


5. Effect of combined application of radiotherapy and Mitomycin C on Sarcoma 180 cells **in vivo**. S.K. Majumdar, S. Mukherji and K.L. Bhattacharya. 4th Indian Photobiology Symposium, Mahabaleswar (Pune), 1981.


In vivo effects of mitomycin-C and gamma radiation on growth and lysosomal enzyme activity of sarcoma-180 tumour cells

Sandip Kr. Majumdar and Sheela Mukherji
Biophysics Department, Chittaranjan National Cancer Research Centre, 37, S.P. Mookerjee Road, Calcutta 700 026, India

In recent years there has been increasing use of anti-tumour drugs such as mitomycin C (MMC) and gamma radiation in different combinations for the management of malignant cell populations [1–3]. Rockwell [4] reported that MMC plus radiation may be useful for the treatment of solid tumours. However, the doses of MMC and radiation used in Rockwell’s work [4] were much larger than are used in clinical therapy. To our knowledge, information regarding the effect of MMC plus gamma radiation on ascites tumour cell growth is scanty. The present communication describes our observation of the in vivo effect of MMC and gamma radiation on sarcoma-180 ascites tumour bearing Swiss albino mice.

Materials and methods: Swiss male albino mice of average weight 20 g, and 8–10 weeks old, were used for the present study. They were kept in alternate light (12 h) and dark (12 h) conditions and were provided with food and water ad libitum. Sarcoma-180 tumour cells were routinely maintained through serial intraperitoneal transplantation in the mice. Mitomycin-C (Hakko Kyowa Co., Tokyo) was dissolved in 0.01 M phosphate buffer (pH 7.2) to the desired concentration immediately before use. Gamma irradiation at a dose rate of 62 R/min was performed using a 137Cs source (Picker, USA) at the Cancer Hospital, Calcutta. Experimental animals were inoculated intraperitoneally with $1 \times 10^7$ sarcoma-180 tumour cells per mouse. The day of tumour transplantation was taken as zero and on the fifth day after tumour transplantation the animals were divided into different groups. One group of animals was kept as control; the other group of animals received a single i.p. injection of MMC (4 mg or 7 mg/kg b.wt) and was given whole body gamma irradiation immediately afterwards with a dose of 400 R or 800 R. There were, therefore, besides the control group, four different groups of animals receiving the following treatments: (i) 4 mg + 400 R; (ii) 7 mg + 400 R; (iii) 4 mg + 800 R; (iv) 7 mg + 800 R. The LD$_{50}$ of MMC is 9 mg/kg b.wt [5] and the LD$_{50}$ of gamma radiation is 550 R [6] in the mouse. Tumour growth was assessed in both control and treated groups on different days by measuring the viable number of tumour cells per ml of ascitic fluid using a Trypan blue dye exclusion test. The number of dead cells per ml of ascitic fluid in all groups of mice remained within 10–12%. The activity of two lysosomal enzymes was measured on the 7th and 10th day after tumour transplantation.

Results and discussion: The sarcoma-180 tumour cells in untreated control mice proliferated rapidly between days 3 and 14 post transplantation, after which the rate of growth decreased (Tables 1 and 2). Some pilot studies were done to

### Table 1: Effect of mitomycin-C (MMC) and gamma radiation on growth of sarcoma-180 tumour cells and on the life span of tumour bearing mice (means ± SD; no. of experiments = 5, with 10 mice in each group for tumour growth and 20 mice in each group for life span)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
<th>Day 10</th>
<th>Day 14</th>
<th>Median survival (days)</th>
<th>Increase in life span (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.20 ± 0.39</td>
<td>3.60 ± 0.14</td>
<td>4.35 ± 0.52</td>
<td>5.12 ± 1.45</td>
<td>10.94 ± 2.24</td>
<td>35.3 ± 0.45</td>
<td>17.74 ± 1.31</td>
<td>—</td>
</tr>
<tr>
<td>400 R</td>
<td>—</td>
<td>—</td>
<td>4.15 ± 0.85a</td>
<td>4.78 ± 1.10a</td>
<td>9.5 ± 1.13a</td>
<td>29.3 ± 1.52</td>
<td>21.04 ± 0.95</td>
<td>18.64</td>
</tr>
<tr>
<td>800 R</td>
<td>—</td>
<td>—</td>
<td>3.78 ± 1.02</td>
<td>4.38 ± 1.27</td>
<td>8.44 ± 1.36</td>
<td>24.3 ± 1.49</td>
<td>14.5 ± 1.04</td>
<td>—18.26</td>
</tr>
<tr>
<td>4 mg MMC</td>
<td>—</td>
<td>—</td>
<td>2.68 ± 0.78</td>
<td>3.80 ± 1.65</td>
<td>7.03 ± 2.92</td>
<td>6.80 ± 0.78</td>
<td>27.5 ± 1.39</td>
<td>55.36</td>
</tr>
<tr>
<td>7 mg MMC</td>
<td>—</td>
<td>—</td>
<td>2.28 ± 1.31</td>
<td>3.71 ± 0.50</td>
<td>6.89 ± 1.47</td>
<td>6.53 ± 1.58</td>
<td>22.4 ± 1.15</td>
<td>26.35</td>
</tr>
</tbody>
</table>

*In comparison with untreated control, p < 0.05, in all other cases p < 0.001. *a The figures in parentheses indicate percentage growth inhibition.

### Table 2: Effect of mitomycin-C (MMC) plus gamma radiation on growth of sarcoma-180 tumour cells (viable no. of cells/ml of ascitic fluid $\times 10^7$; means ± SD; no. of experiments = 5, with 10 mice in each group; percentage growth inhibition in parentheses)

<table>
<thead>
<tr>
<th>Days after tumour transplantation</th>
<th>Treatment</th>
<th>3</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>12</th>
<th>14</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>4.35 ± 0.52</td>
<td>5.12 ± 1.45</td>
<td>7.95 ± 1.01</td>
<td>10.94 ± 2.24</td>
<td>19.9 ± 0.48</td>
<td>35.3 ± 0.45</td>
<td>34.6 ± 0.27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 mg MMC + 400 R</td>
<td></td>
<td>1.98 ± 0.38</td>
<td>2.95 ± 0.58</td>
<td>4.48 ± 0.78</td>
<td>5.80 ± 1.08</td>
<td>5.72 ± 0.34</td>
<td>5.10 ± 0.38</td>
<td>4.75 ± 0.26</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 mg MMC + 400 R</td>
<td></td>
<td>1.74 ± 0.24</td>
<td>2.80 ± 0.65</td>
<td>4.18 ± 0.40</td>
<td>5.62 ± 0.31</td>
<td>4.70 ± 1.34</td>
<td>4.10 ± 0.44</td>
<td>3.74 ± 0.35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 mg MMC + 800 R</td>
<td></td>
<td>1.61 ± 0.35</td>
<td>2.42 ± 1.72</td>
<td>3.72 ± 0.54</td>
<td>4.86 ± 2.32</td>
<td>4.38 ± 1.62</td>
<td>3.30 ± 0.72</td>
<td>3.05 ± 0.49</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 mg MMC + 800 R</td>
<td></td>
<td>1.55 ± 0.45</td>
<td>2.38 ± 0.68</td>
<td>3.65 ± 0.51</td>
<td>4.80 ± 1.35</td>
<td>4.10 ± 0.59</td>
<td>3.25 ± 1.40</td>
<td>2.98 ± 0.20</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*In comparison with untreated control, p < 0.001 in all cases. The analysis of variance shows that there was no interaction between any two effects. All the three main effects are also non-significant between themselves.*

...
see the effects of single agents on sarcoma-180 tumour cell growth, on the lysosomal enzyme activity of tumour cells and on the life span of host mice. The pilot studies 

conformed to the same protocol as outlined above. Table 1 

shows that the application of MMC inhibited the tumour growth more effectively than did radiation only. The change in the tumour cell numbers when MMC was applied just before whole body irradiation is shown in Table 2. It appears that higher doses of radiation in combination with MMC of any dose were more effective in reducing tumour cell number as compared with the results obtained with lower doses of MMC plus radiation. Prior application of MMC in combination with ionizing radiation produced additive cytotoxicity in the case of solid tumours [4, 10] and intestinal crypt cells [3]. Our observations on ascitic tumour cells are in conformity with this result though obtained by using gamma radiation of a much lower dose, viz., 400 R and 800 R.

The activities of two lysosomal enzymes, viz., \( \beta \)-glucuronidase and acid DNase of sarcoma-180 cells, were determined to evaluate the efficacy of the combination of MMC and radiation chosen. The activities of both enzymes increased in in vivo gamma irradiated tumour cells as compared with those for unirradiated control tumours. Enhancement was greater when MMC was applied singly (pilot study, data not shown). The activity of \( \beta \)-glucuronidase and that of acid DNase increased markedly in only two out of four combinations, viz., 7 mg + 400 R and 4 mg + 800 R (Figures A and B). Tables 1 and 3 show the variation in the percent increase of life span in groups of mice subjected to both single and combined treatments. The increase in life span was 83% in the case of 4 mg + 400 R and 71% in the case of 7 mg + 400 R treated mice. Tumour bearing mice receiving MMC + 800 R whole body irradiation failed to survive longer than the control mice.

The outcome of the simultaneous application of MMC and gamma radiation in vivo on sarcoma-180 ascites tumour cells may be summarized as follows: (i) any combination of MMC and gamma radiation within a tolerable dose produced increased antitumour activity; (ii) not all combinations were suitable for the survival of host mice; and (iii) differences shown in the activities of lysosomal enzymes probably signify the heterogeneity in the tumour cell population surviving combined treatments. Reduced survival of host mice possibly occurred due to the toxicity of higher doses of radiation.

Table 3: Effect of mitomycin-C (MMC) plus gamma radiation on sarcoma-180 tumour bearing mice (means ± SD; experiments with 20 mice in each group)

<table>
<thead>
<tr>
<th>Treatment modalities</th>
<th>Median survival (days)</th>
<th>Increase in life span (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (untreated)</td>
<td>17.74 ± 1.31</td>
<td>-</td>
</tr>
<tr>
<td>4 mg MMC + 400 R</td>
<td>32.5 ± 1.73</td>
<td>83.20</td>
</tr>
<tr>
<td>7 mg MMC + 400 R</td>
<td>30.44 ± 1.94*</td>
<td>71.58</td>
</tr>
<tr>
<td>4 mg MMC + 800 R</td>
<td>16.20 ± 2.02</td>
<td>-</td>
</tr>
<tr>
<td>7 mg MMC + 800 R</td>
<td>9.40 ± 0.72</td>
<td>-47.01</td>
</tr>
</tbody>
</table>

*In comparison with control, p < 0.001.

We thank Dr J. Roychowdhury, Director, for her kind encouragement.

Reprint requests to: Dr (Mrs) Sheela Mukherji, Biophysics Dept, Chittaranjan National Cancer Research Centre, 37, S.P. Mukherji Calcutta 700 026, India.

Use of transmission electron microscope to assess the damage to Sarcoma 180 ascites tumour cells following *in vivo* treatment of mitomycin-C and gamma radiation

Sandip Kumar Majumdar, Somenath Bhattacharya, Aruna Mitra and Sheela Mukherji

Biophysics Department, Chittaranjan National Cancer Research Centre, 37, S P Mukherjee Road, Calcutta-700 026, India

**Abstract:** Five day old Sarcoma 180 tumour bearing mice were exposed to different doses of mitomycin-C (4 mg or 7 mg per kg body weight of mouse) and gamma radiation (400 R or 800 R) applied singly or in combination. Surviving populations were collected after 5 days of treatment and processed for transmission electron microscopy. The control Sarcoma 180 tumour cells have the following characteristics: profused microvilli, different sized mitochondria with poorly developed internal structure, distinct endoplasmic reticulum studded with ribosomes, the large nucleus rich in chromatin materials and distinct nucleolus containing closely interwind granular and fibrillar components with associated chromatin. Damage to treated cells were ascertained by the reduction in microvilli, swelling of mitochondria with cloudy appearance, dilation and fragmentation of endoplasmic reticulum, blebbing of nuclear membrane, condensation of heterochromatin,' appearance of perichromatin granules, segregation and fragmentation of nucleolus and invagination of plasma membrane with increased intracellular spaces. With the help of transmission electron microscope it is thus possible to assess the nature of damage to organelles effected by mitomycin-C and radiation both singly and in combination. Growth inhibition and damage in the cellular ultrastructure were maximum among tumour cells which survived with concomitant treatment with 7 mg MMC and 800 R.

**Keywords:** Sarcoma 180, mitomycin-C, gamma radiation, tumour growth, ultrastructure.

**PACS Nos:** 07.80.+x, 87.50.Gi

1. **Introduction**

The role of transmission electron microscope as an useful physical technique in medical sciences is well documented (Carr and Toner 1968, Ghadially 1975, 1985). Because of its extraordinary resolving power (De Robertis and De Robertis 1980), the electron microscope seems to be an ideal instrument for the study of cellular ultrastructure. The purpose of the present study was to investigate whether changes in subcellular organelles of Sarcoma 180 tumour cells could be distingu-
shed electron microscopically when mitomycin-C (MMC, an antitumour antibiotic) and gamma radiation were applied singly or in combination on Swiss mice bearing this neoplasm in ascites form.

2. Materials and methods

Sarcoma 180 ascites tumour was maintained in the laboratory through serial intraperitoneal transplantation (10^7 cells per Swiss albino mice, av. wt. 20-22 g, 8-10 weeks old). After five days of tumour transplantation the animals were divided into four groups. Group A of animals was kept as control, Group B of animals was given a single i.p. injection of MMC in 0.01 M phosphate buffer (pH 7.2) in a dose of 4 mg or 7 mg per kg body weight of mouse. Group C of animals was whole body irradiated with 400 R or 800 R at a dose rate of 62 R/minute (1^8Cs Source, Picker USA, at Cancer Hospital, Calcutta). Group D of animals received MMC and gamma radiation concomitantly. The viable number of tumour cells per ml of ascitic fluid in both control and treated groups were measured after 10 days of transplantation (i.e. 5 days after treatment) using trypan blue dye exclusion test. The percentage growth inhibition was determined (Matsumoto et al 1986) from the relation \( (1 - \frac{T}{C}) \times 100 \), where \( T \) and \( C \) were the viable cell numbers of treated and control groups respectively.

For transmission electron microscopy the tumour cells were collected from control and treated group of animals on the same day and washed with normal saline. The cells were fixed in phosphate buffered (pH 7.2) paraformaldehyde-glutaraldehyde mixture (1.5% : 1%) at room temperature for 30 mins. The cells were rinsed successively in phosphate buffer and veronal acetate (VAC) buffer (pH 7.2) and post-fixed in 1% osmium tetroxide (buffered with veronal acetate) for 18-22 hrs. in dark at room temperature. Following another rinse with VAC buffer, the cells were stained in 0.5% uranyl acetate enblock for 90 mins at room temperature and dehydrated in a graded series (30 to 100%) of alcohol. The samples were infiltrated and embedded in low viscosity epoxy resin (Spurr 1969). Ultrathin sections cut with an ultramicrotome (NOVA, LKB) and stained with lead citrate were examined in Hitachi H-600 transmission electron microscope at an accelerating voltage of 75 KV.

3. Results

The growth of Sarcoma 180 tumour cells was inhibited when they are subjected to different treatment modalities. Table 1 shows that the percentage growth inhibition increased with increased dose of MMC and radiation. While MMC treatment alone inhibited the tumour growth from 35 to 37%, in combination with radiation its effectiveness was increased significantly (47 to 56%). Whole body radiation reduced the growth to an extent of 13 to 22% only. Thus tumoricidal activity of MMC enhanced considerably when it was applied concomitantly with gamma radiation on Sarcoma 180 ascites tumour (Majumdar and Mukherji 1987).
Transmission electron microscopy was carried out with tumour cells which survived after 10 days of transplantation following 5 days of treatment. Since the maximum growth inhibition occurred in 7 mg MMC plus 800 R treatment,

**Table I.** In vivo effect of mitomycin-C and gamma radiation on growth of Sarcoma-180 tumour cells.

<table>
<thead>
<tr>
<th>Treatment modality</th>
<th>Viable cell number per ml of ascitic fluid ($\times 10^7$)</th>
<th>Per cent growth inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>10.84 ± 2.24</td>
<td>—</td>
</tr>
<tr>
<td>400 R</td>
<td>9.50 ± 1.13</td>
<td>13.16</td>
</tr>
<tr>
<td>800 R</td>
<td>8.44 ± 1.36</td>
<td>22.85</td>
</tr>
<tr>
<td>4 mg MMC</td>
<td>7.03 ± 2.92</td>
<td>35.74</td>
</tr>
<tr>
<td>7 mg MMC</td>
<td>6.89 ± 1.47</td>
<td>37.02</td>
</tr>
<tr>
<td>4 mg MMC + 400 R</td>
<td>5.80 ± 1.08</td>
<td>47.00</td>
</tr>
<tr>
<td>7 mg MMC + 400 R</td>
<td>5.62 ± 0.31</td>
<td>48.60</td>
</tr>
<tr>
<td>4 mg MMC + 800 R</td>
<td>4.56 ± 2.32</td>
<td>55.60</td>
</tr>
<tr>
<td>7 mg MMC + 800 R</td>
<td>4.80 ± 1.35</td>
<td>56.10</td>
</tr>
</tbody>
</table>

Figures 1-4 represent the ultrastructures of untreated control cell (Figure 1) and those of cells treated with 800 R (Figure 2), 7 mg MMC (Figure 3) and 7 mg MMC+800 R (Figure 4) respectively. The characteristic features of untreated cells are: different sized mitochondria (M) with poorly developed cristae, distinct endoplasmic reticulum (ER) studded with ribosomes, chromatin rich nucleus containing closely interwind granular and fibrillar components in nucleolus (N). Besides these characteristics the untreated cells possess profused microvilli (not
shown in figure). In the 800 R irradiated cells (Figure 2) microvilli are broken (arrow heads), mitochondria are scattered, endoplasmic reticulum is fragmented (arrows). While nuclear membrane is irregular with foldings, chromatin materials condense at the nuclear periphery with matrix becoming less dense. By treatment with 7 mg MMC (Figure 3) the number of microvilli reduces (arrow heads), the mitochondria (M) get swollen with cloudy appearance, endoplasmic reticulum becomes scanty. While the nuclear membrane becomes irregular with bleb
formation and deep indentation, heterochromatin materials condense at the periphery (arrows). While at low dose of MMC (4 mg MMC, not shown in figure) nucleolus becomes segregated in two zones viz., dark and light, at higher concentration (7 mg MMC) nucleolus becomes devoid of material (Figure 3). Also found few perichromatin granules (PCG) in the nucleus and Intracytoplasmic lumen associated with villi (A) in MMC treated cells.

![Image](image.jpg)

Figure 4. Sarcoma-180 ascites tumour cells after 5 days of in vivo treatment with MMC and gamma radiation concomitantly. 7 mg MMC+800 R X19.200.

Major effects of combined treatment with MMC and radiation (Figure 4) are on the nucleolus which becomes fragmented. Electron dense granules shown by arrows in Figure 4 may represent remnant parts of the nucleolus. While reduction in the number of microvilli (arrow heads) is drastic, plasma membrane invaginates increasing intracellular spaces (S)—a characteristic which is never found with single treatment of either MMC or gamma radiation.

4. Conclusion

While increase in tumour growth inhibition is a measure of increase in cytotoxicity, the changes in the major ultrastructural features of the treated cells with respect to untreated tumour indicate the extent of cellular damage brought by any single or combined treatment of MMC and gamma radiation.

Acknowledgment

We thank Dr (Mrs) J Roy Chowdhury, Director, Chittaranjan National Cancer Research Centre for her kind encouragement.
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Ghadially F N 1975 Ultrastructural Pathology of the Cell (London: Butterworths) p 1-91
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Spurr A R 1969 J. Ultrastruc. Res. 26 31
Concomitant effect of mitomycin C and gamma radiation on sarcoma 180 ascites tumour bearing mice

Sandip Kumar Majumdar, Somenath Bhattacharya, Aruna Mitra and Sheela Mukherji
Biophysics Department, Chittaranjan National Cancer Institute, 37, S P Mukherjee Road, Calcutta-700 026, India

Abstract: Mitomycin-C (MMC) and gamma radiation were administered in different dose combinations on 5 day old Sarcoma 180 ascites tumour bearing swiss albino mice for enhanced tumour cell kill. The MMC dose was maintained below cytotoxicity level and the radiation was also applied within tolerable range for the host. Viable tumour cell numbers with respect to untreated control reduced appreciably during combined treatments. Isobolograms were constructed from dose response curves to find out whether interaction of MMC with gamma radiation was additive or not. The population of the cells that survived the treatments were compared at their ultrastructural level. Haematological parameters of host mice and increase in their life span were determined. Analysis of the data helped to select optimum doses of MMC and gamma radiation for maximum effectivity with minimum toxicity.

Keywords: Sarcoma 180 tumour cell, mitomycin C, gamma radiation, isobolograms, ultrastructure.

PACS No: 87.50. Gi

1. Introduction
In recent years, chemotherapy is used in combination with radiotherapy in both animals and human for management of neoplastic diseases applied either concomitantly or sequentially (Bellamy and Hill 1984, Majumdar and Mukherji 1987, Karuri and Mukherji 1987). Whenever cytotoxic agents are combined for the management of neoplastic diseases, the question arises: Is the effect of combination greater or less than would be expected on the basis of the effects of the agents used alone (Steel and Peckham 1979). To find an answer, Sarcoma 180 ascites tumour bearing mice were exposed to different doses of MMC and/or gamma radiation. Iso-effect plots (isobolograms) were constructed from dose response curves to analyze the interaction between MMC and radiation on tumour cells. The effect on host mice were assessed by determining their mortality rate and haematological parameters. Ultrastructure of tumour cells revealed the changes brought by combined treatment.
2. Materials and methods

Sarcoma 180 ascites tumour cells were maintained by serial intraperitoneal transplantation (10^7 cells per Swiss albino mice, av. wt. 20-22 g, 8-10 wks. old). On the 5th day after tumour transplantation the mice were divided into four groups. Group A of animals was kept as control, Group B of animals was given a single i.p. injection of MMC in 0.01 M phosphate buffer (pH 7.2) in a dose of 4 mg or 7 mg per kg body weight of mouse. Group C of animals was whole body irradiated with 4 Gy or 8 Gy at a dose rate of 0.62 Gy/minutes (137 Cs source, Picker USA, at Cancer Hospital, Calcutta). Group D of animals received MMC and gamma radiation concomitantly in the following sequence i.e. (i) 4 mg MMC + 4 Gy, (ii) 7 mg MMC + 4 Gy, (iii) 4 mg MMC + 8 Gy (iv) 7 mg MMC + 8 Gy. The viable number of tumour cells per ml of ascitic fluid in both untreated and treated groups were measured in different days using trypan blue dye exclusion test. The experiment was repeated for 5 times with 10 mice in each group. Mortality rate R of treated or untreated host mice was determined from the relation

\[ R = \frac{dN}{N} \]  

(Andrews 1974) where N is the number of mice at time t. In the present work unit of time was chosen to be two days. Haematological parameters such as haemoglobin concentration, RBC and WBC count and bone marrow cellularity were measured according to standard procedure (Kolmer et al. 1969).

For electron microscopy tumour cells from treated mice were fixed in 1.5% paraformaldehyde : 1% Glutaraldehyde, post fixed in 1% osmium, enblock with 0.5% uranylacetate and dehydrated in ethanol and embedding was done in epoxy resin (Spurr 1969). Ultrathin sections were stained in lead citrate and examined with Hitachi H-600 transmission electron microscope operated at 75 kv.

3. Results

When mitomycin C and gamma radiation were administered either singly or in different dose combinations on 5 day old Sarcoma 180 Swiss albino mice (as described in materials and methods), the number of viable tumour cells with respect to untreated control reduced appreciably with days (Majumdar and Mukherji 1987). The fraction of tumour cells that survived any treatment on any day was determined from the expression

\[ SF = \frac{T}{C} \]

where C is the viable number of untreated cells and T is the viable number of cells on a particular day after treatment.

For isobologram analysis the cell survival data obtained on 12 day after transplantation were used and they were fitted through least square analysis in three different mathematical models depending on the mode of treatment (MMC, radiation and MMC plus radiation) (Alper 1980, Deen and Williams 1979). Table 1 shows the expression used for different treatment modalities and constants determined from experimental data. Using these constants, complete dose response curves were determined upto level of 15% survival as shown in Figures 1.
Table I. Expressions used to fit cell survival data.

<table>
<thead>
<tr>
<th>Treatment modalties</th>
<th>Expressions used</th>
<th>Experimentally determined constants</th>
</tr>
</thead>
</table>
| Radiation           | \( \ln SF = -\left(\alpha D + \beta D^2\right) \) | \( \alpha = -0.047 \text{ Gy}^{-1} \)  
|                     |                  | \( \beta = 5.687 \times 10^{-4} \text{ Gy}^{-2} \) |
| Mitomycin C         | \( \ln SF = \ln A - K C^a \) | \( A = -0.565 \)  
|                     |                  | \( K = 2.18 \times 10^{-4} \) |
| Mitomycin C Plus Radiation | \( \ln SF = \ln A' - b D \) | For 4 mg MMC  
|                     |                  | \( A' = 0.680 \)  
|                     |                  | \( b = 0.140 \)  
|                     |                  | For 7 mg MMC  
|                     |                  | \( A' = 0.543 \)  
|                     |                  | \( b = 0.120 \)  

and 2, Figure 1 for single treatments and Figure 2 for combined treatments. Isocolorograms (Figure 3) were constructed for two levels of cell survival (IE\(_{40}\) and IE\(_{50}\)).

![Graph](image)

Figure 1. Dose response curves for single treatment (1) Radiation, (2) Mitomycin C.

from Figure 1 following the methods of Steel and Peckham. According to the concept of additivity introduced by these authors, if the experimentally determined
Figure 2. Dose response curves for combined treatment (1) 4 mg MMC plus radiation, (2) 7 mg MMC plus radiation.

Figure 3. Construction of isobolograms at the point of $I_{E_{10}}$ (□) and $I_{E_{50}}$ (○).
data fell inside the envelope of additivity this would mean that interaction between two agents was "additive". On the other hand if it fell left to envelope of additivity this would mean interaction between the same agents was "supra additive". It is apparent from Figure 2 IE40 were produced by the combination of 4 mg MMC+3.6 Gy and 7 mg MMC+2.6 Gy respectively and similarly IEa0 were produced by the combinations of 4 mg MMC+8 Gy and 7 mg MMC+7 Gy respectively. The location of data points all fell to the left of their respective envelope of additivity (Figure 3). The dose combinations used in the present work as mentioned in materials and methods, are almost similar to the dose combinations which produced IEa0 and IEao effects. Hence it is suggested that all the combinations used in the present work produced "supra additive" interaction in tumour cell kill.

Mortality rate of host mice undergoing different treatments are shown in Figure 4 together with that of the untreated group. The slope of mortality curve increased in case of all treatments. However, life span increased appreciably with respect to untreated control in case of 4 Gy and 4 mg MMC+4 Gy respectively. (data not shown)

Table 2 shows the haematological changes of few treated and untreated group of mice on 12th day after transplantation. With respect to untreated control and 7 mg MMC treated group, there is a sharp decrease in haemoglobin concentration, WBC count and bone marrow cellularity in case of 7 mg MMC+8 Gy treatment.
This observation explains the increase in mortality rate in case of 7 mg MMC + 8 Gy treatment which caused toxicity development in the host mice.

The changes brought at the ultrastructural level due to combined treatment (4 mg MMC + 8 Gy) is shown in Figure 5: broken microvilli, swollen mitochondria, increase of intercellular spaces, fragmented nucleus with condensed heterochromatin and segregated nucleolus indicated the degenerating condition of a tumour cell (Ghadially 1985).

Table 2. Effect of mitomycin C and gamma radiation on haematological changes in Sarcoma 180 tumour bearing mice (after 12 days of transplantation). Mean ± S.E., No. of experiments=5, with 5 mice in each group.

<table>
<thead>
<tr>
<th></th>
<th>Untreated control</th>
<th>7 mg MMC</th>
<th>8 Gy Radiation</th>
<th>7 mg MMC + 8 Gy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>9.10 ± 0.45</td>
<td>9.20 ± 0.85</td>
<td>8.95 ± 0.15</td>
<td>7.80 ± 0.60</td>
</tr>
<tr>
<td>RBC (×10^12/l)</td>
<td>4.35 ± 0.62</td>
<td>4.30 ± 0.21</td>
<td>4.32 ± 0.38*</td>
<td>3.55 ± 0.75</td>
</tr>
<tr>
<td>WBC (×10^9/l)</td>
<td>7.90 ± 0.61</td>
<td>7.75 ± 0.24*</td>
<td>7.95 ± 0.20*</td>
<td>3.28 ± 0.48</td>
</tr>
<tr>
<td>Nucleated cells/Femur (×10^6/ml)</td>
<td>16.60 ± 0.27</td>
<td>16.60 ± 0.75*</td>
<td>15.48 ± 0.65*</td>
<td>7.20 ± 0.15</td>
</tr>
</tbody>
</table>

In comparison with control (a) P > 0.05, in all other cases P < 0.05.

4. Conclusion

Concomitant administration of Mitomycin C and of gamma radiation on Sarcoma 180 ascites tumour bearing mice produced supra additive cell kill at different
survival level. Survivality of host mice increased in case of MMC+4 Gy treatment and MMC+8 Gy of radiation developed toxicity.

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