Citrulline as a Biomarker for Gastrointestinal-Acute Radiation Syndrome: Species Differences and Experimental Condition Effects

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ABSTRACT

Acute radiation-induced damage to the gastrointestinal tract results in intestinal crypt cell apoptosis, villous atrophy, and enterocyte mass reduction. Citrulline, a non-coded amino acid produced almost exclusively by enterocytes in the small intestine, has been identified as a suitable biomarker for radiation injury associated with the acute GI-ARS. Here we discuss the effect of radiation on plasma citrulline levels in different species (mice, minipigs and primates) measured by Liquid chromatography mass spectrometry (LC/MS/MS) and the effect of feeding and anesthesia on plasma citrulline levels in the rhesus non-human primates (NHPs). C57BL/6 mice were exposed to five doses of partial body irradiation (13-17 Gy) whereas Göttingen minipigs were exposed to partial body irradiation with dose levels ranging from 8-16 Gy. Rhesus NHPs were exposed to 6.7-13 Gy of total body irradiation. Blood samples were taken and plasma citrulline levels were measured in the three species at baseline and following irradiation. Plasma citrulline concentrations in mice and minipigs with normal intestinal mucosa function were 51.5 μM (SEM ±4.4 μM) and 64.9 μM (SEM ±2.23 μM), respectively. In the Rhesus NHPs, the mean plasma citrulline baseline level was 32.56 μM (SEM ±0.73 μM), closer to the human plasma citrulline levels. Plasma citrulline nadirs were generally radiation dose dependent in the different species and recovery was more rapid at lower doses. Nadir values were observed on Days 3.5 in mice while minipigs and NHPs presented nadirs on Days 5-6. Only partial citrulline level recovery was observed at higher radiation doses in minipigs and Rhesus NHPs. In the postprandial state, citrulline concentrations were significantly decreased by 35.5% (P=0.0017) compared to unanesthetized Rhesus NHPs. In the postprandial state, citrulline concentrations were slightly but significantly decreased by 16.2% (P=0.0074) in the Rhesus NHPs. These results suggest that plasma citrulline is affected by experimental conditions such as anesthesia and feeding.

MATERIALS AND METHODS

All experimental procedures were performed in accordance with Institutional Animal Care and Use Committee (IACUC) and the Canadian Council on Animal Care guidelines for use of experimental animals. Mice were provided with standard certified chow diet ad libitum whereas Göttingen minipigs and Rhesus NHPs were provided with standard certified chow diet twice daily.

Blood samples were obtained from all species prior to and after irradiation. Blood samples were also obtained from Rhesus NHPs after a period at least 12 hours fasting and within 2 hours of feeding from the same animal and from animals that were anesthetized (ketamine 9.09 mg/kg/acepromazin 0.9 mg/kg, intramuscular) compared to plasma citrulline concentrations obtained from animals without anesthesia. C57BL/6 mice, Göttingen minipigs and Rhesus NHPs were exposed to total body irradiation or partial body irradiation as detailed in Table 1. Citrulline was quantified by HILIC-MS/MS (mice) or by LC-MS/MS (Göttingen minipigs and Rhesus NHPs).

Table 1: Summary of Animal Demographics for Citrulline Analysis

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>Age Range Pre-irradiation</th>
<th>Gender</th>
<th>Radiation Dose Range (Gy)</th>
<th>Radiation Dose Rate (cGy/min)</th>
<th>Shielding Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse (C57BL/6)</td>
<td>20</td>
<td>9 weeks</td>
<td>Males</td>
<td>13-17</td>
<td>0.60</td>
<td>Left leg distal to mid-femur</td>
</tr>
<tr>
<td>Mini-pig (Göttingen)</td>
<td>81</td>
<td>5-8 months</td>
<td>Males (N=38) and Females (N=43)</td>
<td>8-16</td>
<td>0.50</td>
<td>Head, thorax and pelvic limb</td>
</tr>
<tr>
<td>Non human primates (Rhesus)</td>
<td>209</td>
<td>2.5-6 years</td>
<td>Males (N=155) and Females (N=56)</td>
<td>6.72-13</td>
<td>0.60-0.80</td>
<td>None</td>
</tr>
</tbody>
</table>

DISCUSSION AND CONCLUSION

Total body irradiation was associated with significant hemorrhage in almost all species except mice where the only organ affected was the brain. Reduced incidence of hemorrhage was noted in animals exposed to partial body irradiation. Hemorrhage was associated with thrombocytopenia where it reached minimal values around Day 11 in Rhesus NHPs. Nadir mean platelet counts were reached on Days 10, 12, 12 in New Zealand White rabbits, Beagle dogs and Rhesus NHPs, respectively. These results suggest species specific differences in the susceptibility to ARS induced hemorrhage and post-ARS platelet kinetics.

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