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Preclinical Evaluation of SOM230 as a Radiation Mitigator in a Mouse Model: Postexposure Time Window and Mechanisms of Action

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Abstract

The somatostatin analog SOM230 has potent radioprophylactic and radiation mitigating properties that are unrelated to cytoprotection but appear to be due to suppression of secretion of pancreatic enzymes into the intestinal lumen. To determine the maximal postirradiation time window for administration, male CD2F1 mice were exposed to 8.5–11 Gy total-body radiation; SOM230 (0.5, 2 or 5 mg/kg) or vehicle was given by twice daily subcutaneous injections for 14 days, beginning 24–72 h after irradiation, and 30-day animal survival was recorded. The contribution of the gut to systemic cytokine levels was estimated by analyzing plasma samples obtained simultaneously from the portal vein and carotid artery. The effect of SOM230 on cell trypsin secretion was assessed *in vitro* and intestinal proteolytic activity was measured *in vivo*. SOM230 was associated with a 40–60% absolute improvement in overall postirradiation survival when treatment was started 48 h after irradiation and even exhibited a statistically significant survival benefit when started at 72 h. SOM230 ameliorated the radiation-induced decrease in chemokine (C-X-C motif) ligand 9 (CXCL9). SOM230 inhibited pancreatic acinar cell trypsin secretion *in vitro* in a dose-dependent fashion and reduced intraluminal and intestinal tissue proteolytic activity *in vivo*. SOM230 is an excellent radiation mitigator with a postirradiation time window in excess of 48 h. The mechanism likely involves preservation of intestinal barrier function due to decreased secretion of pancreatic enzymes into the bowel lumen.

INTRODUCTION

Injuries to the bone marrow and gastrointestinal (GI) tract are the main determinants of lethality after total-body irradiation (TBI) (1, 2). Some progress has been made in the management of radiation injury of the hematopoietic/immune system. In contrast, effective and safe countermeasures against structural injury and dysfunction in the GI tract remain an unmet need (2).

In the intestine, radiation damages crypt stem cells and proliferating transit cells, thereby leading to disruption of the epithelial barrier that normally protects the subepithelial matrix from the influence of the digestive enzymes that are normally present in the intestinal lumen

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(3). Among the various intraluminal factors, pancreatic enzymes exert a particularly prominent influence on development of intestinal radiation toxicity (4). Hence controlling pancreatic enzyme secretion has been explored as a method to attenuate acute mucosal injury and increases survival after abdominal irradiation (5, 6).

Somatostatin analogs are known as universal GH inhibitors and, among many different activities that influence the GI tract, inhibit the secretion of pancreatic enzymes into the bowel lumen (7, 8). Earlier studies performed in our laboratories showed that the “prototype” somatostatin analog, octreotide, markedly ameliorated mucosal injury in the small bowel after localized irradiation (9). Subsequent randomized clinical trials have translated this to patients with severe radiation-induced GI toxicity, thus confirming therapeutic efficacy in humans (10).

While octreotide can be used in the clinical situation to reduce side effects of radiation therapy, its short half-life is a serious limitation in situations where intravenous or multiple subcutaneous (s.c.) administrations are not logistically feasible. A more recently developed synthetic somatostatin analog, SOM230 (pasireotide), however, has much greater metabolic stability and thus circumvents this obstacle (11). We reported in 2009 that SOM230 administered prior to or 4 h after TBI in a mouse model conferred a highly statistically significant survival benefit, even though we also demonstrated that SOM230 did not appear to have direct cytoprotective effects on the bone marrow or the intestine (12). Hence, while post-TBI intestinal crypt survival and hematopoietic injury were similar, the intestinal barrier was structurally and functionally preserved. Moreover, protection was reversed by co-administration of pancreatic enzymes, thus strongly suggesting that reduced secretion of proteolytic enzymes into the bowel lumen was the mechanism of action (12).

To be useful in an actual mass casualty situation, a radiation mitigator must be able to retain its therapeutic efficacy when administration begins 24 h or more after exposure. The study reported here extends our previous findings by prolonging the postirradiation time window for beginning SOM230 administration up to 72 h postexposure while also determining the optimal SOM230 dose for postexposure administration. Our results show that SOM230 retains its full therapeutic efficacy when administered as late as 48 h after TBI and enhances survival and prolongs survival time even when administered at 72 h. Additionally, we confirm that SOM230 inhibits pancreatic enzyme secretion, reduces the intestinal proteolytic activity, and blunts aspects of the postirradiation intestinal cytokine response.

MATERIALS AND METHODS

Animals

Male CD2F1 mice (Harlan Sprague Dawley, Indianapolis, IN) with an initial body weight of 22–25 g were used for this study. Animals were housed in conventional cages under standard conditions of temperature, pressure and humidity and a 12/12-h day/night light cycle. Animals had free access to water and chow (Harlan Teklad laboratory diet 7012, Purina Mills, St. Louis, MO). A total of 648 mice were used for the experiments reported here. The experimental protocols for these studies were reviewed and approved by the Central Arkansas Veterans Healthcare System Institutional Animal Care and Use Committee (IACUC) as well as by the IACUC at the University of Arkansas for Medical Sciences.

Irradiation and Dosimetry

Irradiation was performed with a J. L. Shepherd Mark I model 25 ¹³⁷Cs irradiator (J. L. Shepherd & Associates, San Fernando, CA). Non-anesthetized mice were placed in cylindrical, well-ventilated Plexiglas chambers (J. L. Shepherd & Associates) that were positioned on a turntable rotating at 5 rpm in the position furthest away from the radiation

source. Each chamber contained four mice, and two chambers could be stacked on top of each other, allowing eight mice to be irradiated at a time. The average dose rate was 1.35 Gy/min, and the dose rate was corrected for decay on the day of mice were irradiated.

Mitigating Agent

SOM230 was kindly supplied by Novartis Pharma AG (Basel, Switzerland). Lyophilized SOM230 was stored at 4°C and was reconstituted in 100 µl sterile deionized water just before treatment (see below). Sterile deionized water was used as vehicle.

Survival Experiments

Mice were exposed to graded total-body doses between 8.5 and 11 Gy (the dose range at which previous work with SOM230 had shown it to confer the greatest lethality protection). Mice were randomly assigned to receive vehicle or SOM230 at 0.5mg/kg, 2 mg/kg or 5 mg/kg twice daily (bid) by s.c. injection, beginning 24 h after TBI, with eight animals per dose group.

Once the optimal SOM230 dose was determined at this window of administration, the optimal SOM230 dose was used to determine the postirradiation time window by administering SOM230 at 24 h, 48 h or 72 h after TBI. Again, eight animals per each group were used. For all survival experiments, the study design was optimized for efficiency and statistical power so as to maximally reduce the number of animals required (13).

In all cases treatment was continued for 14 days. Study animals were monitored twice daily by the investigators and veterinary personnel for up to 30 days after TBI, and the number of moribund/dead mice was recorded twice daily. Kaplan-Meier survival curves, median survival times, and lethality at 30 days were recorded.

Selective Sampling of Systemic and Portal Blood

SOM230 reduces the incidence and severity of postirradiation bacterial translocation (12). Bacterial translocation used to be considered a simple process of bacteria or bacterial products transgressing the mucosal barrier, thereby gaining access to lymphatics and/or capillaries/venules. It is now recognized that, in addition, bacterial translocation by virtue of the extremely rich mucosal immune system, transforms the intestine into a large pro-inflammatory organ that produces substantial amounts of cytokines and other inflammatory mediators (14, 15). These “intestinal” cytokines and other inflammatory mediators contribute in a major way to the development of systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS) that develop after TBI (16). To examine the effect of TBI and the influence of SOM230 on the contribution of the intestine to the systemic levels of chemokines and cytokines, an animal model of selective whole blood sample collection that allowed simultaneous collection of blood samples from the carotid artery and portal vein was developed (Fig. 1).

The mouse was anesthetized by intraperitoneal injection of ketamine/xylazine (50/10 mg/kg body weight) and fixed under an operating microscope in the supine position. For carotid sampling, the neck region was disinfected with 70% ethanol and the right carotid artery was exposed and freed from connective tissue. The isolated carotid artery was ligated distally with a 6-0 silk suture (distal ligature) and proximally with a 5-0 catgut suture (stabilizing ligature). The catgut suture was not tied off because it was used to control blood flow during catheterization. A customized catheter, made by connecting a 30-gauge needle to 10 cm of silastic laboratory tubing, was inserted into the right carotid artery, fixed with a small amount of tissue glue, and filled with 10 U/ml heparin saline. To access the portal vein, the mouse abdomen was disinfected with 70% ethanol and the portal vein was exposed by

moving the intestine out of the abdominal cavity. A catheter filled with 10 U/ml heparin saline was then inserted into the portal vein and fixed with a small amount of tissue glue. Using this animal model, approximately 200 μ l of whole blood from the carotid artery and the portal vein can be obtained simultaneously. The blood was collected into EDTA-coated tubes (Fisher Scientific).

Mice were exposed to 8.5 Gy and received treatment with vehicle or 5 mg/kg SOM230 bid (5–7 mice/group) until the last sample collection on day 7 after TBI (samples were obtained at 0, 1 and 7 days after TBI). Plasma was generated by centrifugation (12,000 rpm, 5 min, 4°C) and stored at –80°C until analysis. Plasma levels of 20 cytokines were measured by multiplexing using a Bioplex system (Bio-Rad Laboratories, Hercules, CA) and the BioSource mouse cytokine/chemokine 20-plex panel (Invitrogen, Carlsbad, CA).

Trypsin Activity Measurement in Rat Pancreatic Acinar Cells In Vitro

We have previously shown that administration of pancreatic enzymes by gavage reverses the radiomitigating effects of SOM230 (12). To demonstrate that SOM230 indeed reduces exocrine pancreatic trypsin secretion, the effect of SOM230 on trypsin secretion by pancreatic acinar cells was examined *in vitro*. Trypsin activity was measured by the method described by Kereszturi and Sahin-Tóth (17). Rat pancreatic acinar AR42J cells were purchased from American Type Culture Collection (ATCC) and cultured in F12-K medium containing 20% fetal bovine serum, 100 U/ml penicillin, and 100 μ g/ml streptomycin in 24-well plates (2.5×10^5 cells /plate) in a humidified atmosphere containing 95% air/5% CO₂. The experiments were performed in quadruplicate. Prior to trypsin activity measurement, dexamethasone (100 nM) was added in the culture medium for 48 h to achieve maximum differentiation of cells. SOM230 was added in serum-starved medium 24 h before the measurement. Ten microliters of culture medium was supplemented with 0.1 M Tris-HCl (pH 8.0) and 1 mM CaCl₂ to 50 μ l volume, and trypsinogen was activated with 1 μ l 1.4 μ g/ml enteropeptidase (R&D Systems, Minneapolis, MN) for 1 h at 37°C. Trypsin activity was then measured by adding 150 μ l of the chromogenic substrate *N*-Cbz-Gly-Pro-Arg-*p*-nitroanilide to 0.14 mM final concentration. *P*-Nitroaniline release in 1 min was measured at 405 nm in 0.1 M Tris-HCl (pH 8.0) and 1 mM CaCl₂ at room temperature using a microplate reader (Synergy HT, Bio-Tek Instruments, Winooski, VT).

Measurement of Intraluminal and Intestinal Tissue Proteolytic Activity

To investigate the effect of SOM230 treatment on intestinal proteolytic activity, unirradiated mice were given three doses of 5 mg/kg SOM230 (at 9:00 a.m., 4:00 p.m. and 9:00 a.m.) or vehicle ($n = 5$). Four hours after the last dose, the animals were euthanized and about 10 cm of the proximal jejunum, just distal to the ligament of Treitz, was collected from each mouse. To measure intraluminal proteolytic activity, the intestinal lumen was flushed twice with 100 μ l PBS, the washing fluid was collected and centrifuged at 12,000 rpm for 10 min, and the supernatant was collected and stored at –80°C until analysis. Intestinal tissue proteolytic activity was measured in a separate 1-cm piece of proximal jejunum, just distal to the 10-cm segment used to measure intraluminal proteolytic activity. The 1-cm segment was placed in a 5-ml glass centrifuge tube and 1 ml PBS was added. The intestine was then homogenized and centrifuged at 12,000 rpm for 10 min. The supernatant was collected and frozen as above. Intraluminal and intestinal tissue proteolytic activity was assessed using a protease fluorescence detection kit (Sigma, catalog number PF0100) according to the manufacturer's instructions.

Statistical Analysis

Statistical analyses were performed using NCSS2004 for Windows (NCSS, Kaysville, UT). Data are presented as means $\pm$ SEM, except for postirradiation duration of survival, which

was presented as the median survival with interquartile range as the measure of variability. Two-sided tests were used throughout, and differences were considered statistically significant when the P value was less than 0.05. Survival curves were constructed using the Kaplan-Meier method and were compared using the log-rank test. The Cox proportional hazards multiple regression method was used to determine the influence of SOM230 on postirradiation survival across radiation doses.

RESULTS

SOM230 Confers Lethality Protection and Prolongs Survival with a Postirradiation Window in Excess of 48 h

SOM230, with administration beginning at 24 h after TBI, significantly reduced lethality and enhanced median survival. The mitigating efficacy on both parameters was significantly better after 2 mg/kg bid than after 0.5 mg/kg bid. SOM230, 2 mg/kg bid, started 24 h after radiation exposure improved survival for radiation doses up to 10.5 Gy (Fig. 2, Table 1). There was no additional benefit of increasing the SOM230 dose to 5mg/kg bid (data not shown). Therefore, 2 mg/kg bid was selected as the optimal SOM230 dose and was used for the subsequent time window experiments.

Results showed that the time window for radiation mitigation with SOM230 2 mg/kg bid could be significantly extended to 48 h ($P < 0.001$) and 72 h ($P < 0.05$) after 9 Gy and 9.5 Gy TBI (Fig. 3, Table 2).

SOM230 Administration is Associated with Reduced Intestinal Output of the Chemokine, Monokine Induced by Gamma-Interferon (MIG or CXCL9)

As described above, the purpose of the experiments with selective sampling of blood from the portal vein that drains the intestines and the carotid artery was to assess the effect of SOM230 on the output of cytokines from the gut into the systemic circulation. Among the cytokines and chemokines included in the multiplex assay, the levels of fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), granulocyte macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor alpha (TNF α), and interleukins 5, 6, 10, 12 and 17 (IL5, IL6, IL10, IL12, IL17) as well as the chemokines CXCL9, 10 kDa interferon gamma (IFN- γ)-induced protein (IP-10 or CXCL10), macrophage inflammatory protein 1 (MIP-1) and monocyte chemotactic protein 1 (MCP-1 or CCL2) were above the detection limit. However, only the level of MIG (CXCL9) exhibited changes consistent with differential levels in portal blood and the systemic circulation depending on SOM230 treatment. TBI at 8.5 Gy reduced CXCL9 levels in plasma samples from both vehicle-treated and SOM230-treated mice. The CXCL9 levels in vehicle-treated and SOM230-treated mice were similar at baseline. After irradiation, vehicle-treated mice had significantly higher levels in portal vein blood than in systemic blood on both postirradiation day 1 ($P = 0.0006$) and postirradiation day 7 ($P = 0.001$). In contrast, the difference was not significantly different ($P = 0.26$) in SOM230-treated mice (Fig. 4).

SOM230 Inhibits Trypsin Production by Pancreatic Acinar Cells In Vitro

SOM230 had a highly statistically significant, dose-dependent inhibitory effect on trypsin secretion by rat pancreatic acinar cells ($P = 0.0001$, Fig. 5). Normalized to baseline level, 1 nM concentration was associated with, on average, an almost 50% reduction in trypsin secretion, whereas 10 nM reduced the secretion by more than 70%.

SOM230 Reduces Proteolytic Activity in Intestinal Lumen and Tissue In Vivo

The intraluminal washings and intestinal tissue both exhibited highly statistically significant reductions in response to treatment with SOM230. Intestinal proteolytic activity measured in

mice after treatment with SOM230 (5 mg/kg bid, three doses) or vehicle revealed that SOM230 was associated with decreased intraluminal as well as tissue proteolytic activity ($P = 0.004$ and $P = 0.0003$, respectively), indicating that SOM230 reduced the intestinal “proteolytic burden” (Fig. 6).

DISCUSSION

A wide administration time window is critical for a radiation mitigator to be useful in the field in the event of an actual radiological or nuclear emergency situation. The traditional radioprotective agents (free radical scavengers, vitamin E analogs, immune modulators, etc.) show little mitigation after acute lethal radiation exposure when administration begins postexposure (18). There is clearly a need for safe, effective radiation mitigators with therapeutic window in excess of 24 h.

SOM230 is a novel synthetic somatostatin analog that has a half-life in excess of 11 h and a wide affinity for somatostatin receptors (11, 19). Our previous study showed that SOM230 preserved intestinal mucosa surface area after irradiation, attenuated radiation-induced bacterial translocation, prolonged survival, and reduced lethality after TBI when administration was started 2 days before or 4 h after TBI (12). The present study extends our previous findings by demonstrating that SOM230, at a dose of 2 mg/kg twice daily, significantly enhances survival and prolongs survival time when administration is begun as late as 24 h to 72 h postirradiation. We also provide further evidence as to the specific mechanisms of action of SOM230, as required for regulatory approval under the animal rule.

The intestinal epithelium is one of the most susceptible tissues to radiation injury by virtue of its high turnover rate. Radiation causes death of intestinal crypt cells, which results in insufficient replacement of the villus epithelium and breakdown of the normal mucosal barrier. Under this circumstance, pancreatic enzymes present in the bowel lumen gain access to the intestinal wall, where they initiate a process of autodigestion, the products of which are highly inflammatory and cytotoxic (20–22). The injurious effects of pancreatic enzymes have been demonstrated both in dog studies with abdominal irradiation (4, 5) and in the rat in a model with localized irradiation (6).

There is strong evidence suggesting that SOM230 protects the intestine by inhibiting pancreatic secretion and that this feature may be why the time window for its mitigating effect is so prolonged. As shown previously, SOM230 does not have direct cytoprotective effects; i.e., SOM230 does not enhance crypt survival or reduce hematopoietic injury (12). Moreover, we showed that oral replacement of pancreatic enzymes completely reversed the lethality protection of SOM230 (12). The results of the present study corroborate the previous results by demonstrating that SOM230 suppresses secretion of trypsin by pancreatic acinar cells *in vitro* and inhibits proteolytic activity *in vivo* both in the intestinal lumen and in the intestinal wall. Taken together, our findings thus strongly support the notion that the mechanism by which SOM230 confers lethality protection and prolongs postirradiation survival is related to a reduction in proteolytic enzymes in the bowel lumen at the time of radiation injury.

The stem cell niche also impacts tissue remodeling and repair after radiation exposure (23). Hence, in addition to suppressing autodigestion, it is possible that the decrease in pancreatic enzymes in the intestinal lumen may facilitate the maintenance of an advantageous cellular microenvironment favoring the replication of surviving crypt epithelial stem cells and regeneration of small intestine mucosa (24). On the other hand, somatostatin analogs not only inhibit the secretion of pancreatic enzymes but also, as shown previously (12), reduce the production of insulin-like growth factor 1 (IGF-1) and growth hormone (GH). Both

IGF-1 and GH have been suggested to protect against radiation-induced intestinal and hematopoietic injury by promoting of the proliferation of progenitor cells (25, 26), and somatostatin analogs are known to inhibit of progenitor cell proliferation (27). It is possible that the lack of additional improvement in survival with the “ultra-high” dose of SOM230 (5 mg/kg bid) may have been due to an excess inhibition of hormones, cytokines or stem cells that would otherwise contribute to the regeneration of radiation-injured tissues (28, 29). Further research is clearly needed to investigate the effect of SOM230 on the intestinal crypt stem compartment and on the hematopoietic niche.

The GI tract produces a plethora of inflammatory and anti-inflammatory cytokines (30, 31). Several of these cytokines have hematopoietic or immune stimulatory properties that may protect from radiation-induced tissue injury, such as IL-1, IL-11 and transforming growth factor (TGF)- β (32–34). TBI reduces circulating levels of several cytokines and chemokines (35). Because the intestine is the presumed target organ for mitigation of radiation injury by SOM230, an estimate of the release of cytokines from the intestine was obtained by simultaneously collecting blood samples from the portal vein, which drains the intestine, and the carotid artery at various times before and after radiation exposure with and without SOM230 administration. While several cytokines were detected, consistent differences between portal and systemic blood were seen for CXCL9, a T-cell chemoattractant induced by IFN- γ (36). CXCL9 is an inflammatory chemokine and plays a key role in the accumulation of inflammatory cells at the site of tissue damage. CXCL9 and its receptor, CXCL3, are also important in the recruitment of tissue progenitor cells to damaged tissues and for initiating tissue repair (37, 38). The results of the present study suggest that SOM230 treatment attenuates postirradiation production and/or release of CXCL9 by the bowel. It is tempting to speculate that this may relate to the so-called substance P–somatostatin–IFN- γ immunoregulatory circuit, which is thought to regulate intestinal inflammation in certain situations (39). Somatostatin (analog) ameliorates radiation enteropathy and inhibits IFN- γ release, while substance P has the opposite effect (40). However, the extent to which the substance P–somatostatin–IFN- γ immunoregulatory circuit applies to acute or chronic radiation responses and the possible role of CXCL9 have, yet to be elucidated.

Conclusions

SOM230 is a very promising radiation mitigator with a remarkably wide postirradiation time window between 48 and 72 h postirradiation and extremely low toxicity. The mechanism by which SOM230 exerts its lethality protection is by inhibition of exocrine pancreatic secretion, thus reducing the intraluminal content of proteolytic enzymes. The mitigating effect of SOM230 may also be related to decreased intestinal production and/or release of CXCL9 after irradiation. Although further mechanistic studies are warranted, SOM230 appears ready to undergo advanced development in non-human primates prior to being considered for approval as a medical countermeasure against radiation.

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FIG. 1.

Mouse model for blood collection from the carotid artery and portal vein. Left panel: cartoon of the model. Right panel: photograph from the model showing cannulas in the carotid artery (top) and portal vein (bottom).

FIG. 2.

Dose-dependent radiation-mitigating effects of SOM230. Kaplan-Meier survival curves from mice exposed to 8.5, 9 and 9.5 Gy radiation (SOM230 administration beginning 24 h after TBI). SOM230 significantly reduced lethality in a dose-dependent manner across radiation doses ($P < 0.0001$). $n = 8$.

FIG. 3.

Postexposure time window for SOM230. Kaplan-Meier survival curves from mice exposed to 9 and 9.5 Gy radiation ($n = 8$). SOM230 (2 mg/kg bid) significantly reduced lethality when administration commenced 24 h ($P < 0.004$) or 48 h after TBI ($P = 0.005$). Even when the start of administration was delayed by 72 h, the difference in survival between vehicle-treated and SOM230-treated animals across the two radiation doses was borderline significant ($P = 0.05$).

FIG. 4.

Difference in chemokine (CXCL9) levels from portal vein draining the intestines and carotid artery (systemic circulation) as a function of SOM230 treatment. Difference between portal vein and systemic levels of the chemokine, monokine induced by gamma-interferon (MIG, CXCL9), from vehicle-treated (left) and SOM230-treated (right) mice. SOM230 injection was started 2 days prior to TBI and continued through the termination of the experiment at 7 days. Samples were taken before irradiation and 1 and 7 days after 8.5 Gy TBI. While vehicle-treated mice have significantly higher CXCL9 levels in portal blood than in the systemic circulation after TBI, the difference was not significant in mice treated with SOM230. $**P = 0.006$; $*P = 0.001$; SOM230 dose 5 mg/kg bid. CA, carotid artery; PV, portal vein. $n = 5-7$.

FIG. 5.

Inhibition of trypsin secretion *in vitro* by SOM230. Trypsin (normalized to baseline) secreted by rat pancreatic acinar cells after 24 h treatment with SOM230. Trypsin secretion was inhibited in a highly significant, dose-dependent manner ($P = 0.0001$). $n = 4$.

FIG. 6.

Inhibition of intestinal proteolytic activity *in vivo* by SOM230. Proteolytic activity in the intestinal lumen (luminal washings, left) and in intestinal tissue (right) from unirradiated mice after treatment with vehicle or SOM230. Treatment with SOM230 was associated with a highly significant reduction in proteolytic activity both intraluminally ($P = 0.004$) and in intestinal tissue ($P = 0.0003$). $n = 5$.

TABLE 1

Survival Time (median and interquartile range) in Mice Treated with Vehicle or SOM230 (0.5 mg/kg bid or 2 mg/kg bid) Commencing 24 h after 8.5, 9 and 9.5 Gy TBI

Dose	Median survival time (days)		
	Vehicle	SOM230 0.5 mg/kg bid ($P = 0.05$)	SOM230 2 mg/kg bid ($P = 0.0003$)
8.5 Gy	30 (15–30)	30 (15–30)	30 (30–30)
9.0 Gy	18.5 (12–30)	24.5 (17–30)	30 (30–30)
9.5 Gy	15 (9–19)	17.5 (13–30)	30 (14–30)

Notes. The P values indicate the effect of each of the two doses of SOM230 (compared to vehicle) across the three radiation doses. $n = 8$.

TABLE 2

Survival Time (median and interquartile range) in Mice Treated with Vehicle or SOM230 (2 mg/kg bid)
Starting 24, 48 or 72 h after 9 and 9.5 Gy TBI

	Dose			
	9.0 Gy		9.5 Gy	
	Vehicle	SOM230	Vehicle	SOM230
24 h after TBI ($P = 0.0004$)	23.25 (16–30)	30 (30–30)	14.5 (13–16)	30 (30–30)
48 h after TBI ($P = 0.0005$)	19 (15–30)	30 (30–30)	14.75 (13–16)	30 (30–30)
72 h after TBI ($P = 0.02$)	13.75 (13.5–15)	22.5 (14–30)	12.5 (12–13.5)	14 (13.5–14.5)

Notes. The P values indicate the effect of SOM230 (compared to vehicle) across the two radiation doses for each postexposure time window. $n = 8$.