Mongolian Gerbils are not Susceptible to Induction of Intestinal Metaplasia in Gastric Mucosa by X-Irradiation

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Abstract: This study was designed to determine whether intestinal metaplasia can be induced in Mongolian gerbils (MGS) by X-irradiation. Five-week-old animals of both sexes were X-irradiated with two X-ray doses of 10 Gy each at a 3-day interval (total dose: 20 Gy) and killed 24 or 60 weeks thereafter for histopathological examination. Intestinal metaplasia was not detectable in either 11 male or 10 female MGS at 24 weeks and only two lesions were encountered at 60 weeks after X-irradiation. One was type A in a female and the other of type B in a male. These results demonstrated that the glandular stomach of Mongolian gerbils is not susceptible to induction of intestinal metaplasia by X-irradiation.  

Key words: Mongolian gerbils, X-irradiation, histopathological examination, gastric lesion, intestinal metaplasia

Introduction

Although the overall incidence of gastric cancer has steadily declined over the past 50 years, it is still a major health problem in Japan and remains the second most common cancer in the world¹. Intestinal metaplasia (IM) in the human stomach is considered to be a possible precancerous state, on the basis of epidemiological surveys. However, while several authors have argued that it may play a role in the development of gastric carcinomas²–⁴, others have concluded no association⁵,⁶. Furthermore, in general its pathogenesis remains unclear.

We have reported that intestinal metaplasia can be induced in the glandular stomach of rats by X-irradiation⁷, males being more susceptible than females, along with strain and species differences⁸. It increases with age in humans⁹,¹⁰, but the frequency varies widely in different countries, regions, and races¹¹. Mongolian gerbils (MGS) are regarded as good model animals to study the relation of Helicobacter pylori with stomach neoplasia¹²–¹⁴. Watanabe et al¹⁵ even reported Helicobacter pylori induction of gastric tumors in this species. The main purpose of the present study was to determine whether the intestinal metaplasia can be readily induced by X-irradiation in MGS.

Materials and Methods

Animals

Five-week-old MGS/Sea were used in this experiment. A total of 58 animals were housed four or five to an autoclaved polycarbonate cage with sterilized wood chips for bedding, and kept under conditions of constant temperature (24 ± 5°C) and relative humidity (55 ± 10%) with a 12-h light/12-h dark cycle. They were fed a normal diet (MF; Oriental Yeast, Tokyo, Japan), received tap water ad libitum and were maintained according to the guidelines set forth in the Guide for the Care and Use of Laboratory Animals of Hiroshima University.

X-Irradiation

X-irradiation was performed by the method described previously¹⁶. Briefly, the mice were anesthetized with Nembutal (Dainippon Pharmaceutical CO., LTD. Lot 57988Z7) and placed under an X-ray beam. A 0.6 cm-thick lead cover, with a hole 0.9 cm in diameter, was positioned so that the hole lay over the gastric region of each gerbil. All animals were X-irradiated with two X-ray doses of 10 Gy each at a 3-day interval (total dose: 20 Gy). The X-ray air dose (in R) was then converted to absorbed dose (in cGy), using the factor of 0.95 cGy/R. The scattered dose to other organs was about 5% of this value.
Table 1. Body and Organ Weights (g) 24 Weeks after X-irradiation

<table>
<thead>
<tr>
<th>Group</th>
<th>Effective number</th>
<th>Body weight</th>
<th>Heart</th>
<th>Liver</th>
<th>Kidney</th>
<th>Testis or ovary</th>
<th>Uterus</th>
<th>Adrenal</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
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<tr>
<td>Control</td>
<td>6</td>
<td>93 ± 8</td>
<td>0.52 ± 0.17</td>
<td>3.79 ± 0.44</td>
<td>0.72 ± 0.05</td>
<td>0.03 ± 0.08</td>
<td>0.04 ± 0.01</td>
<td>0.08 ± 0.03</td>
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<td></td>
<td></td>
<td></td>
<td>(5.72 ± 1.96)</td>
<td>(40.80 ± 2.56)</td>
<td>(7.78 ± 0.57)</td>
<td>(11.07 ± 0.35)</td>
<td>(0.51 ± 0.15)</td>
<td>(0.88 ± 0.24)</td>
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</tr>
<tr>
<td>X-ray</td>
<td>5</td>
<td>107 ± 6</td>
<td>0.34 ± 0.03*</td>
<td>5.01 ± 0.50</td>
<td>0.74 ± 0.08</td>
<td>0.04 ± 0.03</td>
<td>0.06 ± 0.01</td>
<td>0.12 ± 0.07</td>
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<td></td>
<td>(3.19 ± 0.20)</td>
<td>(46.96 ± 3.25)</td>
<td>(6.96 ± 0.54)</td>
<td>(10.74 ± 0.51)</td>
<td>(0.56 ± 0.09)</td>
<td>(1.13 ± 0.67)</td>
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<tr>
<td>Females</td>
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<tr>
<td>Control</td>
<td>5</td>
<td>97 ± 10</td>
<td>0.31 ± 0.02</td>
<td>4.61 ± 0.93</td>
<td>0.60 ± 0.05</td>
<td>0.04 ± 0.08</td>
<td>0.10 ± 0.01</td>
<td>0.10 ± 0.01</td>
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<td></td>
<td>(3.18 ± 0.32)</td>
<td>(47.24 ± 5.28)</td>
<td>(6.29 ± 0.80)</td>
<td>(0.43 ± 0.09)</td>
<td>(1.07 ± 0.20)</td>
<td>(0.42 ± 0.17)</td>
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<tr>
<td>X-ray</td>
<td>5</td>
<td>72 ± 6*</td>
<td>0.28 ± 0.03</td>
<td>2.57 ± 0.51</td>
<td>0.47 ± 0.08</td>
<td>0.05 ± 0.01</td>
<td>0.09 ± 0.02</td>
<td>0.04 ± 0.01</td>
<td>0.07 ± 0.01</td>
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<td></td>
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<td></td>
<td>(3.88 ± 0.37)</td>
<td>(35.72 ± 5.09)</td>
<td>(6.58 ± 0.67)</td>
<td>(0.67 ± 0.14)</td>
<td>(1.31 ± 0.29)</td>
<td>(0.59 ± 0.08)</td>
<td>(1.01 ± 0.13)</td>
</tr>
</tbody>
</table>

( ): Relative organ weights (organ weight/body weight multiply 1000).
1Effective number: the number of animal surviving at termination.
*Significantly different from the control value (p<0.01).

Examination of animals

The animals were observed daily and weighed once a month throughout the experimental period. All survivor gerbils were killed under ether anesthesia at the termination of the experiment, 24 weeks or 60 weeks after the X-irradiation. The alimentary canal and other major organs (liver, kidney, adrenal, uterus, ovary, and spleen) were macroscopically examined and prepared for histopathological studies. The stomachs were cut open along the greater curvature, stretched, and pinned on cardboard with the mucosal surface facing upward. Each was washed with physiological saline before gross examination, and fixed in 10% neutral formalin. Alkaline phosphatase (ALP)-positive foci in the gastric mucosa were visualized by the naphthol-AS-MX-phosphate-fast blue RR staining method. The numbers of ALP-positive crypts in both the pylorus and fundus were then counted under a dissection microscope, employing a double-blind protocol.

Strips of stomach, cut perpendicularly to the mucosal surface, two through the lesser curvature and four through the greater curvature, were then routinely embedded in paraffin, serially sectioned at 3 µm, stained with hematoxylin and eosin and, where necessary by the periodic acid-Schiff (PAS)-alcian blue or high iron diamine (HID)-alcian blue staining method. The numbers of ALP-positive crypts in both the pylorus and fundus were then counted under a dissection microscope, employing a double-blind protocol.

Intestinal metaplasias were categorized according to the following histological criteria: type A, gastric mucosa with goblet cells; type B, intestinal type crypt without Paneth cells; and type C, intestinal type crypts with Paneth cells (ALP-positive foci).

Statistical analysis

The significance of differences in numerical data was evaluated by the Student’s t-test, the χ² test, and the Dunnett’s test.

Results

24 weeks

In the animals killed 24 weeks after X-irradiation, the mean body weights in male X-irradiated group were increased compared with the controls, but no significant difference, in the female X-irradiation group were significantly decreased compared with the controls, along with the heart weights in X-irradiated males. The other organs' weights did not differ among the groups (Table 1), and relative organ weights (organ weight/body weight multiply 1000) did not significantly vary among the groups. Stomach ulcers were observed in the 2/6 male of the control group. Intestinal metaplasia was not detected in either the 5 X-irradiated males or the 5 females.

60 weeks

The mean body weights and the organ weights in the X-irradiated animals killed after 60 weeks were not significantly different from the control group values (Table 2). Two intestinal metaplasia lesions were detected. One of type A in a female X-irradiated, and the other of type B in a male of the X-irradiated group. Other stomach lesions are also listed in Table 3. Fibrosis was observed in 3/6 male control, 5/12 in male X-ray, 3/6 female control, and 6/13 female X-irradiated gerbils. Calcification in the fundus was observed in 3/6, 10/12, 5/6, and 9/13, respectively. The lesions in the X-irradiation groups were not significantly different from those in the controls. Polyps were observed in the X-irradiation group, but they were benign lesion without atypical growth. Ovary and liver tumors were observed in females of the X-ray group. Fatty liver, liver and kidney cyst, and inflammatory cell infiltration were also noted, but there were no significant differences between X-irradiation and control groups.
Discussion

In the present experiment, MGS did not appear susceptible to induction of intestinal metaplasia by X-irradiation although the animals were maintained for 60 weeks after treatment, the same period as in an earlier Helicobacter pylori infection experiment. Thus the stomach mucosa sensitivity in this regard is clearly different between X-irradiation and Helicobacter pylori case in the gerbils.

In MGS, Helicobacter pylori infection, chronic active gastritis, peptic ulcers, and intestinal metaplasia closely mimic those in man. Helicobacter pylori infection, chronic active gastritis, peptic ulcers, and intestinal metaplasia closely mimic those in man. MGS is regarded as a model animal for studies of the relation of Helicobacter pylori to stomach cancer development. Induction of both gastric intestinal metaplasia and gastric cancer has been reported in MGS infected with Helicobacter pylori 60 weeks after infection. However, others published reports that infection alone appears insufficient because the majority of Helicobacter pylori-infected individuals do not develop gastric cancer. Shimizu et al suggested that no relationship between intestinal metaplasia and glandular stomach cancers induced by Helicobacter pylori infection and MNNG administration in MGS was found. We suggested that elevation of gastric juice pH due to the disappearance of parietal cells in the fundic gland mucosa is one of the principal factors responsible for the development of intestinal metaplasia. Recently, Kinoshita et al reported that subtotal resection of the fundus combined with X-ray irradiation is an effective induction protocol for intestinal metaplasia. So we considered that intestinal metaplasia is not a precancerous lesion and is reversible.

It can be said that the susceptibility of rats to the induction of intestinal metaplasia by X-irradiation is greatly influenced by the rat strains. It was easy to Donryu, SHR, SD rats, while their ACI counterparts are resistant, although sensitive to MNNG carcinogenesis, but resistant to AOM carcinogenesis. We considered that the basis for sensitivity might be different between chemicals and radiation. Intestinal metaplasia is not induced in mice by X-irradiation, similar to the MGS in this case. The reasons for the difference susceptibility to X-irradiation remain unclear. Further investigation of the factor determining this resistance is clearly warranted.

Acknowledgements: The authors thank Dr. M. A. Moore, Asian Pacific Organization for Cancer Prevention, for critical reading of manuscript and Mr. T. Nishioka and Ms.

Table 2. Body and Organ Weights (g) 60 Weeks after X-irradiation

<table>
<thead>
<tr>
<th>Group</th>
<th>Effective number</th>
<th>Body weight</th>
<th>Heart</th>
<th>Liver</th>
<th>Kidney</th>
<th>Testis or ovary</th>
<th>Uterus</th>
<th>Adrenal</th>
<th>Spleen</th>
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</thead>
<tbody>
<tr>
<td>Males</td>
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<tr>
<td>Control</td>
<td>6</td>
<td>102 ± 11</td>
<td>0.49 ± 0.13</td>
<td>5.36 ± 1.99</td>
<td>0.77 ± 0.08</td>
<td>1.16 ± 0.10</td>
<td>0.04 ± 0.01</td>
<td>0.10 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>X-ray</td>
<td>12</td>
<td>107 ± 13</td>
<td>0.43 ± 0.08</td>
<td>4.77 ± 1.86</td>
<td>0.84 ± 0.20</td>
<td>1.20 ± 0.11</td>
<td>0.06 ± 0.01</td>
<td>0.13 ± 0.04</td>
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<tr>
<td>Females</td>
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</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>80 ± 12</td>
<td>0.33 ± 0.06</td>
<td>3.18 ± 1.14</td>
<td>0.52 ± 0.11</td>
<td>0.03 ± 0.03</td>
<td>0.33 ± 0.11</td>
<td>0.03 ± 0.00</td>
<td>0.07 ± 0.02</td>
</tr>
<tr>
<td>X-ray</td>
<td>13</td>
<td>90 ± 10</td>
<td>0.34 ± 0.03</td>
<td>4.35 ± 0.91</td>
<td>0.58 ± 0.08</td>
<td>0.06 ± 0.02</td>
<td>0.12 ± 0.05</td>
<td>0.05 ± 0.01</td>
<td>0.11 ± 0.06</td>
</tr>
</tbody>
</table>

*: Relative organ weights (organ weight/body weight multiply 1000).
1Effective number: the number of animal surviving at termination.
*Significantly different from the control value (p<0.01).

Table 3. Lesions in Stomachs 60 Weeks after X-irradiation (%)

<table>
<thead>
<tr>
<th>Group</th>
<th>Effective number</th>
<th>Polyp</th>
<th>Erosion</th>
<th>Fibrosis</th>
<th>Calcification</th>
<th>Intestinal metaplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>3(50)</td>
<td>3(50)</td>
<td>0</td>
</tr>
<tr>
<td>X-ray</td>
<td>12</td>
<td>1(8)</td>
<td>0</td>
<td>5(42)</td>
<td>10(83)</td>
<td>1(8.3)a</td>
</tr>
<tr>
<td>Females</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>3(50)</td>
<td>5(83)</td>
<td>0</td>
</tr>
<tr>
<td>X-ray</td>
<td>13</td>
<td>1(8)</td>
<td>10(77)*</td>
<td>6(46)</td>
<td>9(69)</td>
<td>1(7.7)b</td>
</tr>
</tbody>
</table>

a: Type A. b: Type B.
1Effective number: the number of animal surviving at termination.
*Significantly different from the control value (p<0.01).
H. Hamada for their technical assistance.

References


