The effect of radiation therapy on fungal growth: results of in vitro and in vivo studies

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Abstract

Objectives. To investigate the effect of radiation therapy (XRT) on the growth of Candida and Cryptococcus.

Methods. (I) In vitro study: suspensions of Candida and Cryptococcus were irradiated by 0–20 Gy, and colony forming units (CFUs) were counted after 2 and 11 days. (II) In vivo study: (A) The XRT effect—Balb/c mice were injected with Candida or Cryptococcus and irradiated by 5 or 10 Gy. Homogenates of their kidneys or brains were cultured and CFUs were counted two days later. (B) Toxicity—Balb/c mice were injected with Candida and irradiated by 5 Gy. Control mice were either injected or irradiated. Overall survival was documented for all animals.

Results. (I) The CFU counts in the irradiated and non-irradiated mice were similar at days 2 and 11. (II) (A) The CFUs were significantly low for both Candida and Cryptococcus injected and irradiated mice \((p<0.02)\). (II) (B) The overall survival of the injected mice was not affected by the additional irradiation and it was inferior to the irradiated mice only.

Conclusions. XRT has a beneficial inhibitive effect on the in vivo but not the in vitro growth of fungi. It does not decrease the survival rate of injected mice. Clinical studies in selected patients with resistant invasive fungal infection are warranted.

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Introduction

Resistant invasive fungal infection is currently treated by a new generation of anti-fungal medication with a relatively high beneficial effect. Nevertheless, patients, especially those in an immunocompromised state, might have a resistant fungal infection and might succumb to their
disease. Ablative surgery is considered in some patients, but it may be indicated in only a small number of patients who will be left with major deformities.

Radiation therapy (XRT) has successfully replaced ablative surgery in various cancers, such as head and neck and breast tumours. It is being frequently used in various benign disorders when no efficient alternative treatment is available. Its potential implementation in patients with resistant fungal infection raises several questions. Will it decrease the anti-fungal activity of the self-defense processes and thereby enhance fungal growth or will it mainly inhibit the fungal growth and thus assist the self-defenses to overcome the infection?

The aims of this work were (a) to evaluate the effect of low dose XRT on fungal growth in in vitro and in vivo (murine) studies and (b) to monitor whether XRT has a detrimental affect on infected mice.

Material and methods

In vitro study

Suspensions of Candida albicans and Cryptococcus at a concentration of $1 \times 10^5$ (measured by a hemacytometer and colony forming units [CFUs]) in 1 ml were irradiated by a single fraction of 2.5, 5, 7.5, 10, 15 and 20 Gy using a gamma cell cobalt apparatus. Viability was tested by vital staining and colony counts after culture in Sabouraud Dextrose Agar (SDA) before and after XRT. Control cultures were treated identically but without being irradiated. Cultures of 0.1 ml of the suspensions in SDA were performed on the day of XRT and the CFUs were counted on days 2 and 11 following XRT exposure. Three replicates in each XRT dose were used.

In vivo study

(A) The XRT effect: Male balb/c mice, 6-7 weeks old, were injected intravenously with either Candida or Cryptococcus at a dose of $2.5 \times 10^5$ and $1 \times 10^6$, respectively. Before the injection, the viability of the fungi was tested by vital staining and colony count after having been cultured in SDA. Twenty-four hours after the day of injection (day 1), the mice (10 in each group) were either irradiated (5 or 10 Gy) or monitored, and all were sacrificed on the second day after infection (day 2). Homogenates were made of the kidneys (in the Candida injected mice) and the brain (in the Cryptococcal injected mice) and suspended in 5 ml of phosphate buffered solution (PBS). In addition, 0.5 ml of the suspension was cultured in SDA. The CFUs were counted on days 5 and 10. (B) The purpose of this toxicity study was to find out whether delivering XRT might shorten the survival rate of the infected mice. Male balb/c, 6-7 weeks old, were injected intravenously with Candida at a dose of either $2.5 \times 10^5$ or $5 \times 10^5$ and irradiated at a dose of 5 Gy (group A, $n=10$) 24 h later. Similarly injected mice did not receive XRT and served as a control group ($n=10$). A third group of 10 mice was irradiated to a same XRT dose (5 Gy) with no Candida injection. All the mice were monitored for their overall survival.

Statistical analyses and ethical consideration

The two-sided Student’s $t$-test was used for comparison between the groups. Survival was recorded as of the time of fungal injection until the day of expiration. The study was approved by the Hadassah-Hebrew University-School of Medicine Animal Ethical Committee.

Results

In vitro study

The CFU counts of the non-irradiated Candida were 50 and 37 at days 2 and 11, respectively. Similar results were noted in all irradiated groups at day 2 (range 48-63) and at day 11 (range 24-31). The difference between the irradiated and the non-irradiated groups at day 11 did not reach a level of statistical significance.

CFU counts of the non-irradiated Cryptococcus were 58 at day 2 and 28 at day 11. Similar counts of the irradiated groups were seen at day 2 (range 46-61) and at day 11 (range 14-62). Although the number of CFUs did not reach a statistical significance, there was a smaller diameter of CFU in the ≥5 Gy-irradiated groups.

In vivo study

Candida: The median and mean numbers of CFUs in the control mice at day 5 were 30 and 36 (range 22-60) per kidney, respectively. For the irradiated mice, the respective median and mean numbers of CFUs were 13 and 13 (range 8-18) and 14 and 16.7 (range 6-33) for the 5 and 10 Gy doses, respectively. A comparison of CFU counts of the 5 and 10 Gy irradiated groups to the non-irradiated group of
mice revealed a significant statistical difference (p values of 0.02 and 0.05, respectively).

Cryptococcus: The median and mean numbers of CFUs in the control group at day 10 were 40 and 46 (range 27–77) per brain, respectively. The median and mean counts of CFUs in the 5 Gy-irradiated mice were 22 and 29.4 (range 14-52) at day 10, respectively. No CFUs were observed in the mice irradiated with 10 Gy at day 10. A comparison between the CFUs of the non-irradiated and the irradiated groups revealed a p value of 0.025 and 0.005 at 5 and 10 Gy, respectively. No CFUs were observed in the mice irradiated with 10 Gy at day 10. A comparison between the CFUs of the non-irradiated and the irradiated groups revealed a p value of 0.025 and 0.005 at 5 and 10 Gy, respectively.

At a concentration of 5×10⁵, all Candida infected mice (with and without XRT) died within 8 days. No differences of time to death were noted between the injected and irradiated mice and the injected only mice. At a concentration of 2.5×10⁵, two out of five mice in both the injected and irradiated and injected only groups were alive after 2 months. All the irradiated only mice were alive after 2 months.

Discussion

XRT has a documented inhibitory effect on the growth of fungi.²⁻⁵ It had been successfully used to eradicate Tinea Capitis but was later replaced by an active anti-fungal medication that was more convenient to administer. Currently, XRT has a major role in sterilization processes,⁶⁻⁸ but at a high does range. In this study, we evaluated the inhibitory effect of XRT in a low dose range and found that there was an inhibitory effect in the growth of Candida and Cryptococcus in the in vivo but not in the in vitro studies. In mice injected with Candida, the median counts of CFUs after exposure of 5 and 10 Gy were 13 and 14, respectively, while it was 30 in the control arm. In the Cryptococcus injected mice, the CFUs were 22 and 0 after 5 and 10 Gy, respectively, and 40 in the control arm. The differences in both injected groups were statistically significant. Although XRT was reported to have an inhibitory effect on various anti-inflammatory processes⁹ and, by doing so, possibly increase the mortality rate of the injected mice, we found no such excessive mortality rate in the irradiated and injected mice. Our results showed that the overall survival of the injected mice was similar, whether they were irradiated (a total of 5 Gy) or not. A longer survival might probably be expected with XRT does escalation (as it might be suggested for the Cryptococcus), however XRT in a low dose range should only be considered as a temporarily inhibitor of the fungal growth.

In 21 patients who had invasive fungal infection (IFI) before or at the time of hematological stem cell transplantation, we found that those who were exposed to whole body irradiation had a better outcome (unpublished data). Although quite rare, a concern in using XRT in benign disorders is the development of secondary malignancy.¹⁰⁻¹² Nevertheless, a substantial number of benign diseases are being treated with a short duration low total dose XRT¹³ and its beneficial effect seems worthwhile.

We conclude that low dose XRT is effective in the inhibition of fungal growth in mice. This finding might lead to clinical studies in which XRT will be evaluated in patients with end stage resistant IFI.

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