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Marked depression of radiation-induced emesis by olfactory bulbectomy or pre-exposure using low doses

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Abstract. Acute emetic response to relatively low-doses of X-irradiation on suncus (*Suncus murinus*) was examined. The behaviors recorded for each subject, using a video-cassette recorder system, were (1) the number of emesis, during exposure to a dose of 3.0 Gy; (2) emesis threshold. Results showed that the emetic threshold was observed at 0.85 Gy, and the number of radioemesis during exposure was 29. However, the observed threshold dose became 2.22 Gy following olfactory bulbectomy. The emetic number decreased significantly ($p < 0.01$), and reached a value one-fourth of the sham-control. The bulbectomized suncus showed a resistance to X-irradiation. Furthermore, I examined whether the animals could also acquire radio-resistance when they were subjected to a brief of dose X-rays (0.3 Gy) prior to a exposure to 3.0 Gy. Results showed that brief pre-exposure increased the observed threshold, a pattern that was exactly the same as shown in the bulbectomized animals. Increasing the pre-exposure dose further to 0.45–0.60 Gy, however, resulted in the complete disappearance of the effect. These results suggest that only the mice pre-irradiated with 0.30 Gy acquired resistance to radiation-induced emesis.
Key words. X-irradiation; suncus; olfactory bulbectomy; emesis; acute radiation sickness; radio-resistance.

Emesis, along with fatigue, is the most prominent human response to relatively low doses of X-rays, and its occurrence signifies the prodromal phase of acute radiation sickness[1]. At doses of 1.5 Gy, approximately 50% of humans experience nausea and vomiting, while at 3.0 Gy the figure approches 100%[2,3]. Work in this area has been limited to larger animals such as dogs, cats or non-human primates, because laboratory rodents do not vomit. It has been suggested that dogs may be more suitable animal model for comparison with humans, given their similar emetic thresholds to radiation (human 1.8 Gy; dog, 1.5–1.7 Gy)[4–7].

Recently, however, the suncus (*Suncus murinus;* musk shrew, ord. insectivora) has been proposed as a new animal model for research into vomiting. It has been reported that the suncus, which are considered to be closer to primates than rodents, showed dose-dependent emesis when they were given drugs such as nicotine bitartrate or veratrine sulafate by subcutaneous injection[8]. But no report has dealt with changes in emesis behavior induced by low-dose radiation.

We recently reported that brief exposure to X-rays served as an arousal stimulus in mice[9]. Exposure to X-rays (0.04 Gy) induced an immediate change in EEG pattern from low-frequency and high-amplitude (sleep) to high-frequency and low-amplitude (arousal). In olfactory bulbectomized mice, no arousal response could be observed. These results indicate clearly that the olfactory bulb plays an important role in the immediate detection of X-rays.

It would be interesting to examine whether the suncus could show an emesis response to radiation, and if so, whether frequency of radioemesis would change in suncus lacking olfactory bulbs.

Materials and methods

Male suncus (6 weeks old; body weight, 40–43 g) were obtained from Clea Japan Co. Ltd. They were housed in polycarbonate grouped cages (3 mice/cage) for 4 weeks prior to the beginning of the test, in an enviromentally controlled room (temperature: 24 ± 1 °C, humidity: 50 ± 10%), with a lighting schedule of 0.7:30–19:30 hr. Food and water were given ad libitum. The suncus were subjected to a whole-body X-ray exposure of 3.0 Gy with an X-ray machine (MBR 1505-R2, HITACHI) at a dose rate of 0.15 Gy/min (150 kVp, 20 mA, 0.3 mm copper + 0.5 mm aluminium filters). Output was measured with an ionizing chamber (MZ-BD-1, HITACHI). Sham-irradiated animals were treated similarly to the irradiated ones except for receiving no X-ray exposure. The behaviour recorded using a video-cassette recorder system for each subject was (1) the number of emesis and/or retching (nonproductive emesis) during exposure to a dose elevated to 3.0 Gy; (2) the emesis threshold; (3) any other behavior that might be considered a consequence of radiation exposure. Video tapes were scored by two observers who were unaware of the treatment condition of the animals. Statistical analysis of all experimental data was performed by Fisher's exact test.

Table 1. Depressive effects of olfactory bulbectomy on radiation-induced emesis.

	Number of emesis during exposure	Emetic threshold (Gy)	Other observation: number of yawns during exposure
Sham-control suncus (10)	28.8 ± 1.0	0.85 ± 0.04	12.2 ± 1.3
Olfactory bulbectomy (10)	7.7 ± 1.0**	2.22 ± 0.10**	3.9 ± 0.7**

Figures in parentheses represent the number of suncus used. Values are expressed as mean ± S.E. ** significant ($p < 0.01$) when compared with sham-control.

Olfactory bulbectomy. The bulbectomy was done under sodium pentobabital anesthesia by aspirating the bulbs described by Whitten[10]. The cavity was then packed with Gelform, the scalp sutured, and animals were returned to their cages. The surgical shams (normal) received the same anesthesia, incision and bone drilling but the drilling was terminated immediately prior to penetrating the bone.

Pre-exposure using low doses. Many methods of acquiring radio-resistance have been reported. A brief dose of X-rays prior to a second exposure to a sublethal dose, for example, induced resistance to radiation such as inhibiting radiation-induced immunosuppression (enhancing immune-activity)[11-13] or decreasing radiation-induced cancer[14,15]. Therefore we examined whether pre-exposure could induce the same effects as bulbectomy.

Experimental procedure was the same as described in table 1, except that the suncus was subject to split pre-exposures of 0.15 Gy (0.05 Gy/day, 3 consecutive days), 0.30 Gy (0.05 Gy × 6 days), 0.45 Gy (0.05 Gy × 9 days) or 0.60 Gy (0.05 Gy × 12 days) with an X-ray machine at a dose rate of 0.15 Gy/min. 3.0 Gy was then given as a second trial 24 h after the final pre-exposure. Sham-control animals were treated similarly to the irradiated ones, except for receiving no pre-exposure.

Results

Depressive effects of radiation-induced emesis by olfactory bulbectomy. Table 1 summarizes the results of the experiments in which suncus were exposed to X-rays and the effects of olfactory bulbectomy were examined. The observed threshold dose for vomiting was 0.85 Gy. The number of radioemesis was 29 during exposure to 3.0 Gy. The vomiting always began with an oscillatory movement of thoracic and abdominal parts, and then the suncus opened its mouth widely and expelled its

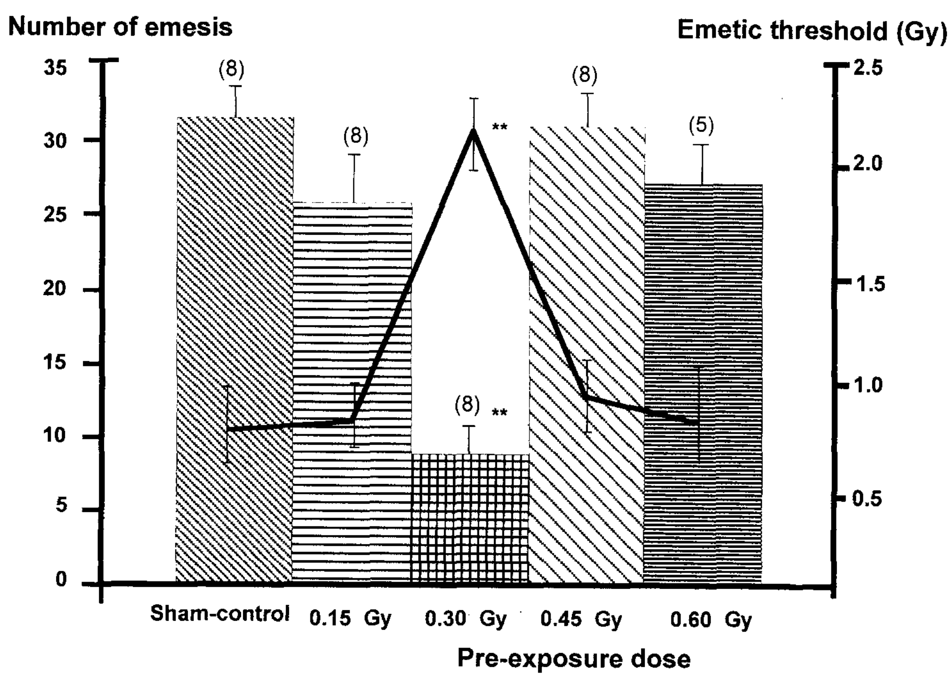

Figure 1. Depressive effects of pre-exposure using low doses on radiation-induced emesis following a 3 Gy challenging dose. Bar chart represents number emesis. Line graph, emetic threshold. **significant ($p < 0.01$) when compared with sham control. Figures in parentheses are numbers of suncus used.

gastric content. The amount of expelled vomitus was the largest for the first vomiting and then decreased sharply, which may reflect the emptying of the stomach. Furthermore, the suncus showed a marked suppression of spontaneous movement (general malaise) before the start of the first vomiting.

The effects of the bulbectomy were remarkable. The observed threshold dose of bulbectomized suncus became 2.22 Gy. The emetic number decreased significantly ($p < 0.01$), and reached a value one-fourth of the sham-control.

General behavior was no different between the control and bulbectomized animals. Only yawning, one of the autonomic signs, decreased signilcantly ($p < 0.01$). These results indicate a distinct loss of radioemesis when the olfactory bulbs, serving as a dectector of low-dose X-rays, are removed. The bulbectomized suncus, that is, showed a resistance to X-irradiation.

Depressive effects of radiation-induced emesis by pre-exposure to low doses of radiation. As shown in fig. 1, the number of radioemesis in 0.15 Gy split pre-exposure groups tended to be slightly lower than that in the sham-control, but the difference was not statistically significant. This tendency became more pronounced when the pre-exposure dose was raised to 0.30 Gy. The emetic number decreased markedly ($p < 0.01$), and the observed threshold was prolonged compared to the sham-control, showing a pattern that was exactly the same as that shown in the table 1.

Increasing the pre-radiation dose further to 0.45–0.60 Gy, however, resulted in the complete disappearance of the protective effects against emesis. In other words, the irradiated animals showed no differences from the control. These results suggest that only suncus pre-irradiated with 0.30 Gy could acquire resistance to radiation-induced emesis.

Discussion

The most important finding reported here is that the suncus shows an emetic response to relatively low doses of radiation. The suncus has a lower emetic threshold than other large animals, such as dogs, although the threshold dose of ferrets reported by King[16] are similar to the values of suncus shown here.

The mechanisms underlying radiation-induced emesis are poorly understood. The emetic chemoreceptor trigger zone, known as the vomiting control system, is located in the area postrema of the lower brain stem[17]. For example, in dogs[18] it has been reported that integrity of the area postrema is critical but that vagal integrity is not. In cats it is reported that the vagus is the primary pathway for radioemesis[19]. Irritation of the mucosa of the upper gastrointestinal tract also causes vomiting. Impulses are relayed from the mucosa to the vomiting center over visceral afferent pathways in the sympathetic nerves and vagi. Other afferents presumably reach the vomiting center from the dicephalon and limbic system, because emetic responses to emotionally charged stimuli also occur. Thus, we speak of 'nauseating smells'. The olfactory bulb, interestingly, has a direct fiber-access to the limbic system[20]. The results shown in table 1 indicate a marked decrease in vomiting behavior when the olfactory bulbs are removed. The olfactory bulbs of suncus might therefore play an important role in detection of X-rays. I think from these results that olfactory bulbectomy could induce subtle changes in neurotransmission to the limbic system, resulting in depression of vomiting behavior.

The other interesting finding is that the effect of pre-exposure on emetic behavior can be seen only within the lower dose range (0.30 Gy), but not at relatively higher doses (0.45–0.60 Gy). These results suggest that the depression in emetic behavior is limited to animals pre-irradiated with smaller doses, and therefore show no correlation with radiation dose. There are many reports indicating that a brief dose of X-rays prior to a second sublethal exposure can induced resistance to radiation, for example inhibiting radiation-induced cancer[14,15]. In recent years concerns over the novel effect of low doses of X-rays have grown. This phenomenon is called the hormetic effect. 'Hormesis' is the name given to the stimulating effect of small doses of substances which in larger doses are inhibitory[21–23]. This term, however, need not be limited to stimulation but may also be applied to any physiological effects which occurs at low doses and which cannot be anticipated by extrapolating from the toxic effects noted at high doses. The fact that only the mice pre-irradiated with 0.30 Gy (0.05 Gy × 6 days) could acquire resistance to radiation-induced emesis might be related to stimulatory effects on the biological defense system.

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