Evaluation of Some Radioprotectors by the Survival Study of Rats Exposed to Lethal Dose of Whole Body Gamma Radiation

S. K. BASU, M. N. SRINIVASAN, K. CHUTTANI and A. GHOSE

Radiation Biology Division, Institute of Nuclear Medicine and Allied Sciences, Probyn Road, Delhi-110007, India
(Received January 31, 1985)
(Revised version, accepted May 27, 1985)

Gamma irradiation/Solcoseryl/AET/MPG/5-HTP

Survival studies were carried out after 8 Gy whole body gamma radiation (WBGR) in rats treated either with Solcoseryl, β-mercaptopropionyl glycine (MPG), 2-aminoethylisothiouronium bromide hydrobromide (AET), 5-hydroxy L-tryptophan (5-HTP), MPG+5-HTP or AET+5-HTP. The drugs were tested after either oral or i.p. administration in fasted rats. Solcoseryl and the combinations of 5-HTP with AET or MPG rendered excellent protection against 8 Gy but lower survival against 10 Gy WBGR. Oral ingestion of these combinations also gave effective protection against 8 Gy whole body gamma radiation.

INTRODUCTION

Aminoethylisothiouronium bromide hydrobromide (AET), Cystamine, Cysteamine, Serotonin and S-2-(3-aminopropylamine)-ethylphosphorothioate (WR-2721) are well known radioprotectors. Due to various considerations such as toxic reactions, non protection of some normal tissues (CNS) and protection of tumor tissue,1-6 continued attempts for new effective radioprotecting drugs are needed. β-mercaptopropionyl glycine (MPG) is claimed to give radioprotection at a nontoxic dose level, though the level of protection is quite low – DRF 1.47.

This laboratory has noted significant protection of survival to whole body gamma irradiated mice by pretreatment with combinations of sub-optimal doses of 5-hydroxy-L-tryptophan (5-HTP) and some thiol radioprotectors7. Such combinations also indicated protection to protein (unpublished) and DNA after whole body irradiation8. The rate of uptake of 22Na into RBC of rats exposed to 11 Gy increased significantly. This was reduced by the administration of 5-HTP combinations with thiol compounds and Solcoseryl prior to irradiation9. Solcoseryl has been reported to be an effective radioprotector in mice10. Combinations of 5-HTP as well as Solcoseryl are likely to be effective and of low toxicity7,10.

The present study was under taken with these drugs in order to determine short term survival after whole body gamma radiation (WBGR) by administering them at pre-selected times before irradiation.
MATERIALS AND METHODS

Drugs:

Solcoseryl\textsuperscript{10} (kindly supplied by SOLCO A. G. Basel) is a low molecular weight compound extracted from deproteinised blood of young calves, has been obtained in saline solution, pH 7.1, 40 mg/ml.

MPG was obtained as a gift from SHANTON PHARMACEUTICALS CO. LTD., JAPAN. AET and 5-HTP were procured from Sigma Chemicals, USA.

Adult male Sprague-Dawley rats (220–225 gm) were housed at 23 ± 2°C and fed ad libitum. Three animals were housed in individual plastic cages and were fasted for 24 hr before drug treatment and exposure. They were given streptomycin (75 μg/ml) in the drinking water for three consecutive days followed by boiled water for drinking purposes to minimise infection of the irradiated animals\textsuperscript{10}. All rats were subsequently given Hindusthan Lever food pellets and boiled water ad libitum after irradiation.

Experimental procedure:

The animals were divided into control and experimental groups consisting of 20 animals each. Groups of rats were injected i.p. or orally with saline or Solcoseryl (400 mg/kg) or MPG (20 mg/kg) or AET (200 mg/kg) or 5-HTP (100 mg/kg) or 5-HTP (100 mg/kg) combined with MPG (20 mg/kg) or 5-HTP (100 mg/kg) combined with AET (20 mg/kg). Solutions were prepared in normal saline for i.p. treatment and in distilled water for oral administration (p.o.) through stomach tube. All MPG containing solutions were adjusted to pH 6.4 with 0.1 N NaOH. The volume administered (i.p. and p.o.) was 1.0 ml/100 gm body weight. Control and treated animals were exposed individually to 8 or 10 Gy WBGR, dose rate 8.5–10.2 Gy per minute from a \textsuperscript{60}Co source in Gamma Cell “220” (Atomic Energy of Canada Ltd.), at pre-selected time intervals. Survival was studied upto 30 days after irradiation.

Statistical evaluation:

Thirty-day survival of control and drug pretreated populations was compared using the help of Kruskall-Wallis test. Survival after i.p. vs p.o. treatment was compared at the 15th and 30th day by Cox’s F test. Effectivity of the drugs (i.p.) against each other was compared by Cox’s F test.

RESULTS AND DISCUSSION

Assessment of effective optimum time of drug administration for radioprotection:

The study reveals that by i.p. route, optimal time relating to the maximal protective effect lies around 40 minutes for most treatments except 5-HTP (30 min) and Solcoseryl (60 min). The effective time range is found to be so broad that effect of each drug is maintained well upto 60 minutes and even at 90 minutes the protective effect persists (Fig. 1). By oral administration the optimum time for protection by each drug lies around 60 minutes, however, the effect is not significantly different from that obtained at 40 or 90 minutes (Fig. 2). All irradiated controls died within 30 days (Figs. 3 and 6).
Fig. 1. The influence of time of administration on the protective effect of different radioprotectors administered intraperitoneally. 30 days per cent survival is plotted against the time interval between i.p. injection and 8 Gy gamma ray exposure.

Fig. 2. The influence of time of administration on the protective effect of different radioprotectors ingested orally. 30 days per cent survival is plotted against the time interval between p.o. administration and 8 Gy gamma ray exposure.
Fig. 3. Survival curves after 8 Gy whole body gamma ray exposure. Effect of AET and 5-HTP+AET combination administered intraperitoneally.

Fig. 4. Survival curves after 8 Gy whole body gamma ray exposure. Effect of MPG, 5-HTP+MPG and Solcoseryl administered intraperitoneally.
Assessment of radioprotection by i.p. administration of combinations of (a) 5-HTP with AET, MPG or (b) Solcoseryl:

Comparison of protection with combinations of 5-HTP has been made against (a) each of the constituents of the combinations, (b) optimum radioprotecting dose of AET and (c) radioprotecting dose of Solcoseryl (Table 2 and Figs. 3, 4). Effect of drugs on survival of rats exposed to 8 Gy or 10 Gy WBGR, was studied by administering drugs i.p. at the optimum times i.e. 5-HTP was administered at 30 minutes, Solcoseryl at 60 minutes, and other drugs at 40 minutes prior to irradiation. Results are presented in Figs. 3–5.

On exposure to 8 Gy all control animals (i.e. saline treated and irradiated) died by 30 days. Treatment with 20 mg/kg of AET was not significantly better though 10% animals survived beyond 30 days. All other treatments rendered protection to survival at different levels (Figs. 3, 4 and Table 1). A 100 mg/kg 5-HTP treatment showed a positive improvement, 50% animals survived beyond 30 days (no deaths recorded after 15 d). 200 mg/kg AET was more effective than 20 mg. The combination of 100 mg/kg 5-HTP and 20 mg/kg AET gave high protection to survival better than either compounds alone, approximately 80% survival beyond 30 days (Fig. 3). Solcoseryl alone and the combination of 100 mg/kg 5-HTP with 20 mg/kg MPG gave almost equivalent protection after 8 Gy irradiation (Fig. 4); both better than 5-HTP alone. Pretreatment with MPG alone yielded 30% survival beyond 30 days and 20% beyond 40 days. When mean survival time up to 60 days are taken into consideration, then the order of survival in days is saline (17.9), AET 20 mg (19.1), MPG (28.1), 5-HTP (34.6), Solcoseryl (45.8), 5-HTP+MPG (47.1), AET 200 mg (48.9) and 5-HTP+AET (50.8). It may be noted that the values for 200 mg/kg AET, Solcoseryl, 5-HTP+AET and 5-HTP+MPG are similar but significantly greater than all other groups. No difference (Tables 1 and 2) in

Table 1. Statistical data on survival. Comparison of chemical protectors vs saline and the drug’s effects by two routes of administration.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (mg/kg)</th>
<th>Comparison of the effect (% survival) Control vs drug (i.p.) by Cox’s F test 15 days</th>
<th>Kruskal-Wallis test 30 days</th>
<th>Comparison of the effects between the two modes of application i.p. vs p.o. by Cox’s F test 15 days</th>
<th>30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline</td>
<td>50</td>
<td>0</td>
<td>50, 50</td>
<td>0</td>
</tr>
<tr>
<td>2.</td>
<td>AET (200)</td>
<td>50, 80</td>
<td>0, 75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80, 55&lt;sup&gt;c&lt;/sup&gt;</td>
<td>75, 45&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>3.</td>
<td>Solcoseryl (400)</td>
<td>50, 75</td>
<td>0, 70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>75, 50</td>
<td>70, 40</td>
</tr>
<tr>
<td>4.</td>
<td>AET (20) + 5-HTP (100)</td>
<td>50, 95&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0, 80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>95, 70&lt;sup&gt;c&lt;/sup&gt;</td>
<td>80, 55</td>
</tr>
<tr>
<td>5.</td>
<td>MPG (20) + 5-HTP (100)</td>
<td>50, 80</td>
<td>0, 70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80, 55</td>
<td>70, 40</td>
</tr>
<tr>
<td>6.</td>
<td>5-HTP (100)</td>
<td>50, 50</td>
<td>0, 50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50, 35</td>
<td>50, 20</td>
</tr>
<tr>
<td>7.</td>
<td>MPG (20)</td>
<td>50, 65</td>
<td>0, 30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65, 35</td>
<td>30, 10</td>
</tr>
<tr>
<td>8.</td>
<td>AET (20)</td>
<td>50, 50</td>
<td>0, 15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>, Highly significant (P < 0.005), <sup>b</sup>, Significant (P < 0.01), <sup>c</sup>, Lowly significant (P < 0.05).
Table 2. Statistical evaluation by Cox's F test of protective effects of different chemical protectors.

<table>
<thead>
<tr>
<th>Group against which significance is sought</th>
<th>MPG</th>
<th>5-HTP</th>
<th>5-HTP+MPG</th>
<th>AET(L)</th>
<th>5-HTP+AET</th>
<th>AET(H)</th>
<th>Solcoseryl</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HTP+MPG</td>
<td>NS&lt;sup&gt;b&lt;/sup&gt;</td>
<td>S</td>
<td>-</td>
<td>HS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>5-HTP+AET</td>
<td>NS&lt;sup&gt;a&lt;/sup&gt;</td>
<td>HS</td>
<td>NS</td>
<td>HS</td>
<td>-</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>AET(H)</td>
<td>NS&lt;sup&gt;b&lt;/sup&gt;</td>
<td>S</td>
<td>NS</td>
<td>HS</td>
<td>NS</td>
<td>-</td>
<td>NS</td>
</tr>
<tr>
<td>Solcoseryl</td>
<td>NS&lt;sup&gt;b&lt;/sup&gt;</td>
<td>LS</td>
<td>NS</td>
<td>HS</td>
<td>NS</td>
<td>NS</td>
<td>-</td>
</tr>
</tbody>
</table>

Highly significant (HS), P < 0.005; Significant (S), P < 0.01; Lowly significant (LS) P < 0.05; a, P < 0.001; b, P < 0.01; c, P < 0.05 by Chi-square test.

![Survival curves after 10 Gy whole body gamma ray exposure. Effect of Solcoseryl, combination of 5-HTP with MPG or AET administered intraperitoneally.](image)

Radioprotecting effects are noted among these four groups; while each of these four groups has significantly greater survival than that treated with 5-HTP, AET (20 mg/kg) and saline (Table 2). Survival in MPG treated group, though way below than that obtained in groups treated with the optimum dose of AET, Solcoseryl and the combinations of 5-HTP with AET or MPG (Figs. 3 and 4), on Cox's F test, surprisingly the differences have turned out to be insignificant (Table 2). From Figs. 3 and 4, this statistical finding is difficult to accept. We hence tried Chi-square test and found that the differences are significant. Fig. 5 shows the protective actions of drugs against 10 Gy WBGR. Saline, MPG and 5-HTP treated groups had similar survival curves, though survival in 5-HTP group was slightly better. Mean survival
times (days) for the respective groups were 7.8, 8.8 and 13.9. The other treatments gave better survival. Thus mean survival time (days) for 200 mg/kg AET group was 21.2, for Solcoseryl group the value was 19.0, for 5-HTP+AET group, 19.8 and for 5-HTP+MPG group 16.0. Thus these four treatments give some protection against 10 Gy WBGR and the levels of protection among these four groups are very close. However, the degree of protection on exposure to 10 Gy are definitely less than the protection obtained after 8 Gy irradiation.

The results obtained by i.p. route generally corroborates with the reported protective effects of these drugs in mice on exposure to low lethal whole body irradiation. However, the combination of 5-HTP with AET or MPG, 200 mg/kg AET and Solcoseryl in the dose used here resulted in better survival in case of 10.5 Gy level of WBGR in mice. Thus the degree of radioprotection by these drugs is lower in rats as compared to that obtained in mice. Radioprotectors are generally known to be less effective in rats as compared to mice.

Assessment of radioprotection by oral treatment with combination of (a) 5-HTP with AET or MPG and (b) Solcoseryl:

Figures 6 and 7 present the effects of the drugs by oral route on survival after 8 Gy WBGR. Positive radioprotection has been obtained when (a) the combinations of 5-HTP, (b) Solcoseryl and (c) 200 mg/kg AET (i.e. optimum radioprotecting dose of AET) have been administered through oral route at optimum time i.e. 60 minutes before WBGR. The degree of protection

![Graph showing survival rates](image-url)

Fig. 6. Survival curves after 8 Gy whole body gamma ray exposure. Effect of AET and 5-HTP+AET combination administered orally.
Fig. 7. Survival curves after 8 Gy whole body gamma ray exposure. Effect of MPG, 5-HTP+MPG and Solcoseryl administered orally.

by these four treatments are close to each other, however combination of 5-HTP with AET seems to have given best result. Lower order of protection has been given by 5-HTP and lowest (10%) protection has been rendered with MPG when administered 60 minutes prior to irradiation (Tables 1 and 2).

When the percentage survivals by the respective treatments through i.p. route and oral route are compared, oral route treatment has produced lower level of protection. The probable explanation may be that of the chemical protectors administered by p.o. route, a portion of the administered amount is resorbed and reaches the radiosensitive organs, considering the slower rate of absorption by the gastrointestinal tract. However, on statistical analysis of the data by Cox's F test and Chi-square test, the differences between the two modes are found to be generally non significant (Table 1).

It may be concluded that radioprotection obtained by the combinations of 5-HTP with AET or MPG is no less than the radioprotection rendered by optimum radioprotecting dose of AET and Solcoseryl. It may also be mentioned that when these combinations were administered consecutively for 3 days in rats and observed for two months, no apparent untoward effect was noted. That Solcoseryl and the combinations of 5-HTP are effective through oral route also, widens the application possibility of these drugs in practical fields.

ACKNOWLEDGEMENTS

The authors are grateful to Brig. N. Lakshmpathi, VSM, Director of the Institute of Nuclear Medicine and Allied Sciences for his interest in the work.
REFERENCES


