Response of intra- and extra-cerebral N29 gliomas in rats to the combination of radiation therapy and immunization

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Introduction

Radiation therapy 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, which may allow for lower radiation doses. In this work, we have studied possible 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 this study, 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 central nervous system of pregnant Fischer rats exposed to ethyl-N-nitrosurea, 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 $3 \cdot 10^6$ IFN-gamma-gene transfected N29 tumour cells. The cells were given a sterilizing dose of 70 Gy just before the injections. Animals with intra-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, $TV=TV_0 \cdot e^{TGR \cdot t}$, where t 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 cm^3, the animals were euthanized.

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

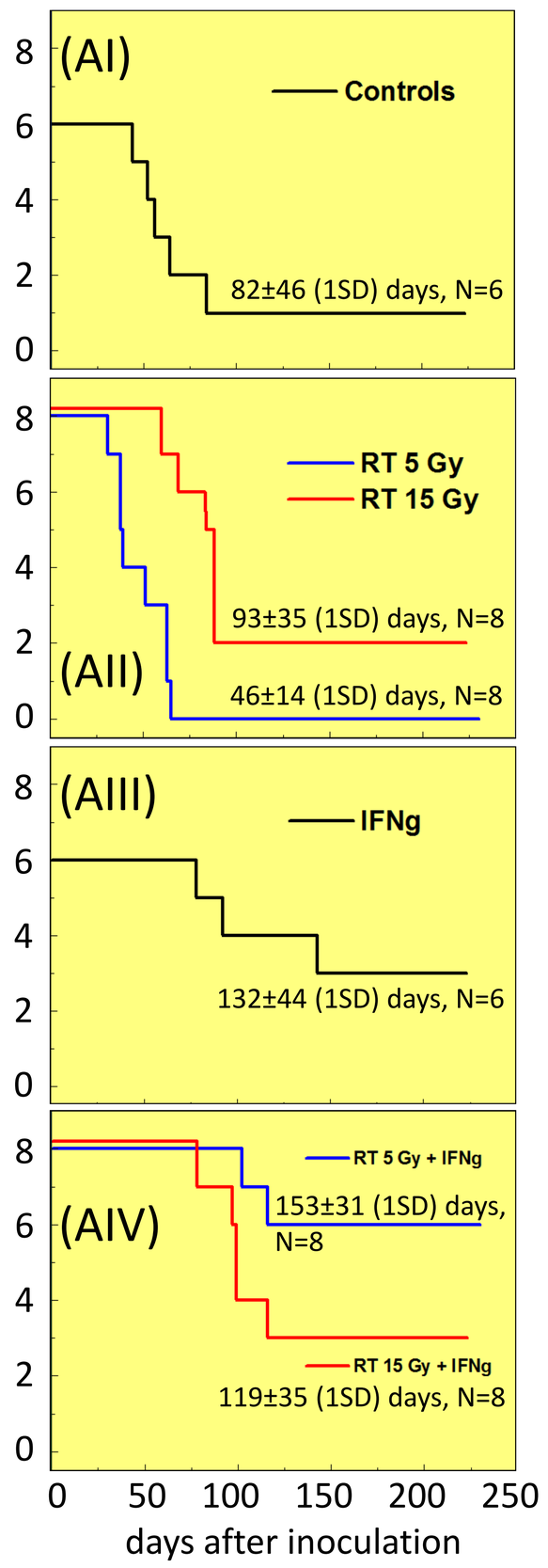

Table 1. Tumour growth rate, TGR, for the rats with extra-cranial N29 gliomas

Treatment group	N	TGR (% $days^{-1}$)
(BI) Untreated controls	40	8.4±0.3 (1SD)
(BII) Radiotherapy alone	15	4.5±0.3 (1SD)
(BIII) Immunotherapy alone	19	7.6±0.6 (1SD)
(BIV) Radiotherapy + immunotherapy	8	5.7±0.5 (1SD)

Results and Discussion

Intra-cranial tumours For the animals with intra-cranial tumours, the number of living animals as a function of time after inoculation is shown in Figure 1 for the 4 different sub-groups. The median survival times are also given in the figure. The survival times of the treated groups were compared to the untreated controls, and analyzed with the Mann-Whitney test using a significance level of α=0.05. It was then found, that RT alone (5 or 15 Gy) had no significant effect on the survival time. IT alone increased the survival time with 60% (p=0.04). The combination of RT at 5 Gy and IT increased the survival time with 87% (p=0.003), yielding 75% complete remissions (p=0.03). Also the 15 Gy RT combined with IT yielded an increased survival rate, although not as effectively as the 5 Gy RT and IT treatment.
Extra-cranial tumours For the animals with extra-cranial tumours, the tumour growth rates (as measured by % per day) derived from the fit of the exponential growth model are displayed in Table 1. The tumour growth rates of the treated groups were compared to the untreated controls, and analyzed with the t-test using a significance level of α=0.05. It was then found, that RT (20 Gy) alone had a significant effect on the tumour growth rate (p<0.0001), while IT alone had no significant effect. The combination of RT and IT also had a significant effect on the tumour growth rate (p=0.001), however, when compared to a hypothetically additive effect of radiotherapy and immunotherapy, there was no observable synergetic effect due to the combined therapy.
Comparison The treatment situations are inevitably quite different in the intra- and extra-cranial cases presented here. Both the dose levels and the timing of the combined treatments differ. In the extra-cranial case, with a large subcutaneous tumour, the immune response is probably systemically suppressed, which may be the reason for the poor effect of immunotherapy in this case. In the intra-cranial case, on the other hand, it is likely that no significant systemic immune suppression had developed, and, hence, there was a larger effect of the immunotherapy and its combination with radiotherapy.

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.

References

B Persson *et al.,* Radiation Research 173:433-440, 2010
B Persson *et al.,* Cancer Therapy 8A, In Press, 2010