WHOLE-BODY LOW DOSE IRRADIATION PROMOTES THE EFFICACY OF CONVENTIONAL RADIOTHERAPY OF CANCER AND IMMUNOLOGICAL MECHANISMS

Jilin University Medical Center, Changchun 130021, China
Correspondence to Prof. Shu-Zheng Liu, email:drliusz@yahoo.com

1. Introduction

Radiotherapy is the most commonly used local treatment of cancer. However, the large dose needed for local control often limits its successful use. In view of the stimulatory effect of low dose radiation (LDR) on anticancer immunity [1] an experimental study of the effect of whole-body irradiation (WBI) of low doses on the outcome of conventional local radiotherapy of cancer was designed with an aim at reducing the total dose and promoting treatment efficacy. Since radiation doses as low as 0.05–0.1 Gy could stimulate the expression of genes downstream of Egr-1 promoter [2, 3] and gene therapy with Egr-IL-18-B7.1 in combination with local X-rays showed better control of mouse melanoma than local radiotherapy alone [3], it is desirable to study the effect of introduction of this plasmid in melanoma and Lewis lung cancer models in combination with conventional radiotherapy combined with WBI of low doses. Here we report on the effect of WBI with low doses combined with conventional radiotherapy or gene radiotherapy on reduction of radiation dose together with better cancer control and its possible immunological mechanisms.

2 Materials and methods

2.1 Animal models

C57BL/6J mice were used in all the experiments. Cancer cell implantation was given subcutaneously in the hind leg with \(10^6\) cells in 50 μl saline using either B16 melanoma (B16) or Lewis lung cancer (LLC) cells. The treatment was started on day 10 after implantation when the tumor size reached 100–150 mm³ with X-irradiation the details of which will be given in the following section.

2.2 Treatment protocols

Local radiotherapy was performed with a deep X-ray machine at the dose rate of 0.287 Gy/min and fraction doses of 2 or 5 Gy. Whole-body irradiation (WBI) with low doses was administered at the dose-rate of 12.5 mGy/min with doses of 0.075 or 0.1 Gy in each session. Combined gene therapy was instituted in certain groups by intratumor injection of 10 μg recombinant plasmid pEgr-mIL-18-B7.1 (E18B) using 50 μl polyethylenimine (PEI) for transfection with the control receiving intratumor injection of 50 μl 0.9% saline. Tumor volume was measured every 2 days after termination of treatment.

2.3 B16 Melanoma modal

A 1-week treatment protocol was first tried in a pilot study with B16 model. Four groups were set up: 1) Control: no treatment; 2) E18B: injection of plasmid pEgr-mIL18-B7.1; 3) E18B+5Gy x 3 and 4) E18B+5Gy x 1+0.1Gy x 2. Radiotherapy was started 24 h after plasmid injection and repeated every other day. Observation was continued for 19 days after termination of treatment.

2.4 Lewis lung cancer modal

Two protocols were set up for the treatment of LLC-bearing mice. The first consists of 5 groups treated for 2 weeks and observed for one month after termination of treatment: 1) Control: no treatment; 2) 2 x (5Gy x 3); 3) 2 x (5Gy x 1+ 0.075Gy x 2); 4) 2 x (E18B +5Gy x 3); 5) 2 x (E18B+5Gy x 1+0.075Gy x 2). The second protocol consists of 5 groups using the same schedule as in the first protocol but using a local dose of 2 Gy instead of 5 Gy.
2.5 Mechanistic studies

Separate groups of mice were used for studies on the possible immunologic mechanisms of the therapeutic effects. Mice were treated as in the different protocols and sacrificed on days 1, 3 and 5 after termination of treatment. The spleen was taken to prepare cell suspensions to examine the NK activity against Yac-1 cells, CTL activity against B16 or LLC as well as secretion of interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) using methods reported previously [4]. The expression of Lamp-1 (CD107a) was assayed with flow cytometry using a FACScan machine as reported [5]. Four to five animals were used in each group.

3. Results and discussion

3.1 B16 model with one week treatment

In the B16 melanoma model gene radiotherapy with transfection of the plasmid pEgr-mIL-18-B7.1 followed by local X-irradiation with 5 Gy every other day for 3 sessions (total dose 15 Gy) resulted in marked retardation of tumor growth in comparison with both the control (no treatment) and the gene only (E18B) groups. Substitution of the second and third local doses of 5 Gy with 0.1 Gy WBI showed a similar effect as demonstrated in the lower two curves in the left panel of figure 1. It is known that IL-18 exerts its anticancer effect through inducing the secretion of IFN-γ [6] and B7.1 (CD80) expression increases the immunogenicity of cancer cells [7]. The plasmid pEgr-mIL-18-B7.1 was used in the present study since it has previously been proven that radiation doses from 0.05 to 5 Gy could stimulate the expression of the genes downstream of Egr-1 promoter [2, 3]. Injection of E18B not followed by radiation had only an insignificant effect on cancer growth (second curve in the left panel of figure 1).
3.2 LLC model treated with 5 Gy x 6

In the LLC model the protocol of gene radiotherapy was compared to local radiotherapy with or without low dose WBI. As seen in figure 2, substitution of 4 local doses of 5 Gy with 4 doses of WBI of 0.075 Gy produced the same effect of retardation of tumor growth. Since the survival rate of tumor-bearing mice in the control group (no treatment) dropped to 1/8 to 2/8 beginning from d20 after termination of treatment, so statistical analysis between the treated groups and the control could only be made up to d18. Among the 4 treated groups there was no statistical difference in the tumor volume in the whole course of observation. That is to say, substitution of 4 local doses of 5 Gy with 4 doses of 0.075 Gy WBI produced the same effect of tumor control in the presence or absence of gene therapy. That is to say, about 1/3 of the total dose produced the same therapeutic effect.

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### Figure 2  Tumor growth curves of Lewis lung cancer treated with a 2-week regimen of 5 Gy sessions

3.3 LLC model treated with 2 Gy x 6

The same setting as that in section 3.2 was followed in another experiment except the change of local doses of 5 Gy to 2 Gy in each treatment session. The results are shown in figure 3. As seen in the left upper panel of this figure, 2 Gy x 6 given to the tumor mass in 2 weeks caused significant retardation of tumor growth after termination of treatment, and substitution of the second, third, fifth and sixth doses of 2 Gy with WBI of 0.075 Gy increased the efficacy of tumor control with the tumor volume on the 30th day after termination of treatment being only 40.6% of that in the 2 Gy x 6 group ($P<0.05$). The right upper panel shows the result of treatment protocol as that in the left upper panel but with introduction of pEgr-mIL-18-B7.1 before irradiation. In this case there was a further increase in cancer control, with the tumor
volume being 61.4%, 53.5%, 56.5% and 60.5% of that in the 2 Gy x 6 group on d10, d12, d14 and d16, respectively (P<0.05). At the end of observation for 30 days after termination of treatment the tumor volume was only 39.1% of that in the 2 x (E18B+2Gy x 3) group (P>0.05 due to greater variation of tumor volume and decrease in number of surviving mice toward the end of this period). In the left and right lower panels of figure 3 the treatment result of the protocols with and without introduction of gene therapy was compared. In the presence of pEgr-mIL-18-B7.1 the tumor control was as a whole better than that in the corresponding protocol without the plasmid, especially on d16 through d24 for the 2 x (E18B+2Gy x 3) group, and on d12 and d14 for the 2 x (E18B+2Gy+0.075Gy x 2) group.

Figure 2  Tumor growth curves of Lewis lung cancer treated with a 2-week regimen of 2Gy sessions with or without gene therapy and low dose WBI (Explanation in text)

Figure 3 shows the immunological changes in the spleen of different groups of the 2 Gy protocol from d1 to d5 after termination of treatment. The NK activity on d1 after termination of treatment is shown in the left upper panel of this figure and CTL activity on d3 is shown in the right upper panel of the figure (no changes were observed on other days). It seems that NK activity was stimulated promptly and briefly after treatment, with the group receiving 2 x (E18B+2Gy+0.075Gy x 2) demonstrating the most prominent activation and CTL activity only became markedly enhanced on d5 after treatment, also with the group receiving 2 x (E18B+2Gy+0.075Gy x 2) showing more marked changes. As shown in the left lower panel TNF-α secretion was up-regulated with time in the tumor control and all the treated groups, and the magnitude of the changes showed the following relationship: 2 x (E18B+2Gy+0.075Gy x 2) > 2 x (E18B+2Gy x 3) > 2 x (2Gy+0.075Gy x 2) > 2 x (2Gy x 3). This may implicate that low dose WBI could stimulate host immunity as well as the plasmid in the cancer cells resulting in significant immune up-regulation. Lamp-1 (lysosomal-associated membrane protein-1) in the NK cells and CD8+ T cells are related to the enzymes perforin and granzyme which are important in cancer killing by these
immune cells [8, 9]. As shown in the right lower panel the expression of Lamp-1 was stimulated in response to tumor implantation (column B) and there was no further up-regulation in all treatment groups (columns C, D, E, F). This is probably due to the fact that the granules are further mobilized when the immune cells are in contact with the targets and in the present study no target cells were added in the assay preparations. But the group with introduction of E18B and substitution of 4 doses of 2 Gy with 0.075 Gy WBI (F) showed the most prominent changes on d5.

From these experimental studies it is evident that WBI with low doses can be made use of in the planning of cancer treatment and other measures such as gene therapy up-regulating host anticancer immune responses could be added on this basis to further promote the efficacy of cancer radiotherapy.

4. Conclusion

From data presented in the above sections a few tentative conclusions may be drawn as follows:

(1) Whole-body X-irradiation with low doses (0.075 to 0.1 Gy) substituting 2 doses of 5 Gy in the 5 Gy x 3 protocol (melanoma) or substituting 4 doses of 5 Gy in the 5 Gy x 6 protocol (Lewis lung cancer) could produce the same anticancer effect as the original 15 Gy or 30 Gy protocols as revealed by the overlapping tumor growth curves (figures 1 and 2).
(2) Whole-body X-irradiation with low doses (0.075 Gy) substituting 4 doses of 2 Gy in the 2 Gy x 6 protocol (Lewis lung cancer) showed a better control of cancer growth than that in the original 12 Gy protocol, suggesting the possibility of optimizing cancer radiotherapy by decreasing the total radiation dose with increase of efficacy at the same time (figure 3).

(3) The introduction of gene therapy in the treatment protocols of 2 Gy x 6 and 2 x (2 Gy+0.075 Gy x 2) could further enhanced the anticancer effect as shown by slower cancer growth rate (lower panels of figure 3).

(4) As a whole, by using WBI with low doses a reduction of total local doses by two thirds could achieve the same or even better therapeutic effect. The improvement of anticancer effect seems to be related to the stimulation of immune response by the treatment modalities, especially in the case of low dose radiation in combination with gene therapy,

(5) Further experimental work with different treatment protocols is needed to optimize radiotherapy of different cancer types in order to translate the results of laboratory studies into possible clinical practice.

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6. References


