Short Communication

Circadian Variation in Lung Tumor Induction with X-rays in Mice

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Many studies on the acute deaths of rodents with ionizing radiation given at various times of day exist in the literatures¹-⁵). The mean survival time of mice exposed to X-rays at night has been reported to be shorter than that of mice exposed in the daytime²). The reason for this fact has been revealed by two hypotheses that (1) rodents are most sensitive during periods of increased bone marrow mitotic activity²), and (2) a daily variation of the content of endogenous radioprotectors is included in the variation of sensitivity⁵). Both hypotheses imply that not only bone marrow cells but also other cells are sensitive at night. On the other hand, circadian rhythm in tumorigenicity has not been studied so far. In the present study we investigated circadian variation in tumorigenesis with X-rays. The C3H lung tumor system was used. Circadian variation of alveolar injury by X-rays was also studied. The alveolar injury was judged by the degree of proliferation of epithelial cells, since proliferation of epithelial cells is a common response to alveolar injury⁶). ³H-labeled thymidine (³H-TdR) was utilized to measure the proliferation of alveolar cells.

The animals used were SPF C3H/HeSle male mice purchased from Shizuoka Laboratory Animal Cooperative Association, Japan. The mice purchased at 6 weeks of age had been kept in conventional condition in our laboratory. They were given unrestricted access to water and MB-1 chow (Funabashi Farm Co., Japan). The light cycle of the animal room was 12-hour light and 12-hour dark and the lights were turned on at 6:00 a.m. Four animals at 8 weeks of age were simultaneously irradiated by using a Toshiba KXC-18 X-ray source operated at 170kVp and 25mA with 0.5mm Cu and 0.5mm Al filter at a dose rate of 0.87Gy/min. The exposure was localized to the thoracic region by shielding other region with a 5-mm thick lead. Prior to irradiation, the animals were anesthetized with pentobarbital (10mg/kg). The mice were divided into two groups and exposed to 10Gy at 2:00 or at 14:00. A part of animals were autopsied.
to measure the labeling index of alveolar cells every 5-day until 20 days following irradiation. The mice were dosed with single ip injection of $^{3}$H-TdR (2mCi/kg) on the day assigned to kill. $^{3}$H-TdR was injected at 2:00 to the mice irradiated at 2:00, and at 14:00 to the mice irradiated at 14:00. The mice were killed by anesthesia 90 minutes after the injection. The lungs were fixed with formalin and embedded in Paraplast Plus (Lancer, St. Louis). The left lung was cut to the sections with 3 μm in thickness. The sections were mounted on glass slides and coated with NR-M2 emulsion (Sakura). The sections were kept for 14 days in light-tight boxes at 4°C, and then stained with hematoxin-l-eosin. In each lung, about 10,000 alveolar epithelial cells were counted, and the percentage of cells with labeled nuclei (labeling index) was calculated. The other animals were autopsied 12-15 months after irradiation. Their lungs were fixed and embedded as the same manner with autoradiography. Semi-serial 5 μm sections were collected from whole lung at intervals of 100 μm in thickness and stained. The tumors were counted to calculate both the percent incidence of tumors and the number of lung tumors per mouse. The statistical comparison were made by using $\chi^2$ test for the incidence and t-test for the number of tumors per mouse.

The initial number of mice used and the number of mice surviving until the time of sacrifice in each experimental group are shown in Table 1. No animal was lost due to X ray-induced acute injury or due to lung injury in any groups. Therefore, the experimental results were not substantially influenced by the level of mortality. The effects of time at irradiation on the percent tumor incidence and the number of tumors per mouse 12-15 months after the treatments are also shown in Table 1. Both the percent tumor incidence and the number of tumors per mouse irradiated at the daytime were significantly higher than those of non-irradiated mice. Furthermore significant difference was seen in both the percent tumor incidence and the number of tumors per mouse between the groups irradiated at the daytime and at night. However, no significant difference was seen between the group irradiated at night and the control. As for the labeling index, following results were obtained (Fig. 1). The labeling index for control group killed at the daytime was 0.16% and that for control group killed at night was 0.18%. There is no statistically significant difference between the two control groups. On day 10, a significant increase in labeling index was seen in the mice irradiated at night. This pro-

**Table 1.** Lung tumor incidence in C3H male mice at 12-15 months after irradiation.

<table>
<thead>
<tr>
<th>Dose (Gy)</th>
<th>Time</th>
<th>No. of mice</th>
<th>Percent incidence</th>
<th>Tumors/mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>Initial 28</td>
<td>Surviving 26</td>
<td>7.7</td>
</tr>
<tr>
<td>10</td>
<td>14:00</td>
<td>36</td>
<td>35</td>
<td>31.4b</td>
</tr>
<tr>
<td>10</td>
<td>2:00</td>
<td>19</td>
<td>18</td>
<td>5.6</td>
</tr>
</tbody>
</table>

- **a**: Mean ± S.E.
- **b**: Significantly higher ($p < 0.1$) compared to control.
- **c**: Significantly higher ($p < 0.01$) compared to the group irradiated at 2:00.
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Fig. 1. Labeling index of alveolar cells. The mice were exposed to 10Gy of X-rays and were sacrificed at 14:00 (Day) or 2:00 (Night). Shaded areas show labeling index range (±S.E.) for non-irradiated mice. Each point represents the mean ±S.E.

Proliferative response may suggest that there happened a compensatory proliferation due to cell killing by irradiation.

It is known that spontaneous incidences of lung tumors are high in RFM\(^7\) and A/J\(^8\) mice. Because of the low incidence of spontaneous lung tumors in C3H, we could provide evidence for the tumorigenicity in X-irradiated mice with statistical significance. Present results suggest that tumorigenic effect of X-rays might vary with circadian rhythm (Table 1). Circadian rhythm of radiosensitivity for cell killing may also be suggested from the difference of proliferative response (Fig. 1). However it is not clear whether the proliferative response prevent the tumor induction or not. To study the mechanisms for the circadian rhythm in X-ray-induced tumorigenicity, radiation response of type II cells, which are thought to be an origin of lung tumor\(^9\), should be studied.
REFERENCES