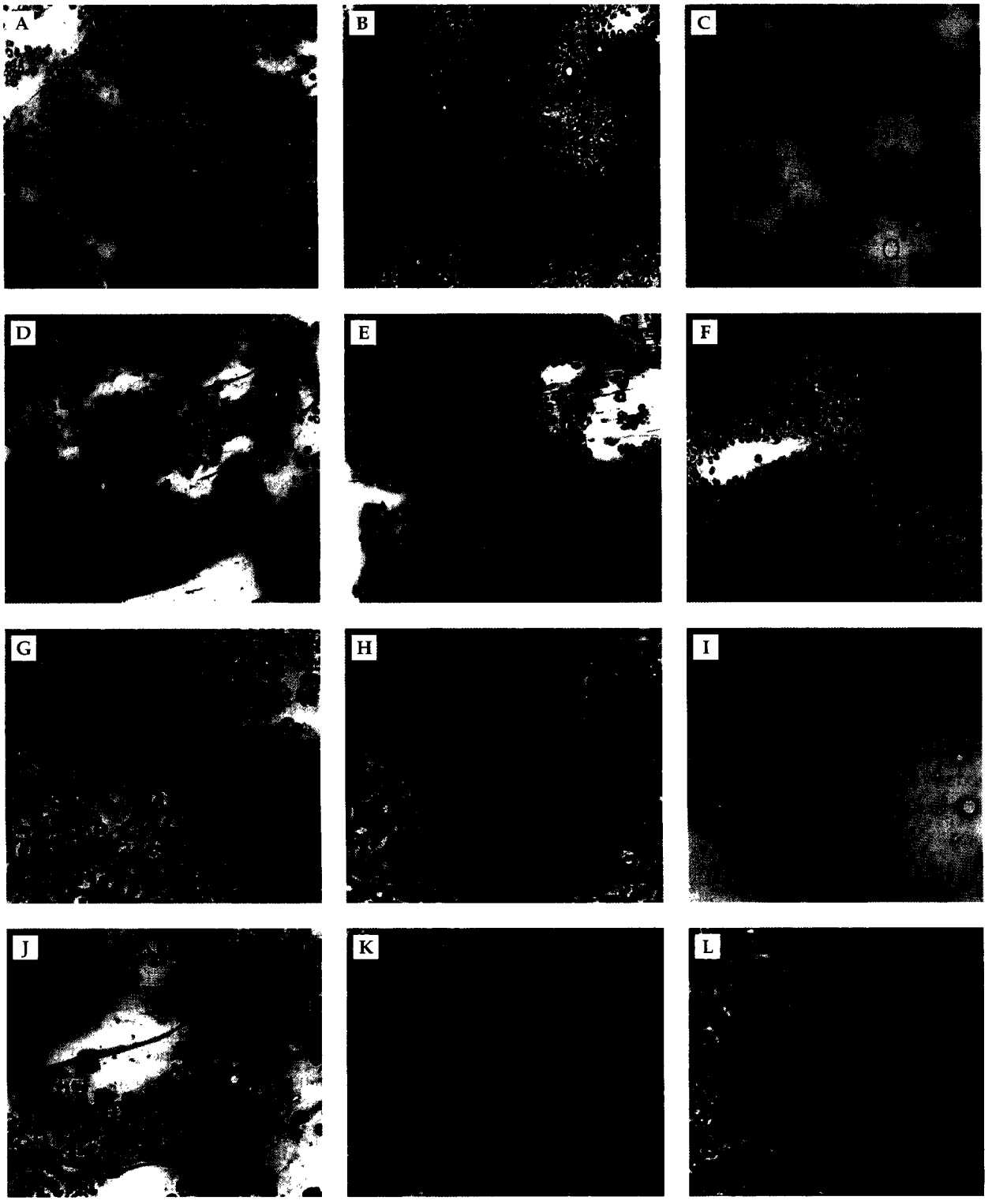


Figure 6. *Histologic examinations of the bone marrow of mice which received different doses of radiation (RT) and water supplement (W) or zinc compound supplement (P) 5 weeks after total body irradiation. Liu's stain, 40× microscopic examination: (A) no RT+W; (B) 3 Gy+W; (C) 6 Gy+W; (D) no RT+P; (E) 3 Gy+P; (F) 6 Gy+P. Liu's stain, 400× microscopic examination: (G) no RT+W; (H) 3 Gy+W; (I) 6 Gy+W; (J) no RT+P; (K) 3 Gy+P; (L) 6 Gy+P. Note that water-supplement mice in the groups receiving higher irradiation dose have significantly less recovery of bone marrow cells 5 weeks after the radiation than those with lower dose radiation.*

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cell proliferation are correlated with the improvement of bone marrow recovery after irradiation in our study. Dublineau et al concluded that functions of the rat distal colon were affected by total body irradiation and may contribute to diarrhea induced by ionizing radiation [24]. According to a previous paper that reported the effect of zinc in maintaining the integrity of intestinal mucosa to reduce or prevent fluid loss in the case of diarrhea [6], it may explain the effects in reducing weight loss in our study.

This study demonstrated that body weight loss was significantly decreased in the groups administered zinc compounds as compared to the groups given RO water alone. These observations suggest that zinc should be considered for testing in humans to reduce body weight loss induced by radiation therapy and to improve bone marrow recovery after total body irradiation. With respect to survival, there was no statistically significant benefit to the mice receiving the same radiation dose and zinc supplement. These results may be partly due to some mice being sacrificed early for bone marrow examination according to this experimental design.

From the results of this investigation, zinc compounds may play a beneficial role in the body weight loss of mice and improve bone marrow recovery after total body irradiation. Further clinical trials are needed to demonstrate if this conclusion is also valid in clinical practice.

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鋅化合物對於接受全身放射線照射小鼠體重與骨髓恢復之影響

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本研究是探討鋅化合物對於放射線所引起體重降低與骨髓抑制之作用是否有影響。研究中之 ICR 小鼠隨機分為兩組，實驗組餵食鋅化合物而對照組餵食逆滲透純水。餵食藥物七天後小鼠再分組接受高能放射線全身照射，照射劑量為 3 Gy 與 6 Gy 並定期紀錄小鼠體重變化。全身照射後第三週與第五週，解剖取出後大腿骨髓進行組織細胞檢測。實驗結果發現，於全身照射後第一週至第五週間，無論餵食藥物與否，6 Gy 組平均體重均較 3 Gy 組輕且存活率較差。(照 6 Gy / 照 3 Gy) 平均存活天數為餵食鋅組 26 天 / 76 天而餵食水組 14 天 / 70 天且 p 值均小於 0.00001。而餵食鋅化合物與否對於接受相同劑量全身照射的小鼠而言，餵食鋅組平均體重於照射後第二與三週比餵食水組重 7 到 8 公克且 p 值小於 0.05。但有無餵食鋅化合物對於接受相同劑量全身照射的小鼠存活率，則未達統計上意義。從組織病理學檢查可見，接受 6 Gy 比接受 3 Gy 全身照射的小鼠骨髓細胞照射後修復較差。餵食水的對照組，接受 3 Gy 的骨髓中有核細胞及巨核細胞於第五週可觀察到已恢復，而 6 Gy 組則尚未恢復。而本研究中餵食鋅化合物的實驗組骨髓細胞均於照射後第三週觀察到已恢復。本研究結論，餵食鋅化合物對於全身照射後小鼠的體重有幫助。而骨髓之組織學檢查亦顯示，餵食鋅化合物的小鼠骨髓細胞照射後修復情況較好。因此我們建議在臨床接受放射治療之病患可以使用鋅化合物，利用鋅化合物之促進細胞組織之恢復作用以期降低放射線副作用。

關鍵詞：骨髓，體重，小鼠，全身照射，鋅化合物
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