Introduction

Radiotherapy is commonly designed to deliver the highest possible dose to the target volume, without exceeding the tolerance of surrounding normal tissues. When combining radiation with other treatments, however, synergistic effects may occur, allowing for lower doses. Here, we report on the results from two parallel studies on synergetic effects of the combination of radiotherapy (RT) and immunotherapy (IT) in rats with intra- or extra-cranial N29 gliomas.

Materials and Methods

Animal model In two parallel studies (groups A and B), we used inbred female and male Fischer 344 rats, inoculated with N29 rat glioma cells. The N29 cells were previously derived from tumours developed in the CNS of pregnant Fischer rats exposed to ethyl-N-nitrosourea, and have since then been successively propagated both in vitro and in vivo.

Intra-cranial tumours (group A), resembling human glioblastoma multiforme, were developed in a total of 44 animals after inoculation by a stereotactic injection of 5000 N29 cells into the head of the right caudate nucleus. Extra-cranial tumours (group B) were induced in a total of 82 animals by a subcutaneous inoculation of 200000 N29 cells into the right hind leg.

Treatment combinations The animals in the two groups were further divided into 4 sub-groups; (I) untreated controls, (II) radiotherapy alone, (III) immunotherapy alone, and (IV) radiotherapy and immunotherapy combined. Due to the different growth pattern and radiation response, the treatment schedule and evaluation methods differed for the intra- and extra-cranial tumours, respectively. Sub-optimal, non-curative dose levels were chosen for each group.

Radiotherapy (RT) was given locally with collimated fields of Co-60 gamma rays. Animals with intra-cranial tumours were treated on day 7 after the inoculation, with a single fraction of 5 Gy or 15 Gy. Animals with extra-cranial tumours were treated around day 30, with 4 daily fractions of 5 Gy each.

Immunotherapy (IT) was given as intraperitoneal injections of 3·10⁶ IFN-gamma-gene transfected N29 tumour cells. The cells were given a sterilizing dose of 70 Gy just before the inoculation. Animals with extra-cranial tumours were immunized within 1 h after the irradiation on day 7 after the inoculation. Animals with extra-cranial tumours were immunized 5 days before RT, and then two more times with 14-day intervals.

Treatment evaluation For the animals in group (A), the effect of the treatment was evaluated in terms of survival time. The animals were observed daily for symptoms of the growing tumour, and euthanized when the pre-set breakpoint symptoms appeared (keeping their heads turned to one side, rotating, or losing weight). For the animals in group (B), the treatment effect was evaluated in terms of tumour growth. The subcutaneous tumours were estimated by an ellipsoid, with length and width as measured by using a caliper. The measured tumour volume, TV, was fitted by an exponential growth model, TV=TV₀eⁿ⁻ⁿ, where n is the time after inoculation in days, and TGR is the tumour growth-rate constant in days⁻¹. When the tumour reached a volume of 9 cm³, the animals were euthanized.

Results and Discussion

For the group of animals carrying intra-cranial N29 gliomas, a highly synergetic effect of the combined radiotherapy and immunotherapy was observed. For the animals with extra-cranial tumours, however, no synergistic effect could be demonstrated for the treatment scheme applied in this particular case. It was hypothesized that this apparent difference was a result of a higher degree of immune suppression in the case of the larger subcutaneous tumours.

Table 1. Tumour growth rate, TGR, for the rats with extra-cranial N29 gliomas

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>N</th>
<th>TGR (% days⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Bi) Untreated controls</td>
<td>40</td>
<td>8.4±0.3 (1SD)</td>
</tr>
<tr>
<td>(Bii) Radiotherapy alone</td>
<td>15</td>
<td>4.5±0.3 (1SD)</td>
</tr>
<tr>
<td>(Biii) Immunotherapy alone</td>
<td>19</td>
<td>7.6±0.6 (1SD)</td>
</tr>
<tr>
<td>(Biv) Radiotherapy + Immunotherapy</td>
<td>8</td>
<td>5.7±0.5 (1SD)</td>
</tr>
</tbody>
</table>

Figure 1. Number of living rats with intra-cranial N29 gliomas, including median survival times

Conclusions

For the group of animals carrying intra-cranial N29 gliomas, a highly synergetic effect of the combined radiotherapy and immunotherapy was observed. For the animals with extra-cranial tumours, however, no synergistic effect could be demonstrated for the treatment scheme applied in this particular case. It was hypothesized that this apparent difference was a result of a higher degree of immune suppression in the case of the larger subcutaneous tumours.