Effect of 2-Mercaptopropionylglycine and 2-Aminoethyl Isothiuronium Bromide Hydrobromide on Leucocytes in Peripheral Blood After Gamma Ray Exposure in Mice

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2-Mercaptopropionyl glycine (MPG) treatment has not improved leucocytopenia in laboratory mice after 760R or 1060R gamma exposure inspite of the fact that reduction in the total leucocyte count was slightly less in MPG treated group on the 10th day after 760R gamma exposure; leucocytopenia was more marked in MPG treated group on the 14th day and 21st day after 760R gamma exposure as compared to that in control animals.

Two hundred mg/kg of 2-aminoethyl isothiuronium bromide hydrobromide (AET) treatment which is half the recommended dose for radioprotection has offered considerable protection to leucopenia after 760R and 1060R gamma exposure. It has also helped substantially in rapid recovery of lymphocytes after 760R exposure.

MPG combined with two hundred mg/kg AET has not offered any extra radioprotection to peripheral blood leucocytes after 760R gamma exposure, on the contrary the combination has been less effective than the respective AET dose given singly. Similar result has been noted with combination treatment of MPG and 40 mg/kg AET in 1060R gamma exposure.

INTRODUCTION

2-Mercaptopropionylglycine (MPG), a non-toxic radioprotector in mice, has been reported to protect peripheral blood leucocytes in cancer patients\(^1\). It is worthwhile to know the status of leucopenia in MPG treated gamma irradiated laboratory mammal under proper control condition. 2-Aminoethyl isothiuronium bromide hydrobromide (AET) is a very effective radioprotector but its toxicity is a practical problem. In the present study peripheral blood leucocytes was studied in mice receiving gamma radiation and treatments with MPG and/or suboptimal doses of AET.

MATERIALS AND METHODS

Groups of inbred Swiss A albino mice properly controlled for sex, age etc., were treated intraperitoneally with water or MPG (20 mg/kg), or AET 1/2 (200 mg/kg) or AET 1/10 (40 mg/kg) or AET 1/10+MPG or AET 1/2+MPG in volume of 0.5 ml. All MPG containing solutions were adjusted to pH 6.4-6.5.
The animal was exposed after half an hour of the i. p. injection individually to 760R or 1060R gamma radiation from $^{60}$Co source, dose rate 16.5 R/sec to 17.0 R/sec, in Gamma Cell 220, Canadian Atomic Energy Commission, Ottawa. Total leucocyte count (TLC) and differential leucocyte count (DLC) were followed after different periods of radiation exposure, blood being collected from tail. For DLC, 100 cells were counted in each sample to find out the percentage of polymorphs and lymphocytes. The peripheral blood film was stained with leishman stain and magnification of microscope was 800 times.

To start with 15 animals were taken in each group when 760R exposures were given while 22 animals were taken in each group in case of 1060R exposure. In case of 760R exposure same groups of animals were tested on the 2nd day, 10th day and 14th day. These were female animals of 3-3.5 months age group. For the 21st day analysis a different set of male animals, age 4.5 months, were taken. There was very few mortality with 760R exposures. In case of 1060R exposure, 22 female animals of 3-3.5 months age group were taken in each group, 11 animals were put in each cage. After the exposure, a batch of 10 animals was analysed after 24 hours and another batch of 10 animals were analysed after 3 days. On the 7th day the same batch of animals that had been analysed on the 24 hours were tested. After 7 days due to high mortality, the surviving animals out of the twenty two animals in each group were taken together for testing.

RESULTS AND DISCUSSION

Normal values in the animal stock for the parameters were as follows. TLC=10,040, SD=1,627 per cu.mm., polymorph %=41, SD 7.8; lymphocytes %=56, SD 7.4. Twenty animals were analysed for getting the normal value.

After 2 days of exposure to 760R, marked leucocytopenia and lymphocytopenia were noted in all groups equally irrespective of treatments (Table 1). After 10 days TLC value was lowest in the water injected group; in MPG treated group it was slightly higher. Due to the very low leucocyte counts, DLC count could not be done in these samples. On the 14th day, control group had improved its TLC count considerably and reached the same level as that obtained on the 2nd day while the improvement in the MPG treated group was considerably less. The difference between the control and MPG treated groups on the 14th day is highly significant (P<.01). On the 21st day the MPG group still had lower TLC count as compared to that of water injected group though substantial recovery happened in both groups. Polymorph % (P%) and lymphocyte % (L%) values were not very different in the two groups. With 760R exposure very low mortality was noted. After 1060R exposure animals could be statistically analysed only upto 7 days for these parameters in water injected and MPG injected groups as very few animals survived on the 14th day (Table 2). In fact mortality was very high during 7-14 day in all groups except in the AET 1/2 group. The acute leucopenia after 1060R was not different either in quantity or in quality in water and MPG treated groups. Thus MPG treatment had not helped in reducing leucocytopenia and lymphocy-
Table 1.
Effect of MPG and AET on leucocytes following 760r gamma irradiation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2 days</th>
<th>10 days</th>
<th>14 days</th>
<th>21 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TLC</td>
<td>P%</td>
<td>L%</td>
<td>TLC</td>
</tr>
<tr>
<td>Water</td>
<td>3800 ±587</td>
<td>84.6±4.3</td>
<td>14.8±4.2</td>
<td>612.8±253</td>
</tr>
<tr>
<td>MPG</td>
<td>3100±850</td>
<td>77.8±3.3</td>
<td>21.3±3.3</td>
<td>1100±346</td>
</tr>
<tr>
<td>AET1/2</td>
<td>3670±703</td>
<td>79.4±3.9</td>
<td>20.1±3.5</td>
<td>3730±519</td>
</tr>
<tr>
<td>+ MPG</td>
<td>3611±271</td>
<td>85.8±4.0</td>
<td>13.7±3.9</td>
<td>3825±1046</td>
</tr>
</tbody>
</table>

± indicates standard deviation
8-10 analysed in each group
TLC = Total leucocyte count
P% = Polymorph %; L% = Lymphocyte %

Table 2.
Effect of MPG and AET on leucocytes after 1060R gamma irradiation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>24 hours</th>
<th>3 day</th>
<th>7 days</th>
<th>14 days</th>
<th>21 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TLC</td>
<td>P%</td>
<td>L%</td>
<td>TLC</td>
<td>TLC</td>
</tr>
<tr>
<td>Water</td>
<td>3290±427</td>
<td>81.9±3.3</td>
<td>17.8±3.3</td>
<td>1020±265</td>
<td>410±99</td>
</tr>
<tr>
<td>MPG</td>
<td>3225±444</td>
<td>82.3±3.3</td>
<td>17.2±3.3</td>
<td>780±215</td>
<td>522±172</td>
</tr>
<tr>
<td>AET1/10</td>
<td>3190±569</td>
<td>79.4±2.8</td>
<td>20.9±3.1</td>
<td>—</td>
<td>488±162</td>
</tr>
<tr>
<td>AET1/10 + MPG</td>
<td>3120±442</td>
<td>77.6±2.1</td>
<td>22.0±2.4</td>
<td>—</td>
<td>592±192</td>
</tr>
<tr>
<td>AET1/2</td>
<td>4030±513</td>
<td>79.2±4.8</td>
<td>20.3±4.5</td>
<td>—</td>
<td>890±191</td>
</tr>
</tbody>
</table>

9-10 animals in each group were analysed unless otherwise indicated in bracket
± indicates standard deviation
TLC = Total leucocyte count;
P% = Polymorph % and L% = Lymphocyte %
topenia after gamma irradiation.

AET 1/2 or AET 1/2 plus MPG treatment failed to render protection to leucocytes on the 2nd day after 760R exposure (Table 1). Positive improvement in leucocytes however was noted equally in both the groups on the 10th day. On the 14th day AET 1/2 group had shown considerably higher TLC count over the control and AET 1/2+ MPG groups, there being no difference in the latter two groups. AET 1/2 treatment has also helped recovery of lymphocytes as is evident from DLC picture on the 14th day after 760R exposure. After 1060R exposure AET 1/2 treatment has shown positive beneficiary role in restoring TLC count on the 14th day and 21st day. It however failed to render preferential quick restoration of lymphocytes as was observed in case of 760R exposure (Table 2). Higher level of leucocytes has prevailed in water or AET 1/10 treated group as compared to that obtained with AET 1/10 plus MPG treatment on the 21st day after 1060R exposure.

The present data does not therefore fall in similar line with the finding obtained in human patients regarding MPG’s beneficiary role in reducing leucocytopenia after ionizing radiation. Present study reveals that in mice MPG treatment either alone or in combination with suboptimal doses of AET is of no help in recovering leucocytopenia due to whole body gamma irradiation; on the contrary it is rather harmful in this aspect. One paper (in abstract form) has reported potentiation of radioprotection by combining MPG with AET5, though it is not known whether they have studied effect on leucocytes. The reason for the different finding is not clear. It is worth mentioning that under similar experimental condition, MPG has not improved 30 days survival also9. Excepting in rare case7, chemical radioprotectors reveal radioprotecting effect on blood leucocytes after ionizing radiation exposure6-17. In the light of present finding therefore, any radioprotecting role of MPG under the present experimental situation thus seems improbable. However, P. Uma Devi and her associates have reported that MPG offers radioprotection to tissues like small intestine18,19, liver20,25, testis18,25, and bone marrow24,25. In their experiments they have irradiated the animals on table top (personal communication) and have used a lower dose rate. Some environmental factors, difference in dose rate etc. are possibly responsible for the different type of findings.

The recommended dose of AET for radioprotection is 400 mg/kg b. wt.9. At this dose level, AET has been found to help leucocytopenia with a small protection to lymphocytes in mice after 900R gamma exposure15. This dose level for AET is too high as it often kills normal mice14,26,27. The present study shows that half the amount of the recommended dose was efficient in restoring leucocytopenia upto 1060R exposure. After 760R exposure AET 1/2 dose also helped in preferential rapid recovery of lymphocytes.

REFERENCES

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