The Effects of Gut Commensal Bacteria Depletion on Mice Exposed to Acute Lethal Irradiation

Bing HOU, Zhi-Wei XU and Cheng-Gang ZHANG*

Gut/Total-body irradiation/Commensal bacteria.

The prevention and management of bacterial infection are the mainstays of therapies for irradiation victims. However, worries about adverse effects arise from gut commensal flora depletion owing to the broad-spectrum antibiotics treatment. In the present study, we investigated the effects of gut bacteria depletion on the mice receiving total-body irradiation (TBI) at a single dose of 12 Gy. One group of mice was merely exposed to TBI but was free of antibiotic treatment throughout the experiment, while the other two groups of mice were additionally given broad-spectrum antibiotics, either from 2 weeks before or immediately after irradiation. The survival time of each animal in each group was recorded for analysis. Results showed that the mean survival time of mice was longest in the group without antibiotic treatment and shortest in the group treated with broad-spectrum antibiotics from 2 weeks before TBI. In conclusion, our data suggested that depletion of gut commensal bacteria with broad-spectrum antibiotics seemed deleterious for mammals receiving lethal TBI.

INTRODUCTION

The potential threat of accidental or terroristic exposure to ionizing irradiation is still a serious social problem in the world. Many efforts have been devoted to deal with acute radiation syndrome in the past decades. It has been demonstrated that ionizing irradiation may suppress host defenses and increase susceptibility to local and systemic bacterial infections. If there was no anti-infection treatment, bacteremia resulting from endogenous organisms would be one of the major reasons of high mortalities, especially after total-body irradiation (TBI) at greater doses. Thereafter, it has been believed that control of bacterial infection probably facilitate survival of irradiation victims. In fact, the anti-infection therapy has been demonstrated to be beneficial for rodents receiving sublethal irradiation and even for human beings receiving lethal irradiation. However, as it was reported recently that recognition of commensal flora by toll-like receptors is required for intestinal homeostasis regulation, worries about adverse effects arise from gut bacteria depletion owing to the broad-spectrum antibiotics treatment. To investigate the effects of gut bacteria depletion on irradiated victims, the mice were exposed to lethal TBI and their survivals were assessed in relation with broad-spectrum antibiotics treatment in the present study.

MATERIALS AND METHODS

Experimental animals

Thirty male Kunming mice (~6 weeks old, 20 ± 3 g weight), an inbred strain developed from Swiss mice, were purchased from the Laboratory Animal Center, Beijing Institute of Radiation Medicine. The study was approved by the Institute’s Committee of Experimental Animal Care and all the regulations were observed. The mice were housed in the specific-parasite-free condition. All efforts were devoted to minimize the number of animals used as well as their suffering.

Grouping of the mice and antibiotic administration

The animals were randomly divided into three groups (n = 10), namely group A, B and C. The mice in group A were merely exposed to TBI without any antibiotic treatment throughout the experiment, while the mice in the other two groups were both exposed to TBI and treated with broad-spectrum antibiotics. To avoid possible mechanical injuries to the upper digestive tract of the animals, the antibiotics were delivered by dissolving them in drinking water other than by the intragastric catheter directly. To ensure depletion or substantial decrease of gut bacteria and their debris, the mice in group B received broad-spectrum antibiotics from 2 weeks before TBI (pre-TBI) and continued after TBI (post-TBI) till died, based on the previous reports. In detail,
the mice in group B were administered metronidazole (1 g/L, aiming at anaerobic bacteria), ampicillin (1 g/L, aiming at gram-positive and negative bacteria) and gentamicin (160 mg/L, aiming at gram-negative aerobacteria) in drinking water, from 14 d to 7 d before TBI. After having found that the mice disliked drinking water containing metronidazole, this group was then treated with the same recipe except that the concentration of metronidazole was reduced to 0.5 g/L, till the mice died. To exclude any possible synergic effect of TBI on anti-infectious treatment, the mice in group C were provided metronidazole (0.5 g/L), ampicillin (1 g/L) and gentamicin (160 mg/L) in drinking water immediately after TBI, until they died. Metronidazole, ampicillin and gentamicin were purchased from the Fourth Shijiazhuang Pharmaceutical Co., Ltd. (Shijiazhuang, China), Sigma (St. Louis, USA), and Jinling Pharmaceutical Co., Ltd. (Nanjing, China), respectively.

**TBI treatment**

The $^{60}$Co irradiator was used for TBI treatment. In detail, unanaesthetized mice were placed in well-ventilated plastic boxes 3 meters away from the irradiator and then a single dose of 12 Gy gamma ray radiations was delivered at the dose rate of 2.44 Gy/min. Each mouse was allowed to move freely during the irradiation. After TBI, the mice were released from the plastic boxes and allowed free access to food and water.

**Assessments on the survival of irradiated animals**

The total amounts of water consumption and food intake of the mice in each group were measured daily from 14 d pre-TBI to 3 d post-TBI. The body weight of each mouse was recorded every 2 days from 7 d pre-TBI to 3 d post-TBI. Diarrhea in each mouse was carefully examined to determine severe gastrointestinal syndrome during the study. The survival time of each animal receiving TBI was recorded exactly in hours for analysis. The difference among the mice in the three groups was analyzed using one-way ANOVA followed by the Dunnett comparison test with SPSS 13.0 for Windows (SPSS Inc, USA), where $P < 0.05$ was considered to be significant difference. To determine whether the survival time of irradiated mice could be predicted by body weight, the correlation between survival time of each mouse with its body weight at death was examined in each group.

**RESULTS**

The mice in group B were orally administered metronidazole (1 g/L), ampicillin (1 g/L) and gentamicin (160 mg/L) in drinking water from 14 d to 7 d before TBI, while the mice in the other two groups were free of any antibiotics. During this period, the average amounts of water consumption and food intake per day in group B significantly dropped down to 12 ml and 28 g respectively, only accounting for ~15% (12 ml/76 ml) and ~40% (28 g/69 g) of that in group A (Fig. 1a–b). Corresponding to this, the mice in group B failed to increase their body weights till 7 d before TBI while substantial increase in weight was found in the
other two groups (Fig. 1c). From 6 d pre-TBI up to death, the mice in group B were administered metronidazole (0.5 g/L), ampicillin (1 g/L) and gentamicin (160 mg/L). During one week before the TBI, the average amounts of both water consumption and food intake in group B increased remarkably, reaching 57 ml/d and 50 g/d, respectively (Fig. 1a–b). Correspondingly, the mice in group B grew gradually during this stage (Fig. 1c).

It was demonstrated that the TBI treatment severely suppressed daily water consumption and food intake and resulted in significant weight loss of the mice in all three groups (Fig. 1a–c). All the mice seemed to be normal in terms of regular activity and kept cleaning around their anus within 2 days after TBI, but explosive membranate diarrhea occurred in all mice from 3 d post-TBI. The mean survival time of the mice in group A was 214.3 ± 20.0 h. However, the value in group B was only 82.6 ± 13.3 h, which is significantly shorter than that in group A (P < 0.01). In contrast, the mean survival time of the mice in group C was 144.0 ± 41.5 h, which is significantly different from that in either group A or B (P < 0.01, respectively). In addition, neither positive nor negative correlation was found between survival time and body weight of the mice in each group (Table 1).

<table>
<thead>
<tr>
<th>Order of the mouse at death</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 1</td>
<td>19.5 (3/10)</td>
<td>21.1 (7/10)</td>
<td>20.6 (3/10)</td>
</tr>
<tr>
<td>No. 2</td>
<td>21.1 (5/9)</td>
<td>23.6 (7/9)</td>
<td>22.7 (8/9)</td>
</tr>
<tr>
<td>No. 3</td>
<td>19.0 (2/8)</td>
<td>16.4 (3/9)</td>
<td>22.1 (7/8)</td>
</tr>
<tr>
<td>No. 4</td>
<td>18.9 (3/7)</td>
<td>17.8 (4/9)</td>
<td>19.8 (2/7)</td>
</tr>
<tr>
<td>No. 5</td>
<td>16.5 (1/6)</td>
<td>10.0 (1/6)</td>
<td>21.2 (4/6)</td>
</tr>
<tr>
<td>No. 6</td>
<td>17.7 (1/5)</td>
<td>19.2 (3/5)</td>
<td>19.4 (2/5)</td>
</tr>
<tr>
<td>No. 7</td>
<td>20.6 (3/4)</td>
<td>23.1 (3/4)</td>
<td>20.0 (2/4)</td>
</tr>
<tr>
<td>No. 8</td>
<td>18.2 (2/4)</td>
<td>15.1 (1/3)</td>
<td>17.2 (1/3)</td>
</tr>
<tr>
<td>No. 9</td>
<td>20.3 (2/2)</td>
<td>16.9 (1/2)</td>
<td>19.8 (2/2)</td>
</tr>
<tr>
<td>No. 10</td>
<td>16.7</td>
<td>23.4</td>
<td>17.7</td>
</tr>
</tbody>
</table>

In each group (n = 10), the body weight of each mouse was shown in ascending order of the time at death. The numbers in parentheses showed the ascending order (data before the slash) of body weight of the certain mouse among the remainders (data after the slash) in each group. The mice in group A were exposed to TBI but without antibiotics treatment while those in group B and C were treated with broad-spectrum antibiotics from 2 weeks before and immediately after TBI respectively. a: The mice died at same time in group A; b: The mice died at same time in group B.

**DISCUSSION**

The anti-infection therapy has been demonstrated to be beneficial for rodents receiving sublethal irradiation and even for human beings receiving lethal irradiation. The present study aimed to investigate the possible effects of gut bacteria depletion on survival of the mice exposed to acute lethal TBI. Since combined use of broad-spectrum antibiotics for at least 2 weeks has been found to be capable to deplete intestinal flora and their debris, the mice in group B were treated with metronidazole, ampicillin and gentamicin (M/A/G) in drinking water for a week in which metronidazole was provided at dose of 1 g/L. However, the mice seemed to dislike the water containing metronidazole, probably because of the heavily bitter taste. As a result, the average water consumption per day in this group sharply dropped down to the level only accounting for approximately 15% of that in the antibiotics-free group. We continued providing these mice with M/A/G treatment for another week and had to reduce the concentration of metronidazole to 0.5 g/L. In general, it is not advisable to reduce the concentration of an antibiotic throughout the whole course of experiment. By this means, however, the average water consumption in this group was increased by 4–5 times, which was believed that the mice in group B had taken enough antibiotics for gut flora depletion. As metronidazole at concentration of 1 g/L suppressed water consumption of the mice and caused growth stagnancy, one may doubt that the M/A/G treatment itself would be deleterious for survival of the mice. Although we could not completely exclude this possibility, combinative use of metronidazole (0.5 g/L), ampicillin (1 g/L) and gentamicin (160 mg/L) did not impede growth of the mice in group B any more, at least before TBI. Accordingly, in group C, the mice received post-TBI M/A/G treatment in which metronidazole was also provided at a dose of 0.5 g/L in drinking water. As shown in the present study, the Kunming mice could survive for almost 9 days after lethal TBI even without any supportive therapy. According to the symptoms exhibited, these mice finally died of severe digestive tract damage. Unexpectedly, the gut bacteria depletion resulted from M/A/G treatment throughout the experiment remarkably shortened the survival time of the mice receiving TBI. To exclude the synergic effect of TBI on anti-infectious treatment, another group of the mice was given M/A/G immediately after TBI. We found that the mean survival time of the mice in the group treated with M/A/G immediately after TBI was also significantly shorter than that in post-TBI M/A/G-treated group. Since the difference in mean body weight was found from each other among the three groups, it was doubted that the survival time of irradiated mice might be associated with their body weight. However, we failed to find any correlation between the survival time of each mouse with its
body weight at death in each group. In other words, it is
unlike that when the body weight of the mouse treated with
TBI was decreased, the survival time will be shorter.
Accordingly, it seemed that the difference in physical con-
ditions of the mice among these groups did not account for
the difference of survival time. Given the fact that recog-
nition of commensal flora is indispensable for gut homeo-
stasis,10) gut bacteria depletion by M/A/G treatment throughout
the experiment might worsen the irradiation-induced dam-
age to digestive tract and then shorten the survival time of
mice receiving TBI.

Taken together with the previous reports13,14) that anti-
microbial agents strictly against anaerobic bacteria could be
deleterious for the animals receiving TBI, it is suggested that
the effects of gut commensal flora on the irradiated mice as
well as the underlying mechanisms need to be further invest-
gigated in the future. Moreover, current anti-infection
therapy should be re-evaluated for victims receiving lethal
irradiation from the viewpoint of benefits of gut commensal
flora to the health.

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