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The Etiology of Treatment-related Lymphopenia in Patients with Malignant Gliomas: Modeling Radiation Dose to Circulating Lymphocytes Explains Clinical Observations and Suggests Methods of Modifying the Impact of Radiation on Immune Cells

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Abstract

Purpose—Severe treatment-related lymphopenia (TRL) occurs in 40% of patients with high grade gliomas (HGG) receiving glucocorticoids, temozolomide, and radiation. This occurs following radiation, persists for months, and is associated with reduced survival. As all three treatment modalities are lymphotoxic, this study was conducted to estimate the radiation dose that lymphocytes receive passing through the radiation field and if this could explain the observed TRL.

Materials and Methods—A typical glioblastoma plan (8-cm tumor, 60 Gy/30 fractions) was constructed using the PinnacleTM radiation planning system. Radiation doses to circulating cells (DCC) were analyzed using MatLabTM. The primary endpoints were mean DCC and percent of circulating cells receiving 0.5 Gy. The model was also used to study how changes in target volumes (PTV), dose rates, and delivery techniques affect DCC.

Results—The modeling determined that while a single radiation fraction delivered 0.5 Gy to 5% of circulating cells, after 30 fractions 99% of circulating blood had received 0.5 Gy. The mean DCC was 2.2 Gy and was similar for IMRT, 3D-conformal techniques, and different dose rates. Major changes in PTV size affected mean DCC and percent of circulating cells receiving 0.5 Gy.

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DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Conclusions—Standard treatment plans for brain tumors deliver potentially lymphotoxic radiation doses to the entire circulating blood pool. Altering dose rates or delivery techniques are unlikely to significantly affect DCC by the end of treatment. Novel approaches are needed to limit radiation to circulating lymphocytes given the association of lymphopenia with poorer survival in patients with HGG.

Keywords

Lymphocytes; Brain Radiation; Normal Tissue Toxicity; High-Grade Gliomas; Glioblastoma; Lymphopenia; Survival

INTRODUCTION

Marked reductions in the lymphocyte count are common following therapy for malignant glioma and can have significant clinical consequences. Severe lymphopenia in this setting has been associated with serious opportunistic infections (1, 2). Perhaps more importantly, emerging data from patients with malignant gliomas and pancreatic cancer demonstrate that patients with severe treatment-induced lymphopenia have significantly worse survival and die from early tumor progression (3, 4).

Interest in treatment-induced lymphopenia was sparked by a cluster of *Pneumocystis jiroveci* pneumonia cases, associated with extremely low CD4 counts, in patients treated with radiation therapy and steroids (without chemotherapy) for brain tumors (1). This led to a prospective study of serial total lymphocyte and CD4 counts in brain tumor patients receiving radiation and corticosteroids. This demonstrated that CD4 counts were $>450/\mu\text{L}$ in all patients before starting therapy, but that during treatment, approximately one-fourth developed CD4 counts $<200/\mu\text{L}$ (5). After temozolomide became standard therapy, a second study prospectively evaluated serial lymphocyte counts in high-grade glioma patients receiving radiation and temozolomide. In this study, over 40% of patients developed CD4 lymphocyte counts under $200/\mu\text{L}$ 2 months after completing treatment and over 70% had CD4 counts under $300/\mu\text{L}$. Participants with CD4 counts $<200/\mu\text{L}$ 2 months after initiating radiation and temozolomide had significantly worse overall survival than those with higher CD4 counts (4).

Although patients with malignant glioma receive a triad of lymphotoxic agents (corticosteroids, temozolomide, and radiation therapy), radiation may play an important role in lymphopenia. Lymphopenia following radiation therapy was first described in the early 20th century, only a few years after x-rays were discovered, and has since been documented to occur after either external beam radiotherapy or brachytherapy directed to virtually every part of the body (6). Radiation can induce lymphopenia regardless of whether chemotherapy or steroids are given concurrently or whether bone marrow or lymphatic tissue is included in the field. For example, irradiation of the brain, which contains neither bone marrow nor lymphatic tissue, can cause over a 60% reduction in the lymphocyte count (7). Furthermore, even radiation of extracorporeal blood in patients undergoing renal dialysis can result in profound and durable lymphopenia (8). These observations suggest that irradiation of

circulating lymphocytes may contribute to the development of radiation-induced lymphopenia.

In order to further evaluate the etiology of treatment related lymphopenia, we used modern radiation planning software and mathematical modeling to estimate the dose of radiation delivered to circulating blood cells during a typical 60-Gy radiation treatment course for glioblastoma, a nonmarrow, nonlymphatic containing site. We subsequently used our model to investigate changing treatment parameters that could affect the dose to circulating lymphocytes such as target volume, dose rate, and radiation delivery technique.

METHODS AND MATERIALS

Model design

A normal CT scan of the head was loaded into the treatment planning system, and a spherical tumor volume centered in the falx superior to the lateral ventricles was created. Dose to the brain was calculated using the PinnacleTM radiation treatment planning system (Version 9.0, Philips Inc., Madison, WI). The entire brain was also delineated as a region of interest. A treatment plan was then generated resulting in a calculation for the dose, voxelized in 0.4 mm³ voxels. Using Pinnacle scripts, the three-dimensional dose grid for the brain was extracted and analyzed using an in-house program written in MatLab (version R2011b, Mathworks, Inc., Natick, MA). Several treatment plans were considered. Unless otherwise noted, the results below are for a four-field conformal plan that treats the PTV to a homogeneous dose. For comparison, a 3D-conformal plan using wedges and five fields was calculated, as was an intensity-modulated radiotherapy (IMRT) plan using a 2-cm wide sliding window and an inverse planning approach. In each case, the dose was extracted beam-by-beam (for IMRT, segment-by-segment).

The model uses as its input the dose to the brain during a normal course of RT then calculates the associated dose to blood circulating through this radiation field using the following assumptions: (a) 16% of cardiac output goes to the brain; (b) blood flow is evenly distributed, flowing inferiorly to superiorly through the dose grid; (c) blood flow velocity is 10 mm/s; and (d) the total volume of blood is 5 L (9). Furthermore, we assumed that blood could pass through the beam multiple times but that during the duration of a single beam and/or segment, blood does not reenter the treatment field. This latter assumption breaks down with beam times that are longer than the 30-s heart-to-heart circulation time, but this only affected our calculations at the very lowest dose rates. Between beams and between treatment fractions, the blood was considered to reenter the treatment field, and the cumulative dose was calculated via a convolution of blood pool dose histograms. Within the MatLab code, we could control the dose rate, expressed in monitor units (MU)/min and the dose delivered per fraction by scaling the total number of monitor units delivered.

The mean dose to the total blood pool was calculated, and the total volume of blood receiving greater than 0.5 Gy was quantified. This latter dose cutoff was chosen based on *in vitro* data on lymphocyte radiosensitivity that showed a D₁₀ (dose required to reduce the surviving lymphocyte population to 10% of initial values) of ~3 Gy, a D₅₀ of ~2 Gy, and a D₉₀ of ~0.5 Gy (10). The model was used to calculate these dose parameters for PTVs of

two sizes: 2-cm diameter (4.2 cm³ volume) and 8-cm diameter (268 cm³ volume), plans administered at varying dose rates (300, 600, and 1,200 MU/min), and radiation techniques (IMRT and 3D-conformal).

RESULTS

Using the technique described above, we modeled the radiation dose delivered to circulating blood during a typical partial intracranial field in an effort to determine the role of radiation in the observed lymphopenia. The effects on dose to circulating blood after varying different treatment-related parameters in the model are described below.

Dose to circulating blood pool as a function of number of fractions

The model indicates that a single fraction (2 Gy) delivered 0.5 Gy to 4.6% of the total blood pool. After 10 fractions (20 Gy), 61.5% of the blood pool received 0.5 Gy, and after 20 fractions (40 Gy), 92.2% of the blood pool received 0.5 Gy. By the end of a typical 60-Gy radiation treatment plan to the brain (2 Gy $\times$ 30 fractions to an 8-cm diameter field), 98.8% of all circulating blood receives at least 0.5 Gy. Mean dose to the blood pool was 2.2 Gy for a 60-Gy course with a PTV of 268 cm² at a dose rate of 600 MU/min. According to the model, as the total dose and the number of fractions increase, the percentage of blood receiving 0.5 Gy increases rapidly. Figure 1 shows the percentage of the blood pool receiving at least 0.5 Gy in terms of the number of fractions and total dose administered (8-cm diameter/268-cm³ PTV, dose rate 600 MU/min).

Dose to circulating blood pool as a function of dose rate

Increasing the dose rate effectively shortens the treatment time, thereby reducing the number of lymphocytes passing through the radiation field. An increased dose rate (i.e., a shorter beam time), therefore, increases the percentage of the blood pool receiving a low dose. As shown in Figure 2, the percent of the blood pool receiving 0.5 Gy was less with higher dose rates at the beginning of treatment. However, once 30 fractions have been administered, the percentage of blood receiving 0.5 Gy approached 100% for all dose rates tested. Only minor dose-rate-dependent differences were observed when using the mean dose as an endpoint; a mean dose of 2.2 Gy was calculated for both 600 MU/min and 1,200 MU/min plans, while a mean dose of 3.1 Gy was calculated for 300 MU/min plans.

Dose to circulating blood pool as a function of treatment technique

The model was used to compare dose to the blood pool for IMRT and 3D-conformal treatment techniques. By the end of a 60 Gy plan administered in 30 2-Gy fractions to an 8-cm diameter PTV, no differences were observed in the mean dose to the blood pool or in the proportion of blood receiving 0.5 Gy. Mean dose to the blood pool was 2.4 Gy for the tested 3D-conformal plan and 2.7 Gy for the tested IMRT plan. In both cases, nearly all of the blood received at least 0.5 Gy after 30 2-Gy fractions.

Dose to circulating blood pool as a function of target volume size

Major differences in target volume size had a significant impact on the mean dose to the blood pool, particularly at higher doses, as shown in Figure 2. For a 60-Gy plan

administered in 30 fractions, the mean blood dose was 2.2Gy for an 8-cm diameter PTV but was 0.3 Gy for a 2-cm diameter PTV. These data were also analyzed in terms of the percent of blood receiving at least 0.5 Gy, as shown in Figure 3. Smaller PTV size decreased CBD. For a 2-cm diameter PTV (volume = 4.2 cm³) receiving 60 Gy, 15% of the blood received 0.5 Gy; however, for the 8-cm diameter PTV (volume = 268 cm³), 99% of blood received 0.5 Gy.

DISCUSSION

This study demonstrates that the entire circulating blood pool receives a potentially lymphotoxic dose of radiation during a typical external beam radiation treatment course for glioblastoma. Our model estimates the mean dose to circulating lymphocytes is approximately 2 Gy, and that nearly all the circulating blood receives at least 0.5 Gy. Even though this radiation dose may be relatively low compared to the total dose received by the targeted tumor, it may still be sufficient to eliminate a large number of lymphocytes. These cells are among the most radiosensitive in the body, with a D₁₀ of approximately 3 Gy and a D₅₀ of about 2 Gy (10). The model also demonstrates that neither increased dose rate nor choice of treatment technique (IMRT vs. 3D-CRT) significantly changes the radiation dose to circulating lymphocytes. However, major decreases in target volume size would result in a significant decrease in the dose to circulating lymphocytes.

It is likely that irradiation of the circulating blood plays a significant role in the pathophysiology of radiation induced lymphopenia. Although irradiation of the bone marrow or lymph nodes may contribute to lymphopenia, irradiation of structures such as the brain that contain neither lymphatic tissue nor bone marrow can also cause lymphopenia. Irradiation of the circulating blood alone via a radioactive source mounted within a dialysis unit can also cause lymphopenia, with decreases of over 60% in the circulating lymphocyte count persisting for over 2 years in patients undergoing irradiation of the circulating blood in preparation for renal transplant (8).

Lymphocytes are well known to be radiosensitive, and are the only highly radiosensitive nonproliferative tissue (11). Even low-dose total body radiation exposures cause decreases in the circulating lymphocyte count and detectable cytogenetic abnormalities in lymphocytes that can persist for up to 10 years following radiation exposures (12). The exact mechanisms underlying lymphocyte radiation sensitivity are not well understood. Lymphocytes appear to have a decreased DNA repair capacity, possibly related to the active DNA recombination that aids in the development of an individual's immune repertoire (12). B-lymphocytes are slightly more radiosensitive than T-lymphocytes, and naïve T lymphocytes appear to be more radiosensitive than memory cells (13). The difference in radiosensitivity between naïve and memory T-cells is a potentially important phenomenon in terms of immune control of cancer. Malignant tumors represent a novel challenge to an individual's immune system, and depleting the T cell population that responds to novel antigens (i.e., naïve T cells) may contribute to decreased immune surveillance of malignant cells and capacity for tumor control.

Lymphocytes are important mediators of the immune response to cancer, and treatment-induced lymphopenia is associated with worse disease-specific survival in high-grade gliomas and in both resectable and locally advanced/unresectable pancreatic cancer (3, 4, 14). Most current immunotherapy strategies rely on modulation of lymphocyte activity for efficacy, and it is plausible that treatment-induced lymphopenia could impede efforts to enhance the immune response to malignancy. For example, sipuleucel-T, the recently approved prototypic cancer cell vaccine, relies on stimulating a patient's own T lymphocytes to attack prostate cancer cells using a chimeric antigen consisting of prostatic acid phosphatase (PAP) fused to the immune stimulant G-CSF (15). Ipilimumab, which inhibits the lymphocyte regulatory receptor CTLA-4, has been found to prolong survival in patients with metastatic melanoma and is the subject of considerable clinical interest. Preliminary data suggest that the efficacy of ipilimumab may be dependent on circulating lymphocyte count, with lymphopenic patients being significantly less likely to respond to ipilimumab (16).

In this study, we sought to identify alterations in radiation treatment techniques that could modify the dose to circulating blood. Changing the dose rate seemed to be a promising technique, as higher dose rates decrease treatment time and thus the length of time that circulating blood would be exposed to the radiation beam. However, altering the dose rate had a limited ability to spare circulating lymphocytes. Although higher dose rates were associated with lower dose to the circulating lymphocytes early in treatment, these differences disappeared by the 10th fraction for changes in the dose rate. This study was also designed to investigate whether IMRT would increase the circulating blood dose compared with three-dimensional conformal radiation therapy (3D-CRT). IMRT creates highly conformal plans at the target but usually results in a larger area of tissue receiving low doses of radiation compared with 3D-CRT (17). However, the model showed that doses to circulating blood appeared to be similar regardless of whether IMRT or 3D-CRT was used. Changing the target size appears to have a larger impact on the dose to circulating blood; a 2-cm diameter PTV (volume = 4.2 cm³) resulted in 15% of the blood receiving 0.5 Gy, whereas an 8-cm diameter PTV (volume = 268 cm³) resulted in 99% of the blood receiving the potentially lymphotoxic dose of 0.5 Gy. However, such small PTVs may not be appropriate for many patients.

The data presented above on field size, dose rates, and delivery techniques suggest that changing the delivery of radiation in an effort to spare circulating blood from exposure to focal radiation will be challenging. However, our data also suggest several strategies to minimize the radiation dose to circulating blood. One of the most promising is hypofractionation, or increasing fraction size while decreasing the total number of fractions given. In the mid-1970s, MacLennan et al. demonstrated that lymphocyte count was inversely proportional to the number of fractions administered when the total radiation dose was held constant (7). This finding is intuitive and is corroborated by our modeling study results. Because of the clear dependence of dose to circulating blood on PTV size and the number of fractions given, combining hypofractionated treatment plans with smaller target volumes (as in stereotactic radiosurgery) is a promising approach to decrease the dose to circulating blood. A detailed model of hypofractionated treatments would be required to confirm the benefit of such treatments because of the long beam-on times, which would

require consideration of reentry of blood into the treatment field during therapy. These strategies also may not be suitable for all patients due to large primary tumor sizes or other factors, including the importance of maintaining appropriate chemotherapy dosing schedules. Other strategies could focus on radioprotective agents or immune reconstitution.

Limitations of this model include the fact that estimating the dose to circulating blood is challenging and can be affected by estimates of blood flow velocity, volume of blood within the target, and recirculation. The model makes simplifying assumptions about all of these factors. The analysis was also unable to account for the continuous regeneration of circulating blood cells from the stem cell compartment (which may be impacted by long-term temozolomide therapy) and the fact that lymphocytes are continually redistributed among different organs, including the spleen, bone marrow, circulating blood, and lymph nodes. Although some regeneration of the lymphocyte population may occur between radiation fractions, it is important to note that radiation-induced lymphopenia is prolonged and can last up to 10 years after irradiation. Other factors, such as abnormal cytokine responses to lymphopenia, may deplete an individual's capacity to regenerate the lymphocyte pool after radiation. Further investigation into the pathophysiology of radiation-induced lymphopenia is clearly needed. Finally, variations in treatment technique (such as the use of a vertex field) may result in an expanded low-dose radiation field and affect lymphocyte counts. Nevertheless, we believe that this model provides an important basis for further investigations. Because standard radiation fields result in lymphotoxic doses of radiation to circulating lymphocytes and because posttreatment lymphopenia is associated with opportunistic infections as well as poorer disease-specific survival in cancer patients, further studies aimed at evaluating new ways to limit the effect of radiotherapy on circulating lymphocytes are critical.

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Figure 1.
Percent of blood receiving >0.5 Gy plotted against total dose/number of fractions administered. Dose rate = 600 MU/min; PTV diameter = 8 cm (volume = 268 cm³); 2 Gy/fraction.

Figure 2.
Percent of blood receiving >0.5 Gy with varying dose rates (300, 600, and 1,200 MU/min).
PTV diameter = 8 cm (volume = 268 cm³); 2 Gy/fraction.

Figure 3. Percent of blood receiving >0.5 Gy with varying PTV sizes of 2-cm diameter (4.2 cm^3) and 8-cm diameter (268 cm^3). Dose rate = 600 MU/min; 2 Gy/fraction.