Evaluation of sodium orthovanadate as a radioprotective agent under total-body irradiation and partial-body irradiation conditions in mice

Yuichi Nishiyama[a], Akinori Morita[a]*, Bing Wang[b], Takuma Sakai[a], Dwi Ramadhani[b,c], Hidetoshi Satoh[d], Kaoru Tanaka[b], Megumi Sasatani[e], Shintaro Ochi[a], Masahide Tominaga[a], Hitoshi Ikushima[a], Junji Ueno[a], Mitsuru Nenoi[b], and Shin Aoki[d].

[a]Graduate School of Biomedical Sciences, Tokushima University, Tokushima, Japan; [b]National Institute of Radiological Sciences, National Institutes for Quantum and Radiological Science and Technology, Chiba, Japan; [c]Center for Radiation Safety Technology and Metrology, National Nuclear Energy Agency of Indonesia, Jakarta, Indonesia; [d]Faculty of Pharmaceutical Sciences, Tokyo University of Science, Chiba, Japan; [e]Research Center for Radiation Genome Medicine, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan

*Correspondence:

Akinori Morita, Ph.D.

Graduate School of Biomedical Sciences, Tokushima University, 3-18-15 Kuramoto-cho, Tokushima 770-8503, Japan

morita@tokushima-u.ac.jp

Biography

Yuichi Nishiyama is an assistant professor in the Department of Biomedical Science and Technology, Graduate School of Biomedical Sciences, Tokushima University.

Akinori Morita is a professor in the Department of Biomedical Science and Technology, Graduate School of Biomedical Sciences, Tokushima University.

Bing Wang is a group leader in the Department of Radiation Effects Research, National Institute of Radiological Sciences, National Institutes for Quantum and Radiological Science and Technology.

Takuma Sakai is a graduate student in the Department of Biomedical Science and Technology, Graduate School of Biomedical Sciences, Tokushima University.

Dwi Ramadhani was a fellow of the Nuclear Researchers Exchange Program 2017 supported by the Ministry of Education, Culture, Sport, Sciences and Technology (MEXT), Japan, and the Nuclear Safety Research Association, Japan.

Hidetoshi Satoh is a graduate student in the Department of Medicinal and Life Sciences, Faculty of Pharmaceutical Sciences, Tokyo University of Science.

Kaoru Tanaka is a professional staff in the Department of Radiation Effects Research, National Institute of Radiological Sciences, National Institutes for Quantum and Radiological Science and Technology.

Megumi Sasatani is an associate professor in the Department of Experimental Oncology, Research Institute for Radiation Biology and Medicine, Hiroshima University.

Shintaro Ochi was a graduate student in the Department of Biomedical Science and Technology, Graduate School of Biomedical Sciences, Tokushima University.

Masahide Tominaga is a lecturer in the Department of Diagnostic Radiology, Graduate School of Biomedical Sciences, Tokushima University.

Hitoshi Ikushima is a professor in the Department of Therapeutic Radiology, Graduate School of Biomedical Sciences, Tokushima University.

Junji Ueno is a professor emeritus at Tokushima University. He was a professor in the Department of Diagnostic Radiology, Graduate School of Biomedical Sciences, Tokushima University.

Mitsuru Nenoi is a director in the Department of Safety Administration, National Institutes for Quantum and Radiological Science and Technology.

Shin Aoki is a professor in the Department of Medicinal and Life Sciences, Faculty of Pharmaceutical Sciences, Tokyo University of Science.

Abstract

Purpose: Our previous study indicated that sodium orthovanadate (vanadate), a strong inhibitor of p53, effectively suppressed the lethality from the hematopoietic (HP) and gastrointestinal (GI) syndromes after 12 Gy total-body irradiation (TBI) in mice. This conclusion, however, was inconsistent with the fact that p53 plays a radioprotective role in the intestinal epithelium. The death after TBI of around 12 Gy was attributed to a combined effect of HP and GI syndromes. To verify the effect from prophylactic administration of p53 inhibitor on protection of HP and GI syndromes, in this study, the radioprotective effects from vanadate were investigated in TBI and lower half-body irradiation (partial-body irradiation: PBI) mouse models.

Methods: Female ICR mice were given a single injection of vanadate or vehicle, followed by a lethal dose of TBI or PBI. Radioprotective effects of vanadate against the irradiations were evaluated by analyzing survival rate, body weight, hematopoietic parameters, and histological changes in the bone marrow and intestinal epithelium.

Results: TBI-induced HP syndrome was effectively suppressed by vanadate treatment. After TBI, the vanadate-treated mice retained better bone marrow cellularity and showed markedly higher survival rate compared to the vehicle-treated animals. In contrast, vanadate did not relieve loss of intestinal crypts and failed to rescue mice from GI death after PBI.

Conclusion: Vanadate is a p53 inhibitor that has been shown to be beneficial as a radiation protective agent against HP but was not effective in protecting against acute GI radiation injury.

Keywords: vanadate; p53 inhibitor; partial-body irradiation; gastrointestinal syndrome; hematopoietic syndrome

Introduction

Bone marrow and the intestinal epithelium are highly sensitive to ionizing radiation. Preventing the development of the hematopoietic (HP) and gastrointestinal (GI) syndromes is critical in terms of reducing mortality after exposure to high-dose radiation. Development of radioprotectors or radiomitigators against the GI syndrome is considered to be particularly important. While the HP syndrome can be prevented by bone marrow transplants (Baranov et al. 1990), there is no effective method for the treatment of the GI syndrome. In abdominopelvic radiotherapy, several side effects, including intestinal enteritis and diarrhea commonly occur (Andreyev 2007), which adversely affect the quality-of-life of patients (Dunberger et al. 2010).

Chemical agents that possess the ability to regulate p53 activity have been attracted interest as a countermeasure for preventing or mitigating acute radiation injuries. The relationship between p53 and radiation cell death has been well documented (Lowe et al. 1993; Ianzini et al. 2006). It has also been reported that p53 causes apoptosis in irradiated hematopoietic lineages, whereas it plays a radioprotective role in the intestinal epithelium (Kirsch et al. 2010). In a series of studies, we identified and characterized some radioprotective p53-regulating compounds based on the different roles of p53 in response to irradiations in hematopoietic and gastrointestinal tissues (Morita et al. 2010, 2014, 2018). Among such compounds, sodium orthovanadate (vanadate) has been shown to be a strong p53 inhibitor, and that it effectively prevented and ameliorated the HP syndrome after total-body irradiation (TBI) in mice (Morita et al. 2010; Wang et al. 2013). Furthermore, since it could rescue mice from GI death induced by 12 Gy-TBI, it would have the potential to be a useful compound to reduce or eliminate radiation-induced HP and GI syndromes. However, the suppressive effect of vanadate on GI death was inconsistent with the fact that p53 acts as a radioresistant factor in the intestinal epithelium (Kirsch et al. 2010).

The radiation GI syndrome can be generated by two irradiation techniques, TBI and abdominal irradiation. The difference between these techniques is whether the HP syndrome arises along with the GI syndrome. Death that occurs within about 10 days after irradiation is widely accepted as GI death (Burdelya et al. 2008; Patil et al. 2015) and this occurs independent of the irradiation technique used (Mason et al. 1989). Bone marrow transplantation increases dose level necessary to cause GI death for a TBI mouse model (Terry and Travis 1989). Bone marrow injury is thought to be a contributing factor to GI death, and the lethality of TBI at around 12 Gy is attributed to the combined effect of the HP and GI syndromes. Therefore, the efficacy of p53 inhibition by vanadate on the GI syndrome should be investigated using irradiated mice that do not develop the HP syndrome.

Accordingly, the objective of this study was to investigate whether the p53 inhibitor, vanadate, is beneficial as a radioprotective agent against both GI syndrome and HP syndrome in abdominally and systemically irradiated mice, respectively, and examine an optimal usage of vanadate as a radioprotector. To induce GI syndrome while avoiding bone marrow death, the mouse abdomen was irradiated using a partial-body irradiation (PBI) (also referred to as subtotal-body irradiation (SBI)) system (Kirsch et al. 2010; Morita et al. 2018). This PBI technique permits the bone marrow in mouse frontal body to be preserved, thus allowing the bone marrow to be re-populated in the irradiated body parts (Kirsch et al. 2010). Mice treated with or without vanadate exhibited severe crypt degeneration and GI death in the PBI model. In contrast, vanadate enabled the rescue mice from bone marrow death after TBI through the protection of myeloid tissue. These findings suggested that vanadate is particularly effective for inhibiting HP syndrome in total-body exposure situation, but ineffective for acute GI syndrome generated by local exposure of abdomen.

Materials and methods

Animals

Specific-pathogen-free female ICR mice (7 weeks of age) were obtained from Japan SLC Inc. (Shizuoka, Japan). They were housed under controlled temperature (22°C) and a preset light-dark cycle. Standard MF diet and normal drinking water were provided *ad libitum* during all experimental periods. Ethics approval for the experimental design was obtained from the animal experimental committee of Tokushima University.

Irradiation

Vanadate was purchased from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in a normal saline (NS) solution. After 7 days of acclimatization, the mice were given a single intraperitoneal injection of vanadate (20 mg/kg body weight, 2 mg/mL in NS solution) or vehicle (NS solution) 30 min before irradiation. TBI and PBI were then performed using an X-ray generator (MBR-1520R-3, Hitachi, Ltd., Tokyo, Japan) under a tube voltage of 150 kV, a tube current of 20 mA, and a dose rate of approximately 1.0 Gy/min. Dosimetry was carried out with a 0.3 cc N31003 ionization chamber (PTW Freiburg, Freiburg, Germany).

The PBI treatment was conducted in a manner that permits bone marrow death to be avoided. Briefly, mice were anesthetized with a mixture of midazolam (4 mg/kg), medetomidine (0.3 mg/kg), and butorphanol (5 mg/kg). Frontal side of the body, including head, chest, and front leg, were shielded with lead layers with thicknesses of 1.8 cm, and X-irradiation was then performed in a dorsal-to-ventral direction. The shielded region extended about 4.5 cm craniocaudally from nasal apex, and the scatter dose rate in the region was 0.09 Gy/min. The survival times for the mice were recorded daily up to 60 days with body weight change being monitored since mice that survived acute radiation GI injury possibly develop fatal vascular

endothelial damage in delayed GI syndrome. The death attributed to the delayed GI injury begins to occur about 30 days after abdominal irradiation (Kirsch et al. 2010; Lee et al. 2019).

Hematopoietic analysis

Mice were sacrificed 7, 9, and 12 days after irradiations, and blood was collected from the heart. The amounts of red blood cells (RBC), hemoglobin (Hgb), white blood cells (WBC), and platelets (PLT) were measured using an automated impedance-based hematology analyzer (Microcemi LC-662, Horiba Ltd., Kyoto, Japan).

Histology

At 4 and 7 days post-irradiation, mice were intraperitoneally injected with 5-Bromo-2'-deoxyuridine (BrdU; 50 mg/kg; Sigma) 1 h prior to euthanasia. The excised ileum was washed with 10 mM phosphate-buffered saline, fixed in 10% formalin, and embedded in paraffin. Cross sections of intestinal tract (5 μm thick) were stained with hematoxylin-eosin (HE) to examine morphological change of intestinal epithelium. Cells incorporating BrdU in the intestinal crypts were detected immunohistochemically using the BrdU *in situ* detection kit (BD Biosciences, San Diego, CA, USA) according to the manufacturer's instructions. Hematoxylin was used as counterstain. Intestinal tissue images were photographed with a BZ-9000 microscope (Keyence, Osaka, Japan), and three observers counted a surviving crypt having 5 or more BrdU-positive cells using a BZ-X analyzer software (Keyence) (Saha et al. 2011). The percentage of surviving crypts relative to the mean crypt number in mice that had been treated with NS only was calculated from a minimum of 3 cross-sections in each mouse.

Femurs were removed from mice 9 and 12 days after receiving 10 Gy-TBI, fixed in 10% formalin, and decalcified with 0.5 M EDTA solution (Decalcifying solution B, Wako, Osaka,

Japan). Longitudinal paraffin sections (5 μm thick) were stained with HE, and the number of megakaryocyte in bone marrow area of the femur was quantified using a BZ-9000 microscope (Keyence).

Statistical analyses

Data are presented as the mean ± standard deviation. Chi-square test was used to determine statistical differences in survival rate between groups. Statistical differences in weight gain, hematopoietic parameters, megakaryocyte number, and crypt survival between groups were determined by Student's *t*-test. $P < 0.05$ was considered significant.

Results

Vanadate reduces TBI lethality but not PBI lethality

Considering the radioprotective role of p53 in the GI tissue, we assumed that vanadate promotes or does not affect radiation GI injury. Thus, the protective and exacerbating effect of vanadate on radiation GI syndrome was investigated at lethal (17 Gy) and sublethal doses (16 Gy) of PBI, respectively. Doses of 17 Gy and 16 Gy were determined corresponding approximately to LD100/15 and LD50/15, respectively. Figure 1(A) clearly demonstrates the border between shielded and unshielded regions. As shown in Figure 1(B), the NS-treated mice had healthy and severely damaged bone marrow in the humerus and femur, respectively, on day 7 after 16 Gy-PBI. In contrast, the NS-treated mice that survived for 60 days after the irradiation had mildly repopulated bone marrow in the femur. These findings indicated that the PBI technique successfully avoided the systemic loss of bone marrow leading to bone marrow death.

A 60-day survival test was performed to examine how vanadate affects the lethalities of TBI and PBI (Figure 2(A)). The survival rate for the vanadate-treated mice was 90% on day 60 after 10 Gy-TBI, which was significantly higher than that in NS-treated mice. In contrast, vanadate could not rescue mice from death after abdominal irradiations of 16 Gy and 17 Gy, there were no significant differences in the survival rate between NS- and vanadate-treated mice.

Body weight was monitored as an indicator of the physiological condition (Figure 2(B)). In both the NS- and vanadate-treated mice, body weight gradually decreased after PBI. However, in the TBI model, body weights of the NS-treated mice decreased more rapidly than that of the vanadate-treated mice. These results indicate that vanadate could improve the physiological condition in mice receiving TBI but not PBI.

Vanadate suppresses TBI-induced HP syndrome

Hematopoietic parameters were analyzed to examine radioprotective effect of vanadate in the TBI model. As shown in Figure 3(A), WBC and PLT counts decreased by 95% and 94% in NS-treated mice on day 7 after 10 Gy-TBI, respectively, which is a typical sign of the early HP syndrome (Patchen et al. 1991). RBC count and Hgb level showed a delayed reduction, resulting in 70% and 73% reductions on day 12 after the irradiation, respectively (Figure 3(B)). Vanadate-treated mice also showed a reduced HP capacity, but compared with NS-treated mice, significantly higher RBC count and Hgb level were observed from day 9 to 12 after the irradiation. Although significantly higher PLT count was also observed from the day 12, the difference was small.

Histological examination was performed for further assessment of the radioprotective efficacy of vanadate in the TBI model (Figure 4). The 10 Gy-TBI caused bone marrow cells to disappear from the femur of NS-treated mice. Vanadate effectively reduced the loss of bone marrow cells and megakaryocytes, indicating protective effect on the myeloid tissue. These results are correlated with higher survival rate in the vanadate-treated mice.

The TBI treatment caused severe HP syndrome but not fatal GI injury. In the TBI model, mild GI injury, namely, villus atrophy with sparse crypts, was observed in mice treated with or without vanadate on day 4, but returned almost healthy on day 7 after the irradiation (Figures 5 and 6). These data indicated that the cause of death in this model was HP syndrome due to severe myelosuppression.

Vanadate does not suppress PBI-induced GI syndrome

As shown in Figure 3(A), 10 Gy-TBI induced drastic declines in WBC and PLT counts in the case of the NS-treated mice. On the other hand, 17 Gy-PBI induced 22% and 48% reductions in WBC and PLT, respectively, indicating the HP syndrome was not severe enough to induce

bone marrow death. In fact, PBI treatment led to the retention of HP capacity in the shielded bone marrow and induced a milder, non-lethal HP syndrome which can be attributed to radiation damage to the unshielded bone marrow (Figure 1(B)).

Figure 5 clearly showed that the PBI treatment caused severe GI syndrome and vanadate could not protect intestinal tissue. The PBI treatment destroyed almost all of the intestinal crypts in both the NS- and vanadate-treated mice. Severe collapse of villous structure was also observed. For further assessment of the radioprotective effect of vanadate, BrdU incorporation assay was performed (Figure 6). In both NS- and vanadate-treated mice, surviving crypt was hardly observed on day 4 after the PBI treatment. NS-treated mice showed a slight, but significant regeneration of crypts on day 7 after PBI. Although the crypt regeneration was also observed in vanadate-treated mice, it was not statistically significant. Of note, similar results were obtained in the TBI model. These findings indicate that vanadate has no radioprotective effect against acute radiation-induced intestinal injury.

Discussion

The findings reported herein show that vanadate is highly effective on TBI-induced HP syndrome, but ineffective on GI syndrome caused by acute abdominal irradiation. Vanadate treatment prior to PBI could not inhibit loss of intestinal crypts and reduced gain of body weight, resulting in a failure to rescue mice from GI death. On the other hand, the vanadate treatment provided an improved survival rate after TBI through the retention of bone marrow cellularity.

Radiation GI death mostly occurs around day 10 of post-irradiation, and the day 10 has been widely accepted as a good end-point for acute GI syndrome in abdominally irradiated mice (Mason et al. 1989). Hematopoietic (Figure 3) and histological (Figures 1, 5, and 6) assays provided a clear evidence that our PBI technique using X-rays of maximum energy 150 keV induced lethal GI injury without causing severe HP syndrome in ICR mice, but some of them died later than the typical end-point (10 days) (Figure 2(A)). Although cesium-137 (^{137}Cs) is a 662 keV gamma-ray source and has been standardly used for inducing radiation GI syndrome, our PBI model was constructed using an X-ray generator due to the laboratory equipment constraints. With almost the same PBI technique as using ^{137}Cs, our previous study demonstrated GI death around 10 days of post-irradiation in ICR mice (Morita et al. 2018). The end-point may vary depending on various factors including photon energy (Poirier et al. 2020).

Different from other p53 inhibitors such as pifithrin α (Komarov et al. 1999) and pifithrin μ (Strom et al. 2006), vanadate strongly suppresses radiation-induced apoptosis by inhibiting both transcription-dependent and transcription-independent p53 apoptotic pathways (Morita et al. 2010). However, its marked p53-inhibiting effect was not a benefit in regard to preventing radiation GI syndrome. The 60-day survival test revealed that vanadate is ineffective against the GI syndrome caused by acute abdominal irradiation. All the mice treated with vanadate died within 15 days after 17 Gy-PBI. One of 10 mice treated with NS survived acute GI syndrome

but died on day 32 after the PBI treatment (Figure 2(A)), which may be attributed to the delayed GI injury (Lee et al. 2019). The ineffectiveness was also demonstrated in mice abdominally irradiated with high-LET carbon-ion that causes more complex DNA damage than low-LET photon (Morita et al. 2020). However, the failure of vanadate to prevent GI death in the PBI model differed from the findings in our previous study in a TBI mouse model, where vanadate was shown to rescue 60% of the mice from a lethal dose of 12 Gy-TBI (Morita et al. 2010). The protective effect of vanadate in this TBI model was attributed to suppression of bone marrow injury coincident with the HP syndrome in the TBI model.

The fact that the constitutive lack of p53 in the intestinal epithelium increases sensitivity to abdominal irradiation raises concerns that the use of p53 inhibitors for cancer therapy promotes GI injury (Kirsch et al. 2010). However, vanadate treatment did not increase the sensitivity (Figure 2(A)). This may be somewhat reasonable considering the pharmacological characteristics of vanadate in the p53 inhibition. A single intraperitoneal injection of vanadate 2 hours before 12 Gy-TBI had no radioprotective effect (Morita et al. 2010), which indicates a transient p53-inhibiting effect of vanadate. These data suggest that the p53-inhibiting effect of vanadate disappears within 4 days after irradiation after which intestinal disorders become prominent. A similar result was also demonstrated using pifithrin α by Komarova *et al.* (Komarova et al. 2004). They compared the influence of temporal p53 inhibition by a single injection of pifithrin α with a constant p53 deficiency (p53 knockout) on the lethality from TBI-induced GI syndrome and showed that pifithrin α had no effects on the lethality whereas p53 knockout mouse became more sensitive to the TBI.

The histological assessments support our conclusion that vanadate has no protective effect on GI injury after abdominal irradiation. NS-treated mice showed a statistically significant regeneration of crypts after 17 Gy-PBI (Figure 6(C)), which may be attributed to

dedifferentiated cells in damaged crypts. Crypt base columnar (CBC) cells at crypt bottoms are essential for epithelial formation of the intestine and succumb to dose above 15 Gy (Hua et al. 2012). The PBI treatment was considered to be sufficient to kill CBC cells. However, differentiating cells at +4 to +6 positions in the crypt are more radioresistant than CBC cells and dedifferentiate as backups of damaged CBC cells to promote intestinal recovery (Pant et al. 2019). Vanadate did not suppress degeneration of crypts in either the TBI or PBI models, suggesting that its use will not effectively work against acute radiation GI injury. Kirsch *et al.* noted that the radiation GI syndrome occurs due to the death of GI epithelial cells, and the cell death may be mainly attributed to non-apoptotic processes (*e.g.,* a mitotic catastrophe) (Kirsch et al. 2010). Subsequently, they showed that the GI syndrome can be attenuated by p53 and its target gene p21 (Sullivan et al. 2012). The induction of p21 through the activation of p53 promotes cell cycle arrest, which may provide adequate time to repair radiation DNA damage (Bunz et al. 1998). Although the detailed mechanism of radiation GI injury remains unclear, modulating the p53-p21 pathway appears to be one of the most useful strategies for developing radioprotectors or radiomitigators against the GI syndrome. For instance, 5-chloro-8-quinolinol (5CHQ) has been shown to act as a modulator of p53 transactivation without inhibiting the activity of p53 and contributes to the effective suppression of GI death after a lethal dose of 24 Gy-PBI (Morita et al. 2018). It enhances the radioresistance of the intestinal epithelium through the upregulation of *Cdkn1a* encoding radioprotective p21, and downregulation of *Bbc3* and *Pmaip1* encoding proapoptotic Puma and Noxa, respectively, in the irradiated intestinal epithelium. Similar results were reported by using RG7112, a chemical compound that activates p53-p21 pathway by inhibiting p53-Mdm2 interaction (Pant et al. 2019).

Consistent with our previous work (Morita et al. 2010), vanadate reduced loss of bone marrow cellularity (Figure 4), improved HP capacity (Figure 3(B)), and successfully rescued

mice from TBI-induced bone marrow death (Figure 2(A)). Irradiated hematopoietic lineage cells undergo p53-mediated apoptotic death (Radford et al. 1994), and several studies demonstrated the efficacy of p53 inhibition for preventing radiation HP syndrome (Komarova et al. 2004; Strom et al. 2006; Kirsch et al. 2010; Morita et al. 2010). The property of vanadate that inhibits both transcription-dependent and transcription-independent p53 apoptotic pathways would provide a great benefit in total-body exposure situation, particularly in terms of reducing HP syndrome. The HP syndrome is involved in the progression of radiation GI death (Terry and Travis 1989). Therefore, suppressing bone marrow injury by a p53 inhibitor should be expected to reduce or prevent GI death depending on the dose of the total-body exposure that caused a combined GI and HP syndrome. However, vanadate failed to rescue mice from GI death after 13 Gy-TBI in ICR (Morita et al. 2010). The pharmacological p53-inhibiting efficacy of vanadate against TBI appears to reach the limit at around 12 Gy, and within such dose levels (< 12 Gy), it would be effective for reducing GI death in addition to bone marrow death. As another use of vanadate, it may be beneficial for protecting normal tissues during cancer chemotherapy (Basu et al. 2015). Myelosuppression is a serious complication due to the low specificity of anticancer drugs to cancer cells (Crawford et al. 2004; Kuter 2015). Vanadate possibly reduces the systemic adverse effect on HP system without influencing the sensitivity of cancer cells to the drugs, because the anti-cell death effect of vanadate is p53-dependent (Morita et al. 2010) and human cancers frequently have impaired p53 function (Petitjean et al. 2007).

Vanadate is also known to inactivate protein tyrosine phosphatases (PTPase) (Gordon 1991) and adenosine triphosphatases (ATPase) (Aureliano and Crans 2009). Our previous study compared antiapoptotic effect of vanadate in irradiated MOLT-4 cells with several PTPase inhibitors, and the results suggested that the suppression of radiation-induced MOLT-4

apoptosis of vanadate is not associated with its PTPase-inhibiting effect (Morita et al. 2006). Therefore, the inhibiting effect of vanadate may not a contributing factor to the lethalities of TBI and PBI. Sodium-potassium ATPase (Na^+/K^+-ATPase), transmembrane protein that is effectively inhibited by vanadate (Aureliano and Crans 2009; Jiang et al. 2018), plays a central role in water and glucose absorptions in the intestine. Lebrun *et al.* demonstrated the reduction of Na^+/K^+-ATPase activity in rats treated with 8 Gy-TBI (Lebrun et al. 1998), and loss of the activity could result in radiation malabsorptive diarrhea (MacNaughton 2000). Since weight gain, stool consistency, and survival rate showed no differences for 60 days between unirradiated mice treated with or without a single injection of 20 mg/kg vanadate (data not shown), the dose was considered to have no effect on physiological condition. Detailed studies are needed to examine the vanadate requirement sufficient to inactivate Na^+/K^+-ATPase in GI tissue and how the inactivation affect in the development of radiation GI syndrome.

In this study, we examined the protective effects of the p53 inhibitor vanadate on HP syndrome and GI syndrome caused by total-body and abdominal irradiations, respectively. The use of vanadate as a radioprotective agent was highly effective at preventing HP syndrome in total-body exposure solution. However, vanadate was found not to have a radioprotective benefit against GI syndrome caused by local acute radiation exposure of the abdomen, suggesting that p53 inhibition may not be a valid prophylactic strategy for protecting against acute radiation GI syndrome.

Acknowledgements

We are grateful to staff of the Support Center for Advanced Medical Sciences, Tokushima University Graduate School of Biomedical Sciences, for their technical supports.

Disclosure statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding

This research was supported by KAKENHI (grant number 16K10396, 19K17143, and 19H03604) from the Japan Society for the Promotion of Science. Dwi Ramadhani was a fellow of the Nuclear Researchers Exchange Program 2017 supported by the Ministry of Education, Culture, Sport, Sciences and Technology (MEXT), Japan, and the Nuclear Safety Research Association, Japan.

Data availability

The data that support the findings of this study are available from the corresponding author, Akinori Morita, upon reasonable request.

References

1. Andreyev J. 2007. Gastrointestinal symptoms after pelvic radiotherapy: a new understanding to improve management of symptomatic patients. Lancet Oncol. 8(11):1007−1017.
2. Aureliano M, Crans DC. 2009. Decavanadate (V10 O28 6-) and oxovanadates: oxometalates with many biological activities. J Inorg Biochem. 103(4):536−546.
3. Baranov A, Gale RP, Guskova A, Piatkin E, Selidovkin G, Muravyova L, Champlin RE, Danilova N, Yevseeva L, Petrosyan L. 1990. Bone marrow transplantation after the Chernobyl nuclear accident. N Engl J Med. 321(4):205−212.
4. Basu A, Ghosh P, Bhattacharjee A, Patra AR, Bhattacharya S. 2015. Prevention of myelosuppression and genotoxicity induced by cisplatin in murine bone marrow cells: effect of an organovanadium compound vanadium(III)-l-cysteine. Mutagenesis. 30(4):509−517.
5. Bunz F, Dutriaux A, Lengauer C, Waldman T, Zhou S, Brown JP, Sedivy JM, Kinzler KW, Vogelstein B. 1998. Requirement for p53 and p21 to sustain G2 arrest after DNA damage. Science. 282(5393):1497−1501.
6. Burdelya LG, Krivokrysenko VI, Tallant TC, Strom E, Gleiberman AS, Gupta D, Kurnasov OV, Fort FL, Osterman AL, Didonato JA, et al. 2008. An agonist of toll-like receptor 5 has radioprotective activity in mouse and primate models. Science. 320(5873):226−230.
7. Crawford J, Dale DC, Lyman GH. 2004. Chemotherapy-induced neutropenia: risks, consequences, and new directions for its management. Cancer. 100:228−237.
8. Dunberger G, Lind H, Steineck G, Waldenström A-C, Nyberg T, Al-Abany M, Nyberg U, Vall-Lundqvist E. 2010. Self-reported symptoms of faecal incontinence among long-term gynaecological cancer survivors and population-based controls. Eur J Cancer.

46(3):606−615.

9. Gordon JA. 1991. Use of vanadate as protein-phosphotyrosine phosphatase inhibitor. Methods Enzymol. 201:477−482.
10. Hua G, Thin TH, Feldman R, Haimovitz-Friedman A, Clevers H, Fuks Z, Kolesnick R. 2012. Crypt base columnar stem cells in small intestines of mice are radioresistant. Gastroenterology. 143(5):1266−1276.
11. Ianzini F, Bertoldo A, Kosmacek EA, Phillips SL, Mackey MA. 2006. Lack of p53 function promotes radiation-induced mitotic catastrophe in mouse embryonic fibroblast cells. Cancer Cell Int. 6:11.
12. Jiang W, Li G, Li W, Wang P, Xiu P, Jiang X, Liu B, Sun X, Jiang H. 2018. Sodium orthovanadate overcomes sorafenib resistance of hepatocellular carcinoma cells by inhibiting Na^+/K^+-ATPase activity and hypoxia-inducible pathways. Sci Rep. 8(1):9706.
13. Kirsch DG, Santiago PM, di Tomaso E, Sullivan JM, Hou W-S, Dayton T, Jeffords LB, Sodha P, Mercer KL, Cohen R, et al. 2010. p53 controls radiation-induced gastrointestinal syndrome in mice independent of apoptosis. Science. 327(5965):593−596.
14. Komarov PG, Komarova EA, Kondratov RV, Christov-Tselkov K, Coon JS, Chernov MV, Gudkov AV. 1999. A chemical inhibitor of p53 that protects mice from the side effects of cancer therapy. Science. 285(5434):1733−1737.
15. Komarova EA, Kondratov RV, Wang K, Christov K, Golovkina TV, Goldblum JR, Gudkov AV. 2004. Dual effect of p53 on radiation sensitivity in vivo: p53 promotes hematopoietic injury, but protects from gastro-intestinal syndrome in mice. Oncogene. 23(19):3265−3271.
16. Kuter DJ. 2015. Managing thrombocytopenia associated with cancer chemotherapy. Oncology. 29(4):282−294.
17. Lebrun F, Francois A, Vergnet M, Lebaron-Jacobs L, Gourmelon P, Griffiths NM. 1998.

Ionizing radiation stimulates muscarinic regulation of rat intestinal mucosal function. Am J Physiol. 275(6):G1333−1340.

18. Lee CL, Daniel AR, Holbrook M, Brownstein J, Silva Campos LD, Hasapis S, Ma Y, Borst LB, Badea CT, Kirsch DG. 2019. Sensitization of vascular endothelial cells to ionizing radiation promotes the development of delayed intestinal injury in mice. Radiat Res. 192(3):258−266.
19. Lowe SW, Schmitt EM, Smith SW, Osborne BA, Jacks T. 1993. p53 is required for radiation-induced apoptosis in mouse thymocytes. Nature. 362(6423):847−849.
20. MacNaughton WK. 2000. Review article: new insights into the pathogenesis of radiation-induced intestinal dysfunction. Aliment Pharmacol Ther. 14(5):523−528.
21. Mason KA, Withers HR, McBride WH, Davis CA, Smathers JB. 1989. Comparison of the gastrointestinal syndrome after total-body or total-abdominal irradiation. Radiat Res. 117(3):480−488.
22. Morita A, Ariyasu S, Wang B, Asanuma T, Onoda T, Sawa A, Tanaka K, Takahashi I, Togami S, Nenoi M, et al. 2014. AS-2, a novel inhibitor of p53-dependent apoptosis, prevents apoptotic mitochondrial dysfunction in a transcription-independent manner and protects mice from a lethal dose of ionizing radiation. Biochem Biophys Res Commun. 450(4):1498−1504.
23. Morita A, Takahashi I, Sasatani M, Aoki S, Wang B, Ariyasu S, Tanaka K, Yamaguchi T, Sawa A, Nishi Y, et al. 2018. A chemical modulator of p53 transactivation that acts as a radioprotective agonist. Mol Cancer Ther. 17(2):432−442.
24. Morita A, Yamamoto S, Wang B, Tanaka K, Suzuki N, Aoki S, Ito A, Nanao T, Ohya S, Yoshino M, et al. 2010. Sodium orthovanadate inhibits p53-mediated apoptosis. Cancer Res. 70(1):257−265.

25. Morita A, Wang B, Tanaka K, Katsube T, Murakami M, Shimokawa T, Nishiyama Y, Ochi S, Satoh H, Nenoi M, et al. 2020. Protective effects of p53 regulatory agents against high-LET radiation-induced injury in mice. Front Public Health. 8:601124.
26. Morita A, Zhu J, Suzuki N, Enomoto A, Matsumoto Y, Tomita M, Suzuki T, Ohtomo K, Hosoi Y. 2006. Sodium orthovanadate suppresses DNA damage-induced caspase activation and apoptosis by inactivating p53. Cell Death Differ.13(3):499−511.
27. Pant V, Xiong S, Wasylishen AR, Larsson CA, Aryal NK, Chau G, Tailor RC, Lozano G. 2019. Transient enhancement of p53 activity protects from radiation-induced gastrointestinal toxicity. Proc Natl Acad Sci USA. 116(35):17429−17437.
28. Patchen ML, MacVittie TJ, Williams JL, Schwartz GN, Souza LM. 1991. Administration of interleukin-6 stimulates multilineage hematopoiesis and accelerates recovery from radiation-induced hematopoietic depression. Blood. 77(3):472−480.
29. Patil R, Szabó E, Fells JI, Balogh A, Lim KG, Fujiwara Y, Norman DD, Lee SC, Balazs L, Thomas F, et al. 2015. Combined mitigation of the gastrointestinal and hematopoietic acute radiation syndromes by an LPA_2 receptor-specific nonlipid agonist. Chem Biol. 22(2):206−216.
30. Petitjean A, Mathe E, Kato S, Ishioka C, Tavtigian SV, Hainaut P, Olivier M. 2007. Impact of mutant p53 functional properties on TP53 mutation patterns and tumor phenotype: lessons from recent developments in the IARC TP53 database. Hum Mutat. 28(6):622−629.
31. Poirier Y, Belley MD, Dewhirst MW, Yoshizumic TT, Down JD. 2020. Transitioning from gamma rays to X rays for comparable biomedical research irradiations: energy matters. Radiat Res. 193(6):506−511.
32. Radford IR, Murphy TK, Radley JM, Ellis SL. 1994. Radiation response of mouse lymphoid and myeloid cell lines. Part II. Apoptotic death is shown by all lines examined.

Int J Radiat Biol. 65(2):217−227.

33. Saha S, Bhanja P, Kabarriti R, Liu L, Alfieri AA, Guha C. 2011. Bone marrow stromal cell transplantation mitigates radiation-induced gastrointestinal syndrome in mice. PLoS One. 6(9):e24072.
34. Strom E, Sathe S, Komarov PG, Chernova OB, Pavlovska I, Shyshynova I, Bosykh DA, Burdelya LG, Macklis RM, Skaliter R, et al. 2006. Small-molecule inhibitor of p53 binding to mitochondria protects mice from gamma radiation. Nat Chem Biol. 2(9):474−479.
35. Sullivan JM, Jeffords LB, Lee CL, Rodrigues R, Ma Y, Kirsch DG. 2012. p21 protects "Super p53" mice from the radiation-induced gastrointestinal syndrome. Radiat Res. 177(3):307−310.
36. Terry NH, Travis EL. 1989. The influence of bone marrow depletion on intestinal radiation damage. Int J Radiat Oncol Biol Phys. 17(3):569−573.
37. Wang B, Tanaka K, Morita A, Ninomiya Y, Maruyama K, Fujita K, Hosoi Y, Nenoi M. 2013. Sodium orthovanadate (vanadate), a potent mitigator of radiation-induced damage to the hematopoietic system in mice. J Radiat Res. 54(4):620−629.

Figure legends

Figure 1 PBI technique preserves bone marrow in the foreleg of mice. (A) Exterior appearance of the mouse that survived for 60 days after 16 Gy-PBI. Discolored body hair represents the irradiated region in the PBI treatment. (B) HE-stained sections of bone marrow of the humerus and femur on day 7 and 60 after 16 Gy-PBI. Magnification, 20×. *PBI* partial-body irradiation.

Figure 2 Vanadate rescues mice from bone marrow death after TBI, but not from GI death after PBI. (A) 60-day survival test after 10 Gy-TBI, 16 Gy-PBI, and 17 Gy-PBI (n = 10 in each group). (B) Body weight changes after irradiations (n = 10 in each group). Data represent the mean ± standard deviation. Asterisks indicate a significant difference ($^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$) between groups. *TBI* total-body irradiation, *PBI* partial-body irradiation.

Figure 3 Vanadate improves hematopoietic capacity after TBI. (A) Hematopoietic parameters measured at 7 days after 10 Gy-TBI and 17 Gy-PBI (n = 5 in each group). (B) Changes of the parameters in TBI-treated mice were further examined on day 9 and 12 after irradiation (n = 2−5 in each group). Data represent the mean ± standard deviation. An asterisk indicates a significant difference ($^{*}P < 0.05$) between groups treated with TBI. *V* vanadate, *NS* normal saline, *TBI* total-body irradiation, *PBI* partial-body irradiation, *RBC* red blood cells, *Hgb* hemoglobin, *WBC* white blood cells, *PLT* platelets.

Figure 4 Radioprotective effect of vanadate on the myeloid tissue. HE-stained sections of bone marrow of the femur on day 9 (A) and 12 (B) after 10 Gy-TBI. Arrows indicate megakaryocytes. Magnification, 40×. (C) Quantitation of megakaryocytes in the region of

femur bone marrow on day 12 after the TBI (n = 6− 7 in each group). An asterisk indicates a significant difference ($^{*}P < 0.05$) between groups. *V* vanadate, *NS* normal saline, *TBI* total-body irradiation.

Figure 5 Vanadate has no radioprotective effect on PBI-induced intestinal damage. HE-stained sections of intestinal epithelium on day 4 (A) and 7 (B) after 10 Gy-TBI and 17 Gy-PBI. Magnification, 40×. *NS* normal saline, *TBI* total-body irradiation, *PBI* partial-body irradiation.

Figure 6 Vanadate could not suppress PBI-induced crypt degeneration in the intestinal epithelium. BrdU-labeled cells were detected immunohistochemically on day 4 (A) and 7 (B) after 10 Gy-TBI and 17 Gy-PBI. Magnification, 40×. (C) The percentage of surviving crypts relative to the mean crypt number in mice that had been treated with NS only was estimated quantitatively (n = 3− 5 in each group). Data represent the mean ± standard deviation. Asterisks indicate a significant difference ($^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$) between groups. *V* vanadate, *NS* normal saline, *TBI* total-body irradiation, *PBI* partial-body irradiation.