Radiation Modulated Immune-Response and Abscopal Effect in Rats with Contra-lateral tumors

Bertil R.R. Persson,1,4 Catrin Bauréus-Koch,1,4 Gustav Grafström,1,4 Per Munck of Rosenschöld,1 Crister Ceberg,2,4 Henrietta Nittby,4 Bengt Widergren,3,4 and Leif G. Sal福德,2,4

1Medical Radiation Physics, 2Neurosurgery, 3Tumour Immunology, 4Raising Laboratory, Biomedical Centre, Lund University, 221 85 LUND, Sweden

Correspondence to: Bertil R.R. Persson, bertil_r.persson@med.lu.se

Introduction

We studied radiation modulated immune response in intracranial N29 tumors by combining a single fraction of radiation therapy (5 as well as 15 Gy) and immunization with interferon-gamma (IFN) transfected immunoogenic tumor cells. In a parallel study we also studied the abscopal effect of radiation therapy in a model of collaterally implanted N29 tumors on both hind legs in rats, with and without the combination of immunization.

Materials and Methods

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

Intracranial tumors, 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.

Extracranial tumors were induced in a total of 81 animals by a subcutaneous inoculation of 200 000 N29 cells into the right hind leg, and 50 000 cells in the left hind leg.

Tumor response 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 extracranial, 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 intracranial tumors were treated on day 7 after inoculation, with a single fraction of 5 Gy or 15 Gy. Animals with extracranial tumors were treated around day 30, with 4 daily fractions of 5 Gy each.

Immunization (IFN) was given as intraperitoneal injections of 3 x 10^7 IFN-gamma-gene transfected N29 tumor cells. The cells were given a sterilizing dose of 70 Gy just before the injections. Animals with intracranial tumors were immunized 1 h after irradiation on day 7 after inoculation. Animals with extracranial tumors were immunized 5 days before RT, and then two more times with 14-day intervals.

Tumor regression. Treatment for the intracranial tumors, the effect of the treatment was evaluated in terms of survival time. The animals were observed daily for symptoms of the growing tumor, and euthanized when the predescribed symptoms appeared (keeping their heads turned to one side, rotating, or losing weight). For the extracranial tumors, the treatment effect was evaluated in terms of tumor growth.

Results and Discussion

Intracranial tumors For the animals with intracranial tumors, the number of living animals as a function of time after inoculation is shown in Figure 1 for the rats given combined treatment with RT and immunization. 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. IFN, alone increased the survival time with 60% (p=0.04). The combination of RT at 5 Gy and IFN, increased the survival time with 87% (p=0.003), yielding 75% complete remissions (p<0.03). Also the 15 Gy RT combined with IFN, yielded an increased survival rate, although not as effectively as the 5 Gy RT and IFN treatment.

Extracranial tumors For the animals with extracranial tumors, see Figure 2, the tumor growth rates (as measured by % per day) derived from the fit of the exponential growth model are displayed in Figures 3 and 4. The tumor 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 tumor growth rate (p<0.0001), while IFN, alone had no significant effect. The combination of RT and IFN, also had a significant effect on the tumor growth rate (p<0.001), however, when compared to a hypothetically additive effect of radiotherapy and immunotherapy, there was no observable synergistic effect due to the combined therapy.

Comparison By treatment of single intracranial N29 glioma tumors with RT in combination with immunization using IFN transfected tumor cells, 75% complete tumor remissions has previously been demonstrated (3). That effect of radiation was related to diminishing of the tumor’s immune-suppression and enhanced infiltration of activated T-cells affecting the tumor. In the present extracranial model with two contra-lateral tumors it was hypothesized that activated T-cells should also affect the left lateral un-irradiated tumor. The results of RT alone appear as an abscopal effect with TGR decrease also in the contra-lateral un-irradiated tumor which account for the abscopal effect. But in this model no significant abscopal effect was found by radiotherapy combined with immunization using transfected tumor cells. This might indicate that factors other than immunological are responsible for the radiation induced abscopal effect.

References

18 Persson et al., Radiation Research 173:433-440, 2010