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Effects of Solar Particle Event Proton Radiation on Parameters Related to Ferret Emesis

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Abstract

The effectiveness of simulated solar particle event (SPE) proton radiation to induce retching and vomiting was evaluated in the ferret experimental animal model. The endpoints measured in the study included: (1) the fraction of animals that retched or vomited, (2) the number of retches or vomits observed, (3) the latency period before the first retch or vomit and (4) the duration between the first and last retching or vomiting events. The results demonstrated that γ ray and proton irradiation delivered at a high dose rate of 0.5 Gy/min induced dose-dependent changes in the endpoints related to retching and vomiting. The minimum radiation doses required to induce statistically significant changes in retching- and vomiting-related endpoints were 0.75 and 1.0 Gy, respectively, and the relative biological effectiveness (RBE) of proton radiation at the high dose rate did not significantly differ from 1. Similar but less consistent and smaller changes in the retching- and vomiting-related endpoints were observed for groups irradiated with γ rays and protons delivered at a low dose rate of 0.5 Gy/h. Since this low dose rate is similar to a radiation dose rate expected during a SPE, these results suggest that the risk of SPE radiation-induced vomiting is low and may reach statistical significance only when the radiation dose reaches 1 Gy or higher.

INTRODUCTION

As reviewed by Hellweg and Baumstark-Khan (1), the primary components of radiation in interplanetary space are galactic cosmic rays (GCR) and solar cosmic radiation (SCR). GCR originate from outside of our solar system and consist of 98% baryons and 2% electrons. The baryonic component consists of 87% protons (hydrogen nuclei), 12% α particles (helium nuclei) and approximately 1% of heavier nuclei with atomic numbers (Z) up to 92 (uranium). In contrast, SCR is predominately composed of low-energy solar wind particles containing primarily protons. SCR flows constantly from the Sun and the highly energetic solar particle events (SPEs) that originate from magnetically disturbed regions of the Sun sporadically emit bursts of energetic charged particles (2, 3). SPEs are unpredictable, develop rapidly and usually last no longer than several hours. However, some SPEs may

continue for several days, with variable tissue dose-rates and (deep) doses ranging from 0–0.5 Gy/h and 0–2 Gy, respectively.

Acute radiation syndrome (ARS) or radiation sickness is an acute illness caused by whole-body irradiation. Exposure to radiation during space missions, including SPE or combined SPE and GCR radiation, may immediately affect the probability for successful mission completion (mission critical) or result in late radiation effects in individual astronauts (1). While avoidance of the radiation risk is the best protective strategy, it is nearly impossible for astronauts to avoid the risk completely.

Early symptoms of ARS vary with radiation dose, dose rate, quality, individual radiation sensitivity and the presence of additional injury (such as trauma or burns) (1). The early phase, known as the prodromal syndrome, may include nausea, retching, vomiting, diarrhea and fatigue. These effects manifest within 1–72 h after exposure at sublethal doses, with a latency time inversely correlated with dose. The prodromal phase is followed by a manifest illness stage in which symptoms may last up to several months, followed by recovery or death. The four major organs and classic ARS syndromes are the bone marrow or hematopoietic syndrome, gastrointestinal (GI) syndrome, cardiovascular/central nervous system syndrome and the cutaneous (skin) radiation syndrome.

Vomiting is the reflexive act of forcefully ejecting the stomach contents through the mouth by coordinated muscle contraction. Previous clinical studies have shown that patients receiving total body irradiation or upper-abdominal irradiation often show nausea, retching and vomiting as side effects (4, 5). During vomiting, the muscles of the abdomen and chest contract and the diaphragm spasms downward and inward, thereby putting pressure on the stomach. The part of the diaphragm surrounding the esophagus relaxes and helps to open the esophagus, then the longitudinal muscle of the esophagus contracts and further opens the junction between the stomach and esophagus. The pressure placed on the stomach then forces the contents of the stomach up into the esophagus and out of the mouth, with a closed glottis to prevent pulmonary aspiration of stomach contents (6, 7). The act of vomiting is coordinated by neuronal circuitry located in the brain stem between the obex and the retrofacial nucleus. Other central nervous system (CNS) regions, e.g., area postrema, medullary midline and certain higher brain centers are also important for vomiting. The sensation of nausea is thought to involve the cerebral cortex (7).

As described by Andrews *et al.* (8), data from 24 emetic stimuli in the ferret show a strong correlation between retching and vomiting events. The numbers of retches precede vomits for many of the stimuli (including radiation), with retching being present in 40% of all events. Moreover, their study and a literature review support the concept that retching allows the body to overcome gastroesophageal anti-reflux mechanisms to provide afferent feedback and enable the central (brainstem) circuit to determine whether gastric contents should be expelled.

The emetic responses to various pharmacological agents, cytotoxins and radiation have been compared previously among humans and various animal species including nonhuman primates, dogs, cats and ferrets (8). Ferrets are considered to be a useful species in emesis research (9), in particular for radiation and cytotoxic drug-induced emesis (10). The effective dose at which 50% of ferrets exhibited an emetic response (ED₅₀) to radiation is similar to that of humans (G. Anno and G. McClellan, personal communication) and data from the ferret have been used by the Department of Defense (DOD) to develop a mathematical model for the human emetic response to radiation (11). Another advantage of using ferrets as a model system for radiation-induced vomiting is that the prodromal response appears at lower doses and with an earlier onset time compared to other species,

including humans (12). The aim of this study was to determine the effectiveness of protons at the energy, doses and dose rate ranges relevant to those expected for SPEs to induce retching and vomiting using ferrets as an experimental model system.

MATERIALS AND METHODS

Animals and Radiation Experiments

Female descended, Fitch ferrets (*Mustela putorius furo*) of 12 to 16 weeks of age and 0.6 to 0.8 kg of body weight were obtained from Marshall Farms (North Rose, NY) and housed in the Loma Linda University Medical Center (LLUMC) animal facility. The animals were group-housed and had access to ferret chow and water *ad libitum* with a 12 h light-dark cycle. The animals were maintained under standard husbandry conditions and all procedures for the animal care and treatment were approved by the Institutional Animal Care and Use Committees of the LLUMC and the University of Pennsylvania.

Upon acclimation for 7 to 10 days, the animals were randomly assigned to groups with 6–22 animals per group. Nineteen animals were used as controls with no radiation exposure, and the rest of the animals were exposed to γ rays or a fully modulated proton beam with maximum range of 11 cm in water at an average dose rate of 0.5 Gy/min (high dose rate; HDR) or 0.5 Gy/h (low dose rate; LDR) with a single dose of 0.25, 0.5 (not performed for LDR), 0.75, 1 or 2 Gy. The animals were observed and recorded during the radiation exposure period with radiation-hardened cameras (CID8825DX6; Thermo Fisher Scientific, Liverpool, NY). At the end of the radiation exposure, all animals were observed and recorded with Sony® digital Handycams for an additional 3 h. The control animals were observed for a total of 7 h. All recordings were rendered to CD for analyses using software developed at the Armed Forces Radiobiology Research Institute (AFRRI).

Physics and Dosimetry

Gamma radiation was chosen as the reference radiation for the determination of relative biological effectiveness (RBE) values and was delivered using a ^{60}Co source (Eldorado Model 'G' machine, Atomic Energy of Canada Ltd, Commercial Products Division, Ottawa, Canada) at LLUMC. For radiation exposures at the HDR, the source to target distance was 150 cm, with a usable radiation field of $40 \times 40 \text{ cm}^2$ and a field flatness of 5.4% in the horizontal direction and 3.6% in the vertical direction. No additional material was placed between the source and target to modify the dose rate. For the LDR irradiations, the source to target distance was 195 cm with a useable field size of $40 \times 40 \text{ cm}^2$ and a field flatness of 4.0% in the horizontal direction and 2.3% in the vertical direction. To achieve the LDR, an additional 12.7 cm of steel plating was placed between the source and the target to attenuate the flux of the beam. Depth dose measurements were made using a calibrated Markus ionization chamber while field flatness and symmetry was evaluated using Kodak X-Omat V film.

For the proton radiation exposures, experiments were performed in the LLUMC horizontal clinical beam-line using a tuned energy proton beam of 155 MeV. The incident protons were scattered into a uniform field using the clinical 2-stage scattering system and modulated in depth using an 11-cm clinical modulator wheel. At the exit of the beam-line, a polystyrene degrader was tuned to achieve a fully modulated (uniform dose as a function of depth) SOBP proton beam inside of the irradiation chamber. The benefit of using a fully modulated beam of this energy was that regardless of the ferret position or orientation during the experiment, a uniform dose would be delivered to the animal. Depth dose profiles were measured for the optimized polystyrene degrader thickness using a calibrated Markus

ionization chamber while field flatness and symmetry were evaluated using Kodak X-Omat V film.

The HDR and LDR proton irradiations used two different apertures to target distances and two different polystyrene thicknesses (to account for energy loss variations in air between the two experimental setups) to achieve the desired dose rates and beam sizes. For the LDR (0.5 Gy/h), the animal cages were placed 122 cm downstream of isocenter. The useable beam at this distance was measured as $22 \times 322 \text{ cm}^2$ while achieving a flatness of $\pm 10\%$. This field size allowed only one ferret, in the radiation chamber (approximately $24 \text{ long} \times 16 \text{ wide} \times 9 \text{ high cm}^3$) to be irradiated. For the HDR (0.5 Gy/min), the animal cage was placed at isocenter. The usable beam at this distance was experimentally verified and measured as $19 \times 19 \text{ cm}^2$ allowing only one ferret in the radiation chamber to be irradiated. To provide more efficient proton irradiation of multiple ferrets (up to 4 at a time) at the LDR, an alternate scattering system was developed and commissioned in the LLUMC proton research room. This system delivered a 50 cm diameter useable proton radiation field with the flatness, depth dose profile and dose rate optimized to match the previous irradiation conditions on the LLUMC horizontal clinical beam-line.

Animal Immobilization for the γ -Ray or Proton Radiation Exposure

For the γ -ray and proton radiation experiments, the animals were placed in custom-made, aerated Plexiglas[®] radiation chambers (as described above). Animals were provided with NapaNectar hydrating gel (SE Lab Group Inc., Napa, CA) during irradiation and observation periods. For the LDR experiments, the chambers were slightly modified to contain a watering system on the outside of each chamber that did not compromise the radiation dose delivered to each animal, so these animals were not provided NapaNectar gel. The LDR chambers also contained a thermistor, which enabled real-time monitoring of temperature within the radiation chamber during the long exposures of the LDR irradiation. The animals were conscious during the radiation exposures and were able to express normal postural movements throughout. For the HDR experiments, although the actual radiation exposures were only minutes long (as opposed to hours, as in the case of the LDR experiments), the HDR animals were restrained in the radiation chamber for the equal amount of time as the LDR animals, corresponding to the appropriate dose.

Animal Behavior Analyses

The endpoints measured were the fraction of animals that retched or vomited, the number of retching or vomiting events observed, the latency period before the first retching or vomiting event recorded and the duration between the first and last retching or vomiting events. For the animals that did not retch or vomit during the entire observation period, the length of the observation period is used as the most conservative estimate of the latency period since retching or vomiting could have occurred immediately after the recording device was turned off. For the animals that did not retch or vomit more than once during the observation period, the duration of retching or vomiting was assigned as 0 min since no additional retching or vomiting events were observed for these animals. These value assignments were made to facilitate statistical analyses, since a non-numeric value for the latency period or duration would not be amenable to statistical analyses.

The results for the measured endpoints (described above) of each γ -ray or proton irradiated group were compared with the respective control results. Due to the extended time period required for the radiation exposure at the LDR (up to 4 h to deliver a 2 Gy dose at the dose rate of 0.5 Gy/h), the results of the groups irradiated at the LDR were compared to the control results over the same length of observation time as the irradiated groups [e.g., 5 h for the 1 Gy LDR group (2 h for the radiation exposure followed by a 3 h observation period)]

and 7 h for the 2 Gy LDR group (4 h for the radiation exposure followed by a 3 h observation period)]. In other words, in efforts to normalize the time periods of observations between the experimental and control group to be compared, the 1 Gy LDR group (observed during radiation exposure of 2 h plus an additional 3 h observation period) was compared to a control group that was observed for 5 h, while the 2 Gy LDR group was compared to a control group that was observed for 7 h. Further, the 1 Gy HDR (irradiated for 2 min and restrained and observed for 5 h, as in the LDR group) was compared to the control group that was observed for 5 h. The results were also compared between the two types of radiation (γ rays and protons) at each radiation dose and dose rate evaluated. The fractions of animals that retched or vomited were compared by the Fisher's exact test. The effective doses of γ rays or protons required to induce retching or vomiting in 10% (ED_{10}), 50% (ED_{50}) and 90% (ED_{90}) of the animals were calculated using a Weibull nonlinear regression model. The results for the number of retching and vomiting events were analyzed by the Mann-Whitney test. This nonparametric statistical method was selected to avoid a possible violation of the normality assumption. A P value of <0.05 is considered statistically significant.

The RBE values for the proton irradiation to induce retching and/or vomiting were determined separately and in combination. The relationship between the number of retching/vomiting events and radiation dose was established by a nonlinear regression analysis using the radiation dose as the independent variable and the number of the retching/vomiting events as the dependent variable, using the equation $N = ae^{bD}$, where D is dose and a and b are fitted parameters. For each value of the retching/vomiting event count obtained from the proton-irradiated animals, a corresponding γ radiation dose required to produce the same value for the retching/vomiting event count was interpolated from the nonlinear regression line for the γ -irradiated animals, and the ratio of γ ray to proton radiation doses was calculated as the RBE value. The RBE values were plotted against proton radiation doses to illustrate the relationship between the RBE value and the proton radiation dose.

The method chosen to calculate RBE estimates is one of several models that could be used for the data reported here. As it is known that RBE values can vary with the dose of radiation used and that higher RBE values are often observed at lower doses of radiation. Thus, it is now recommended by NASA that RBE values be estimated for the entire range of doses evaluated so that accurate risk estimations can be calculated. The specific model used in this report for the analysis of data allows the estimation of a range of RBE values (denoted in the Results section as the confidence interval) over several radiation doses evaluated.

RESULTS

This study was undertaken to assess the effectiveness of proton irradiation to induce retching and vomiting using ferrets as an experimental model system and γ rays as the reference radiation. For the control group that was not irradiated, the fractions of animals that retched and vomited were 0.37 (7/19) and 0.05 (1/19), respectively. In two of the control animals, the first retching episode occurred within 15–20 min after being placed in the restraint chamber while for all other control animals, retching began 1.5–3 h after the start of the confinement. The ferret chamber temperatures were recorded before and after radiation exposure as well as during and at the end of the post-irradiation observation period. Prior to the irradiations, the average recorded temperature of an empty chamber was 70.0°F. The average temperature during the high and low dose rate observations was 75.8°F and at the end of observations, 74.9°F. The temperatures of the chambers did not vary significantly between preirradiation, during irradiation and post-irradiation periods nor did the temperature of the chamber differ significantly from room temperature.

HDR γ Rays

For the animals irradiated at the HDR, the fraction of animals that retched was 1.00, 0.50, 0.50, 0.65 and 1.00, respectively, for the 0.25, 0.5, 0.75, 1 and 2 Gy dose groups (Table 1). The results in the 0.25 and 2 Gy γ -ray groups were significantly greater than that of the control group, which was 0.37 ($P = 0.015$). ED₁₀, ED₅₀ and ED₉₀ could not be calculated for γ rays at the HDR because the fraction of animals retched did not increase monotonically with the radiation dose. The average number of retching events was 4.7, 2.7, 8.0, 13.2 and 42.0, respectively, for the 0.25, 0.5, 0.75, 1 and 2 Gy γ -ray groups (Fig. 1A), and the results in the 0.25 Gy and the two highest γ -ray groups were significantly greater than that of the control group, which was 0.7 ($P = 0.01$). The average retching latency period was 50.0, 175.5, 147.6, 31.9 and 23.6 min, respectively, for the 0.25, 0.5, 0.75, 1 and 2 Gy γ -ray groups (Fig. 2A). The latency periods in the 0.25 and 2 Gy HDR γ -ray groups were significantly shorter than that of the control group, which was 184.3 min, by the Mann-Whitney test ($P = 0.005$). The latency period in the 1 Gy HDR γ -ray group was significantly shorter than that of the control group by the Mann-Whitney test ($P = 0.033$). The average retching duration was 52.7, 21.9, 27.3, 14.8 and 68.8 min, respectively, for the 0.25, 0.5, 0.75, 1 and 2 Gy γ -ray groups (Fig. 3A), and the average retching duration in the 0.25, 1 and 2 Gy γ -ray groups was significantly longer than that of the control group, which was 11.5 min ($P = 0.016$). Since the statistically significant results for the fraction of animals that retched and the retching duration in the 0.25 Gy γ -ray group was not confirmed by the corresponding results of animals irradiated with γ rays at the next two higher doses of 0.5 and 0.75 Gy, 1 Gy was taken as the minimum dose of γ rays required to induce retching in ferrets.

The fraction of animals that vomited was 0.00, 0.00, 0.25, 0.47 and 1.00, respectively, for the animals irradiated with 0.25, 0.5, 0.75, 1 and 2 Gy of γ rays at the HDR (Table 2). The results in the 1 and 2 Gy γ -ray groups were significantly greater than of the results for the control group, which was 0.05 ($P = 0.006$). The ED₁₀, ED₅₀ and ED₉₀ for the HDR γ rays to induce vomiting were 0.60, 1.01 and 1.42, respectively (Table 3). The average number of vomiting events was 0.0 for the 0.25 and 0.5 Gy γ -ray groups and 0.3, 1.1 and 5.7 for the 0.75, 1 and 2 Gy γ -ray groups, respectively (Fig. 1C). The results in the 1 and 2 Gy γ -ray groups were significantly greater than that of the control group, which was 0.1 ($P = 0.004$). The average vomiting latency period was longer than 240 min for the 0.25 and 0.5 HDR γ -ray groups and 188.8, 147.3 and 23.2 min for the 0.75, 1 and 2 Gy (Fig. 2C) HDR γ -ray groups, respectively. The latency period in the 1 and 2 Gy γ -ray group was significantly shorter than that of the control group, which was 229.7 min, by the Mann-Whitney test ($P = 0.005$). The average vomiting duration was 0.00 for the 0.25 and 0.5 Gy γ -ray groups and 0.1, 5.2 and 22.4 min for the 0.75, 1 and 2 Gy γ -ray groups, respectively (Fig. 3C), and the average vomiting duration in the 1 and 2 Gy γ -ray groups was significantly longer than that of the control group, which was 0.0 min ($P = 0.019$). Since none of the vomiting related results for the 0.25, 0.5 and 0.75 Gy γ -ray groups reached statistical significance, 1 Gy was taken as the minimum dose of HDR γ rays required to induce vomiting in ferrets.

HDR Protons

For the animals irradiated with protons at the HDR, the fraction of animals that retched was 0.33, 0.33, 0.91, 0.86 and 0.88, respectively, for the 0.25, 0.5, 0.75, 1 and 2 Gy proton groups (Table 1). The results in the 0.75, 1 and 2 Gy proton groups were significantly higher than that of the control group ($P = 0.007$). The ED₁₀, ED₅₀ and ED₉₀ for the HDR protons to induce retching were 0.15, 0.48 and 1.02, respectively (Table 3). The average number of retching events was 2.0, 2.5, 11.5, 10.8 and 27.8 for the 0.25, 0.5, 0.75, 1 and 2 Gy proton groups, respectively (Fig. 1A), and the results in the three highest proton groups were significantly greater than that of the control group ($P = 0.001$). The average retching latency

period was 224.0, 182.6, 49.0, 63.7 and 49.3 min for the 0.25, 0.5, 0.75, 1 and 2 Gy (Fig. 2A) proton radiation groups, respectively. The average latency period in the three highest proton groups was significantly shorter than the control group, determined by the Mann-Whitney test ($P = 0.001$). The average retching duration was 19.6, 12.2, 23.8, 16.3 and 79.4 min, respectively, for the 0.25, 0.5, 0.75, 1 and 2 Gy proton groups (Fig. 3A), and the average retching duration in the 0.75, 1 and 2 Gy proton groups was significantly longer than that of the control group ($P = 0.010$). Since the results in the 0.5 Gy proton group did not significantly differ from the control groups and most of the retching-related results in the three highest proton groups were significantly different from the results in the control groups, 0.75 Gy was taken as the minimum dose of protons required to induce retching in ferrets.

The fraction of animals that vomited was 0.00, 0.00, 0.27, 0.64 and 0.82, respectively, for the animals irradiated with 0.25, 0.5, 0.75, 1 and 2 Gy of protons at the high dose rate (Table 2). The results in the 1 and 2 Gy proton groups were significantly greater than the control group ($P < 0.001$). The ED_{10} , ED_{50} and ED_{90} for the HDR protons to induce vomiting were 0.53, 0.89 and 1.22, respectively (Table 3). The ED_{50} for the HDR protons to induce vomiting was approximately 85% higher than the ED_{50} for the HDR protons to induce retching. The average number of vomiting events was 0.0 for the 0.25 and 0.5 Gy proton groups and 0.5, 2.0 and 4.1 for the 0.75, 1 and 2 Gy proton groups, respectively (Fig. 1B), and the average number of vomiting events in the 1 and 2 Gy proton groups was significantly greater than that of the control group ($P < 0.001$). The average vomiting latency period was longer than 240 min for the 0.25 and 0.5 Gy proton groups and 181.5, 109.0 and 55.6 min for the 0.75, 1 and 2 Gy (Fig. 2C) proton groups, respectively. The average latency period in the 1 and 2 Gy proton group was significantly shorter than that of the control group by the Mann-Whitney test ($P < 0.001$). The average vomiting duration was 0.0 for the 0.25 and 0.5 Gy proton groups and 0.1, 6.7 and 20.9 min for the 0.75, 1 and 2 Gy proton groups, respectively (Fig. 3C), and the average vomiting duration in the 1 and 2 Gy proton groups was significantly longer than that of the control group ($P = 0.001$). Since none of the vomiting related results for the 0.25, 0.5 and 0.75 Gy proton groups reached statistical significance, 1 Gy was taken as the minimum dose of protons required to induce vomiting in ferrets.

LDR, γ Rays or Protons

For the animals irradiated with γ rays or protons at the LDR, the fraction of animals that retched ranged from 0.27 to 1.00 (Table 1) and was significantly greater in the 0.25 and 1 Gy proton groups ($P = 0.024$) compared to the control group. ED_{10} , ED_{50} and ED_{90} could not be calculated for either γ rays or protons at the LDR because the fraction of animals retched or vomited did not increase monotonically with the γ or proton radiation dose. The average number of retching events ranged from 1.2 to 14.8 (Fig. 1B) and was significantly higher in the 0.25 and 1 Gy proton group ($P = 0.002$) as compared with the control group. The average retching latency period ranged from 39.5 to 339.6 min and was significantly shorter only in the 0.25 and 1 Gy LDR proton groups by the Mann-Whitney test (Fig. 2B, $P = 0.008$) compared with the control group. The average retching duration ranged from 4.6 to 81.2 min (Fig. 3B) and was significantly longer in the 0.25 Gy γ -ray group as well as the 0.25 and 1 Gy proton groups ($P = 0.013$) compared with the control group. The statistically significant results observed in the groups irradiated with 0.25 and 1 Gy of γ rays or protons at the LDR were not confirmed by the corresponding results observed in the next higher γ -ray or proton radiation groups.

The fraction of animals that vomited ranged from 0.00 to 0.57 for the γ -ray or proton dose groups irradiated at the LDR (Table 2). With exception of the 1 Gy γ -ray group, the fraction of animals that vomited was not significantly different for any of the LDR γ -ray or proton

irradiated groups as compared with the control group ($P = 0.105$). The average number of vomiting events ranged from 0.0 to 0.9 and appeared to increase with the γ -ray or proton radiation dose (Fig. 1D) but the difference did not reach statistical significance as compared with the control group ($P = 0.060$). With exception of the 1 Gy γ -ray group, the vomiting latency period (Fig. 2D) and duration (Fig. 3D) for animals irradiated with γ -rays or protons at the LDR did not significantly differ from the results in the control groups ($P = 0.100$ and 0.061 , respectively) for the LDR γ -ray or proton irradiated groups, although the vomiting latency period was shortened and the vomiting duration was extended in a few animals irradiated with LDR γ -rays or protons at doses of 0.75 Gy or higher.

γ Rays vs. Protons

For comparison between matching γ -ray and proton groups at each dose and dose rate, the differences in results were generally not statistically significant for the retching and vomiting-related endpoints evaluated. The only exceptions were the differences in the fraction of animals that retched for the 0.25 Gy groups irradiated at the HDR (Table 1, $P = 0.013$), the average number of retching events for the 0.25 Gy groups irradiated at the HDR (Fig. 1A, $P = 0.027$), and the average retching latency period for the 0.25 Gy groups irradiated at the HDR (Fig. 2A, $P < 0.001$). None of these significant differences were confirmed by the corresponding results in the higher radiation dose groups.

RBE

For the HDR results, the RBE values were calculated separately for the numbers of retches (Fig. 4A) and vomits (Fig. 4B). The fitted RBE values for the HDR proton irradiation were 0.6, 0.7, 0.8, 0.8 and 0.6, respectively, for 0.25, 0.5, 0.75, 1 and 2 Gy of proton doses using number of retches as the endpoint. The 95% confidence interval for the RBE included 1.0 in the proton radiation dose range of 0.25 to 2 Gy evaluated. The fitted RBE values for the HDR proton irradiation were 0.0 (−0.1 but a negative RBE value is meaningless), 0.3, 0.7, 0.9 and 0.8, respectively, for 0.25, 0.5, 0.75, 1 and 2 Gy of proton doses using number of vomits as the endpoint (Fig. 4B). The 95% confidence interval for the RBE included 1.0 for the proton radiation dose of 1 Gy or higher, but the upper limit of the 95% confidence interval was below 1.0 for proton radiation doses of 0.25, 0.5 and 0.75 Gy.

RBE values for HDR proton radiation were also determined using the numbers of retches and vomits combined as the endpoints. The fitted RBE values for the HDR proton irradiation retches and vomits combined were 0.7, 0.8, 0.9, 0.9 and 0.7, respectively, for 0.25, 0.5, 0.75, 1 and 2 Gy of proton doses (Fig. 4C). The 95% confidence interval for the RBE was relatively broad and included 1 in the HDR proton radiation dose range of 0.25 to 2 Gy evaluated.

The RBE values for LDR proton irradiation for retches or vomits were calculated but the results are not presented since the LDR results did not exhibit statistically significant differences when compared to the results from the appropriate control groups, which rendered the RBE analyses meaningless for the LDR data.

DISCUSSION

The objective of this study was to quantitatively assess the effectiveness of simulated solar proton radiation (compared with ^{60}Co radiation) to induce retching and vomiting, which could seriously impair the ability of astronauts to function in the space environment or even be life threatening. In accordance with previous experiments (12, 13), the event frequency, latency and duration of the retching and vomiting events are characteristics defining the prodromal period of ARS and are reported here. While others have evaluated proton-induced

retching and vomiting previously (14), the RBE for SPE-like proton irradiation on these behavioral endpoints has not been determined. A vomit is usually preceded by several retching responses, even though retching and vomiting can occur separately (14) and involve different sets of muscles (15). Since retching frequently precedes vomiting, it was also used as a biological endpoint so that the effects of the radiation on emesis induction could be determined with a relatively small number of animals. In the current study, all animals that vomited also retched, whereas some animals retched but did not vomit. Thus, retching occurred much more frequently than vomiting, which is consistent with the results of Andrews *et al.* (16) who observed a large ratio of retches to vomits after exposure of ferrets to varied doses of X rays. The minimum radiation dose required to induce significant changes in the vomiting-related endpoints in the present study is up to 33% higher than the minimum radiation dose required to induce significant changes in the retching-related endpoints, and the ED₅₀ for protons to induce vomiting is approximately 85% higher than the ED₅₀ to induce retching, indicating that retching is a more sensitive biological indicator of radiation exposure than vomiting. It is noted that while some of the retching-related endpoints (fraction of animals that retched, number of retching events per animal) were also observed in the control group and dose-dependent changes were observed more consistently for the vomiting-related endpoints than for the retching-related endpoints. Nevertheless, most of the retching-related endpoints (i.e., fraction of animals that retched and retching latency period and duration) displayed statistically significant changes in the groups irradiated with γ rays and protons at the HDR with doses equal to or higher than 0.75 Gy. Thus, retching is still a very useful biological indicator for the evaluation of radiation-induced emesis. However, retching also occurred in 7 out of 19 control animals that were not exposed to γ or proton radiation and the fraction of animals that retched did not increase monotonically with the dose of γ rays at HDR in the present study (Table 1), indicating that retching is probably a less reliable biological indicator of radiation exposure and more prone to experimental variation than vomiting.

Behavioral studies in response to radiation have shown results indicating that increasing or decreasing dose does not always produce a linear response, but more of a monotonic response (77). The data reported here suggest that the retching response does not display a normal distribution of the data. For example, the HDR γ -ray and LDR proton exposures resulted in higher retching counts at the 0.25 and 2 Gy doses (γ ray) and 0.25 and 1 Gy doses (protons), compared to the controls and intermediate doses (0.5 and 0.75 Gy). Neuronal responses detected by electrophysiological methods have been shown to produce a similar trend in terms of nonlinear responses to radiation dose (18). The retching response is clearly sensitive and may be a behavioral response evoked by anatomical and neural circuitry elements (i.e., receptor, neurotransmitter or pathway activation), which may be sensitive to high or low doses of radiation with intermediate doses resulting in a less robust response.

Since ferrets are outbred, larger sample groups may delineate the interindividual response to radiation observed. The heterogeneity of the population may also explain the range of the retching and vomiting response. The reason why the incidence of retching was observed more frequently than the incident of vomiting and why the episodes lasted longer cannot be explained and may be due to the pathophysiological mechanisms of radiation-induced retching and/or vomiting, which are not clearly defined. It has been reported that resection of the abdominal vagus nerve in ferrets prevents the emetic response only in response to exposure to ionizing radiation and cytotoxic drugs, but not to morphine or apomorphine (19). This observation suggests separate mechanisms of activation of the emetic response. It is not unlikely that while different toxins stimulate different mechanisms to evoke the emetic response, the mechanisms of the retching and vomiting responses may at least be partially separate; however, it is not clear how a range of clinical stimuli and specifically various doses of radiation exposure, impacts the emetic response. Further research is warranted in

this field to delineate the mechanisms attributing to the sensitivity of ferrets to ionizing radiation.

The results of the present study have clearly demonstrated that HDR γ rays and protons are effective in inducing retching and vomiting, as evidenced by the increase in the fraction of animals that retched and/or vomited, the number of retches or vomits observed and the duration of retching or vomiting, as well as the decrease in the latency period for retching or vomiting. A similar but much weaker and less consistent trend was also observed for animals irradiated with γ rays and protons at the LDR, indicating a clear dose-rate effect of γ rays and protons in inducing retching and vomiting. Since the SPE radiation dose rate is expected to be similar to or lower than the LDR used in these studies, and far below the HDR used in these studies, the results presented here suggest that the risk of space radiation-induced vomiting is low and may reach statistical significance only when the radiation dose reaches 1 Gy or higher.

The estimated RBE value for the HDR proton irradiation ranged from 0.6 to 0.8 for retching and 0.0 to 0.9 for vomiting in the dose range evaluated in this study and the 95% confidence interval for the RBE included 1.0 for retching in the proton radiation dose range of 0.25 to 2 Gy evaluated and for vomiting in the proton radiation dose range of 1 to 2 Gy. These results indicate that HDR proton irradiation is generally not more effective than HDR γ irradiation in inducing either retching or vomiting. These results are corroborated by the observations that the minimum doses required to induce significant changes in the retching and/or vomiting related biological endpoints were similar between γ rays and protons delivered at the HDR.

The estimated RBE values for the LDR proton irradiation showed a dose-dependent decline from 3.7 to 0.4 for retching in the 0.25 to 2 Gy proton dose range evaluated in the study, and the lower limit of the 95% confidence interval was greater than 1 for the two lowest proton doses (0.25 and 0.5 Gy) used in the study. These results suggest that LDR proton irradiation might be significantly more effective than LDR γ irradiation in inducing retching at the low end of the proton dose range evaluated (< 0.5 Gy); however, these results should be interpreted with caution and need to be confirmed with a large sample size since the statistically significant changes in some of the retching related biological endpoints in the treatment groups irradiated with 0.25 and 1 Gy γ rays and/or protons at the LDR were not confirmed by the corresponding results in the treatment groups irradiated with the higher doses (0.75 and 2 Gy) of γ rays and/or protons delivered at the LDR. In contrast to the downward trend of RBE determined in animals irradiated at the LDR using the number of retches as the end point, the RBE value determined in the same animals using the number of vomits as the endpoint did not differ significantly from 1.0 and did not show a dose dependent trend.

The photon energy spectrum was evaluated taking into consideration the effect of the steel plating that was used for the LDR γ -ray experiment. Higher energy photons tend to lose more energy to electrons by Compton scattering, and so the net effect downstream of the steel plating is that the photon energy spectrum will be shifted to lower energies. A Monte Carlo simulation of the ^{60}Co irradiation experiments was performed and it was found that the mean photon energy for the HDR experiment is 1.0 MeV [similar to ref. (20)] and for the LDR experiment, 0.8 MeV. The RBE of ^{60}Co and ^{137}Cs photons is defined by the International Commission on Radiation Protection (ICRP) as 1 (21) and this is supported by experimental observations of DNA damage (22). Therefore, the experimentally observed differences in emesis observed in our experiments cannot be explained by the difference in RBE between the LDR and HDR exposures.

Ferrets have previously been used to study emesis induced by radiation with (^{60}Co) rays (12, 23), 0.6 GeV/n iron ions, neutrons (23) and 200 MeV protons (24, 25), and the emetic response in the ferret was found to be dependent on the type and dose of radiation. High-LET iron particles and fission neutrons were found to be similar in producing an emetic response (retching or vomiting) in ferrets with an ED₅₀ of 0.35 and 0.40 Gy, respectively (23), whereas γ rays were shown to be intermediately effective with an ED₅₀ of 0.77 Gy (12) to 0.95 Gy (23) and high energy electrons were the least effective, with an ED₅₀ of 1.38 Gy (23). The ED₁₀, ED₅₀ and ED₉₀ values estimated for fraction of animals that vomited after proton irradiation at HDR were comparable to the ED₁₀, ED₅₀ and ED₉₀ values after irradiation at HDR in the current study (Table 2) or at a dose of rate of 1 Gy/min reported previously (12), since the ED₁₀, ED₅₀ and ED₉₀ for HDR proton irradiation were all within the ranges defined by the upper and lower limits of the 95% confidence intervals for the respective ED₁₀, ED₅₀ and ED₉₀ values for γ irradiation. The ED₁₀ and ED₅₀, but not the ED₉₀, values estimated in the present study for the fraction of animals that retched (or vomited) after irradiation with protons at the HDR were smaller than the lower limits of the respective 95% confidence intervals previously reported for γ rays (12), which could suggest that HDR proton irradiation was more effective than HDR γ irradiation in inducing retching and vomiting.

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FIG. 1.

Number of retches and vomits observed in ferrets irradiated with γ rays and protons. The ferrets were irradiated with γ rays and protons at the high (0.5 Gy/min) or low (0.5 Gy/h) dose rate. The animals irradiated at the high dose rate were observed for at least 3 h after irradiation to record retching (panel A) and vomiting (panel B) events. The ferrets irradiated at the low dose rate were observed for at least 4 h after irradiation to record retching (panel C) and vomiting (panel D) events. The results were compared between each irradiated group and the corresponding control group by the Mann-Whitney Rank Sum test. The statistical significance of the differences are indicated with * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

FIG. 2.

Retching and vomiting latency period observed in ferrets irradiated with γ rays and protons. The animals irradiated at the high dose rate were observed for at least 3 h after irradiation and the latency periods before the first retching (panel A) and vomiting (panel B) events were recorded for each animal. The ferrets irradiated at the low dose rate were observed for at least 4 h after irradiation and the latency periods before the first retching (panel C) and vomiting (panel D) events were also recorded for each animal. The results were compared between each irradiated group and the corresponding control group by the Logrank test, as described in the legends for Figs. 2– 6. The statistical significance of the differences are indicated with * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

FIG. 3.

Retching and vomiting duration observed in ferrets irradiated with γ rays and protons. The animals irradiated at the high dose rate were observed for at least 3 h after exposure and the durations between the first and last events of retching (panel A) and vomiting (panel B) were recorded for each animal. The ferrets irradiated at the low dose rate were observed for at least 4 h after exposure and the durations between the first and last events of retching (panel C) and vomiting (panel D) were also recorded for each animal. The results were compared between each irradiated group and the corresponding control group by the Mann-Whitney Rank Sum test. The statistical significance of the differences were indicated with * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

FIG. 4.

RBEs of proton irradiation determined in ferrets. The RBE values were calculated separately for the numbers of retches (panel A) or vomits (panel B) as well as the combined number of retches and vomits (panel C). The ferrets were irradiated with γ rays and protons at the high dose rate. The numbers of individual retches (panel A), individual vomits (panel B), or combined retches and vomits (panel C) were plotted against radiation dose, and the relationship between the number of retching and vomiting events and radiation dose was established for γ rays by a nonlinear regression analysis. The fitted number of retches or vomits for γ rays was calculated from the nonlinear regression curve at a dose interval of 0.05 Gy. The dose of γ rays (D_{γ}) required to produce the same biological effect observed in each proton irradiated animal was calculated by linear approximation using the fitted number of retches and vomits for γ radiation. The relatively small dose interval used to calculate the fitted survival values for γ radiation was selected empirically to assure acceptable precision of the linear approximation. The RBE value for each animal irradiated with protons at a given dose (D_{proton}) was determined using the following equation: $\text{RBE} = D_{\gamma} / D_{\text{proton}}$. The RBE values were plotted against the proton radiation dose and analyzed by nonlinear regression analysis using a quadratic model to show the relationship between RBE value and proton radiation dose.

TABLE 1
Comparison of Results for the Fraction of Animals that Retched after Irradiation by the Fisher's Exact Test

Radiation dose (Gy)	Dose rate	Irradiated with rays		Irradiated with protons		Protons vs. rays (<i>P</i> value)
		Fraction of animals that retched	<i>P</i> value	Fraction of animals that retched	<i>P</i> value	
0 (Control)		0.37 (7/19)	N/A	0.37 (7/19)	N/A	
0.25	0.5 Gy/min	1.00 (6/6)	0.015	0.33 (4/12)	1.000	0.013
0.5	0.5 Gy/min	0.50 (3/6)	0.653	0.33 (2/6)	1.000	1.000
0.75	0.5 Gy/min	0.50 (6/12)	0.710	0.91 (10/11)	0.007	0.069
1	0.5 Gy/min	0.65 (11/17)	0.181	0.86 (19/22)	0.001	0.142
2	0.5 Gy/min	1.00(11/11)	<0.001	0.88 (15/17)	0.002	0.505
0.25	0.5 Gy/h	0.67 (4/6)	0.350	0.83 (10/12)	0.024	0.569
0.5	0.5 Gy/h	Not done	N/A	Not done	N/A	N/A
0.75	0.5 Gy/h	0.4 (4/10)	1.000	0.27 (3/11)	0.702	0.659
1	0.5 Gy/h	0.71 (5/7)	0.190	1.00 (10/10)	0.001	0.154
2	0.5 Gy/h	0.36 (5/14)	1.000	0.50 (6/12)	0.710	0.692

TABLE 2
Comparison of Results for the Fractions of Animals that Vomited after Irradiation by the Fisher's Exact Test

Radiation dose (Gy)	Dose rate	Irradiated with rays		Irradiated with protons		Protons vs. rays (<i>P</i> value)
		Fraction of animals that vomited	<i>P</i> value	Fraction of animals that vomited	<i>P</i> value	
0 (Control)		0.05 (1/19)	N/A	0.05 (1/19)	N/A	
0.25	0.5 Gy/min	0.00 (0/6)	1.000	0.00 (0/12)	1.000	N/A ^a
0.5	0.5 Gy/min	0.00 (0/6)	1.000	0.00 (0/6)	1.000	N/A ^a
0.75	0.5 Gy/min	0.25 (3/12)	0.272	0.27 (3/11)	0.126	1.000
1	0.5 Gy/min	0.47 (8/17)	0.006	0.64 (14/22)	<0.001	0.345
2	0.5 Gy/min	1.00 (11/11)	<0.001	0.82 (14/17)	<0.001	0.258
0.25	0.5 Gy/h	0.00 (0/6)	1.000	0.08 (1/12)	1.000	1.000
0.5	0.5 Gy/h	Not done	N/A	Not done	N/A	N/A
0.75	0.5 Gy/h	0.00 (0/10)	1.000	0.18 (2/11)	0.537	0.476
1	0.5 Gy/h	0.57 (4/7)	0.010	0.30 (3/10)	0.105	0.350
2	0.5 Gy/h	0.21 (3/14)	0.288	0.08 (1/12)	1.000	0.598

^aFisher's exact test could not be performed because the data contingency table is not a 2 × 2 square (no count in either the control or treatment group).

TABLE 3Dose of Gamma and Proton Radiation Required to Induce Retching or Vomiting in Ferrets^a

Effective dose of radiation	HDR		
	Retching and/or vomiting Protons	Vomiting only	
		rays	Protons
ED ₁₀ (95% CI)	0.15 (0.00–0.47)	0.60 (0.43–0.76)	0.53 (0.14–0.92)
ED ₅₀ (95% CI)	0.48 (0.16–0.81)	1.01 (0.91–1.12)	0.89 (0.69–1.08)
ED ₉₀ (95% CI)	1.02 (0.02–2.02)	1.42 (1.03–1.82)	1.22 (0.64–1.81)

^aED₁₀, ED₅₀ and ED₉₀ were not calculated for retching and/or vomiting induced by rays at HDR or for retching and/or vomiting or vomiting only induced by rays and protons at LDR because the fraction of animals retched and/or vomited or vomited only did not increase monotonically with radiation dose.