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Lifetime increased cancer risk in mice following exposure to clinical proton beam generated neutrons

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Abstract

Purpose—To evaluate the lifespan and risk of cancer following whole-body exposure of mice to neutrons generated by a passively scattered clinical SOBP proton beam.

Methods and Materials—Three hundred young adult female FVB/N mice, 152 test and 148 control, were entered into the experiment. Mice were placed in an annular cassette around a cylindrical phantom, which was positioned lateral to the mid SOBP of a 165 MeV, clinical proton beam. The average distance from the edge of the mid SOBP to the conscious active mice was 21.5 cm. The phantom was irradiated with once daily fractions of 25 Gy, 4 days per week, for 6 weeks. The age at death and cause of death, i.e., cancer and type vs. non-cancer causes, were assessed over the lifespan of the mice.

Results—Exposure of mice to a dose of 600 Gy of proton beam generated neutrons, reduced the median lifespan of the mice by 4.2% (Kaplan-Meier cumulative survival, $P = 0.053$). The relative risk of death from cancer in neutron exposed vs. control mice was 1.40 for cancer of all types ($P = 0.0006$) and 1.22 for solid cancers ($P = 0.09$). For a typical 60 Gy dose of clinical protons, the observed 22% increased risk of solid cancer would be expected to decrease by a factor of 10.

Conclusions—Exposure of mice to neutrons generated by a proton dose which exceeds a typical course of radiotherapy by a factor of 10, resulted in a statistically significant increase in the background incidence of leukemia and a marginally significant increase in solid cancer. The results indicate that the risk of out-of-field 2nd solid cancers from SOBP proton generated neutrons and typical treatment schedules, is 6 - 10 times less than is suggested by current neutron risk estimates.

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INTRODUCTION

Spread-out Bragg peak (SOBP) proton beams are increasingly being used for the treatment of patients with cancer. The volume of in-field irradiated normal tissue is significantly reduced in proton vs. X-ray treated patients; however, neutrons are generated via the interaction of protons with the hardware involved in the shaping of beams for clinical applications, and by proton interactions in the patient. The use of magnets to appropriately shape and “scan” or sweep the virgin proton beam through the target volume, a modality of proton delivery known as active scanning or spot scanning, substantially reduces the production of neutrons (1). However, most clinical proton facilities employ beam scattering or a combination of scattering and active scanning systems (2), and both methods of beam shaping will likely be extensively employed in the future.

Neutron-induced 2nd cancer risk in patients treated with protons has been the subject of substantial discussion (3,4). Current neutron risk estimates are primarily derived from experimental studies, most commonly in rodents (5-18). Risk estimates vary substantially, but generally are in the range of ~ 10 to 30 times greater than the risk from the same dose of \times or gamma (photon) irradiation (5-12). However, most neutron risk estimates are based on the response to relatively low energy fission neutrons, and none have evaluated the response to neutrons whose energy spectrum matches the spectrum produced by typical clinical SOBP proton beams.

The transferability of risk estimates obtained in animals to humans is based on the similarity in their response to photon irradiation. As pertains to humans, animal studies indicate that the risk of developing cancer is dependent on: dose, sex, age of the test subject at the time of exposure and attained age. In addition, when exposed to the same dose of photon irradiation, the factor increase in solid cancer i.e., 1.5-1.75 at 1 Gy, is similar across strains of mice, dogs, and humans (9, 13-17, 19). In addition to animal based studies, in vitro studies show that the induction of mutations, chromosome aberrations and carcinogenic transformation is neutron energy dependent (20-22). Relative to the risk of cancer from exposure to photons, the International Commission on Radiological Protection (ICRP) recommended weighting (risk) factor for neutrons, ranges from a high of 20 for 1 MeV neutrons to 5 for neutrons > 300 MeV (23).

The present study evaluates the risk of cancer in mice exposed to neutrons generated by a 165 MeV passively scattered proton beam over 6 weeks. The relative risk is the factor increase above the natural background risk of cancer per unit dose. The excess relative risk is the relative risk minus 1.

METHODS AND MATERIALS

One hundred fifty-two ten-week old adult female FVB/N mice were exposed to SOBP proton generated neutrons; 148 mice served as controls. Irradiated and control mice were placed in top and sidewall perforated Lucite annuli measuring 20 cm inner radius, 30 cm outer radius, and 6.25 cm height. Control mice were transported to and from the treatment facility but remained outside the treatment area. For irradiation, an annulus containing 35-40

mice was placed around a Lucite cylindrical phantom (35 cm diameter, 20 cm length), with the mice positioned lateral to the mid SOBP of a 165 MeV (16.1 cm range in Lucite), 7 cm diameter, 10 cm modulation width beam. The average distance from the edge of the mid SOBP to the conscious active mice was 21.5 cm (range 16.5-26.5 cm). A schematic of the set-up is shown in Figure 1. The proton and resulting neutron dose to the mice was chosen with the intent of avoiding extremes, i.e., no change in the background cancer incidence, or all mice dying of cancer. Were no change in the background incidence of cancer to occur, an estimate of the upper limit of risk per treatment course could be estimated, but the numerical value of the risk could not be defined. Similarly, if all exposed mice died of cancer, the increased risk would be equal to or greater than that resulting from the administered dose, but also undefined.

Three principle factors were considered when choosing the proton dose to the phantom: (1) the neutron dose to the mice per proton dose to the phantom, (2) the relative risk of cancer per Gy in mice exposed to photons as well as the carcinogenicity of fission neutrons vs. photons, and (3) the energy spectrum differences between neutrons generated by clinical proton beams vs. fission neutrons.

Monte Carlo calculations utilizing the TOPAS platform were used to estimate the neutron dose to the mice per proton dose to the phantom (24). The calculated dose to mice randomly positioned mice in the annulus was 3.6×10^{-4} Gy neutrons per Gy protons to the phantom. The neutron dose was also measured with PB-PND neutron dosimeters (Bubble Technology Industries, Chalk River, Ontario, Canada). At a position midway between the inner and outer wall of the annulus, the dosimeters yielded a dose equivalence of 2.3×10^{-3} Sv neutrons per Gy protons to the phantom. As was used by the chamber manufacturer to convert Gy to Sv, NCRP 38 and the fluence-weighted neutron energy spectrum of the 165 MeV beam was used to convert Sv neutrons to Gy neutrons. This conversion resulted in a dose of 3.2×10^{-4} Gy neutrons per Gy protons, i.e. the agreement between the Monte Carlo simulations and measurements is well within the expected uncertainties. Additional details pertaining to neutron dose and equivalent dose are provided in the Supplemental material: Suppl neutron dose and dose equivalent at www.redjournal.org.

As previously noted, the lifetime relative risk of solid cancer following acute doses of photons, i.e., approximately $1.5-1.75 \text{ Gy}^{-1}$, does not significantly vary across 5 mouse strains, even though the background incidence of total cancers and particular cancer types is strain dependent (9,13-17,19).

The energy-weighted fluence spectrum of neutrons generated by typical clinical proton beams including the 165 MeV beam used in this study, substantially exceeds the energy-weighted fluence of fission neutrons employed for prior experimental cancer risk estimates (25). The higher energy neutrons give rise to substantially higher energy protons upon interacting in the exposed subject, than pertains to protons arising from fission neutrons (26). As the LET of protons is inversely related to proton energy, we speculated that the lower LET protons more closely approximate the carcinogenicity of photons than pertains to protons generated by fission neutrons. Thus, for planning purposes it was hypothesized that the relative carcinogenic risk of 165 MeV proton generated neutrons administered over six

weeks, may be substantially lower than pertains to current fission neutron based risk estimates, and thus in the range of 5 or less vs. photons. Based on a relative risk factor of solid cancer of 1.6, from exposure to 1 Gy of photons, and a speculated neutron relative biological effect of 5, administration of 600 Gy protons to the phantom resulting in 216 mGy neutrons to the mice, would increase the lifetime background incidence of cancer from approximately 50% (27) to 80%, i.e. by a factor of 1.6.

Following their entry into the experiment and with infrequent exceptions, all mice were examined once daily until natural death or sacrifice: 13.7% were sacrificed when moribund and not expected to live for an additional 24 hours; 3.7% were sacrificed due to the development of ulcerative dermatitis, subcutaneous lipomas exceeding 14 mm in diameter, and 1 (0.3%) with a subcutaneous fibroma. Greater than 97% of all dying or sacrificed mice were autopsied, at which time an initial assessment of the cause of death was made. Tissue was collected for histopathological evaluation to further confirm or establish the cause of death, i.e., cancer and type or not cancer.

RESULTS

The mean lifespan, regardless of the cause of death, was 794 days in the control and 756 days in the neutron exposed mice ($P=0.03$ Kaplan-Meier, log-rank). In addition to the intentionally sacrificed non-moribund mice ($P=0.3$ for control vs. neutron exposed mice), ten exposed and two control mice abruptly died during the last two weeks of neutron exposure and up to 30 days thereafter (90 to 144 days of age). The mice were active and of normal body-weight prior to and at the time of death. Autopsy and histological examination of tissues collected from the mice were unrevealing, and the cause of death was not resolved. Similar rates of abrupt death without resolved cause, and in the absence of imposed hazards, have been previously noted in FVB/N mice (28). Following the censoring of intentionally sacrificed and early abruptly dying mice, the P value for difference in cumulative survival was 0.053 (Kaplan-Meier, log-rank), Figure 2. Neutron exposure reduced the median lifespan of control mice by 4.8% regardless of the cause of death (no censoring), and 4.2% following censoring. Uroschisis was the leading cause of non-cancer deaths in control and exposed mice.

Cancer Deaths

For the calculation of cancer deaths, mice for which the cause of death (cancer vs. non-cancer) could not be determined due to cannibalism or deterioration of tissue i.e., 4 control and 5 neutron exposed, were censored. Following all censoring, 135 control and 133 neutron exposed mice remained at risk.

The cumulative fraction of mice dying from cancer is shown in Figure 3A. During the first 400 days following irradiation, relatively few and a comparable number of cancer deaths were observed in the control and neutron exposed mice. Beginning at about 500 days of age, the number of cancer deaths in exposed mice began to exceed the number of deaths in control mice. Over their lifespan, 69 control mice died of cancer and 66 from non-cancer causes; for the neutron-exposed mice, 95 died from cancer and 38 from non-cancer causes. Neutron exposure increased the number of cancer deaths by 40% ($P = 0.0006$, Chi-square).

Three cancer types: lymphocytic leukemia (LL), alveolar bronchiolar carcinoma and histiocytic sarcoma accounted for approximately 75% of all cancer deaths in both control and neutron exposed mice. Of these, LL was the most radiogenic cancer with a lethal incidence of 4% in control mice (5 deaths), and 14% (18 deaths) in neutron-exposed mice, $P = 0.004$ (Figure 3B). Other hematopoietic cancer subtypes were not found in control or exposed FVBN/N mice. The increased number and shortened median age at death from LL in exposed mice, 667.5 days vs. 804 days in control mice, substantially contributed to life shortening. Alveolar bronchiolar carcinoma was the most common tumor in control (30/135) and neutron exposed mice (41/133), $P = 0.10$. Thirty-three cancer deaths (17 control and 16 neutron exposed) were attributed to histiocytic sarcoma, a non-radiogenic and rare cancer in humans. In four mice (2 control and 2 exposed), tumors of two histological types were found at the time of death. This included 9 histologically benign tumors in the control and 9 in the neutron exposed mice. The majority of the benign but lethal tumors in both groups were teratomas. Although histologically benign, these tumors grew to a huge mass in the abdominal cavity with severe organ compression leading to early death in the host mice, as seen in Supplemental Figure 7B and 7C. A listing of all tumors leading to the death of the mice is provided in Supplemental Table 1; histopathological slides are provided in Supplemental Figures 1-8 and their associated legends in the Supplemental Figure Legends. Additional histopathological details are provided in: Supplemental histopathology findings at www.redjournal.org.

Cumulative deaths from solid cancer is shown in Figure 3C. For control mice, 64 of 135 mice at risk or 0.47 (0.39 - 0.56, 95% CI), and 77 of 133 neutron exposed mice or 0.58 (0.50 - 0.66, 95% CI) died of solid cancer. The relative risk of 1.22 was of marginal statistical significance ($P = 0.09$). Reclassification of all histologically benign but lethal tumors from the “cancer” cause of death category to the “other” cause of death category, leads to a minor change in the relative risk factor from 1.22 to 1.25, without a decrease in the P value.

DISCUSSION

The principle finding of this study is that a dose of 216 mGy of clinical proton beam generated neutrons over 6 weeks, gives rise to a 1.4 fold increase in the lifetime incidence of solid cancer plus lymphocytic leukemia, a 3.6 fold increase in lymphocytic leukemia, and a marginally significant 1.22 fold increase in the incidence of solid cancer, the latter of which constitute greater than 90% of all cancers in humans. As previously noted, the relative risk of solid cancer from 1 Gy acute photons in rodents, dogs, and humans is similar, i.e. 1.5-1.75. Based on a risk factor of 1.6 for photons at 1 Gy, the carcinogenicity of 216 mGy neutrons is equivalent to 0.36 Gy photons, i.e., 1.7 times more carcinogenic than photons. The 1.7 greater risk from neutrons generated by the 165 MeV clinical proton beam is thus 6 to 10 times less than pertains to the 10 to 30 fold greater risk observed in prior studies employing fission energy neutrons (5-12). It similarly contrasts with the 9.9 calculated risk factor of neutrons produced by 165 MeV proton beam in this study vs. photons, based on ICRP report 92 (23). Had either the experimentally measured fission neutron risk factors, or the ICRP 92 recommended risk factors pertained to the present study, all or nearly all of the neutron-exposed mice in this study likely would have died of solid cancer or leukemia.

As seen in the present study, the radiogenicity of lymphocytic leukemia substantially exceeds the radiogenicity of solid cancer, as also pertains to acute lymphocytic leukemia and myeloid leukemia subtypes in the human population (19, 29). A recent comprehensive report notes that a precise estimate of the risk factor for acute lymphocytic leukemia in the survivors of Hiroshima and Nagasaki is not possible due to the absence of incidence and mortality data from 1945 to 1950 (30). The absence of this data, as well as a somewhat different equivalent age of peak incidence of acute lymphocytic leukemia in humans and lymphocytic leukemia in mice, suggests caution in using the results of the present study for the prediction of acute lymphocytic leukemia risk in the human population.

As a dose of 600 Gy to the phantom and resulting neutron dose to the mice is substantially greater than pertains to a patient receiving proton treatment, the question of how risk from 216 mGy neutrons extrapolates to risk at lower doses arises. In exhaustive studies, Storer and Fry (31), and Heidenreich et al (9) examined the relationship between dose and cancer risk in male and female mice exposed to single and fractionated doses of fission neutrons. These studies showed that a linear no-threshold dose-response relationship pertained for fractionated doses as low as 2.5 mGy per fraction. Thus, as 600 Gy protons give rise to a 22% increased cancer incidence, 60 Gy protons may be expected to give rise to a 2.2% increase in lifetime out-of-field solid cancer risk.

The results of the present study indicate that the risk of cancer from clinical SOBP generated neutrons is 1.7 times greater than pertains to an equivalent acute dose of gamma rays. This conclusion is based on the assumption that the FVB/N mouse is not uniquely sensitive or resistant to radiation-induced cancer. Interestingly, in one of the few studies to evaluate cancer risk from higher energy, i.e., d(50)-Be neutrons, the RBE for cancer induction relative to photons was estimated to lie “probably between 2 and 3”, i.e., substantially lower than studies utilizing fission neutrons and similar to the results found in the present study (11). Additional studies with lower energy proton beam generated neutrons are needed to further interrogate and validate the relationship between neutron energy and neutron carcinogenicity.

Data pertaining to the risk of out-of-field 2nd cancer in proton treated patients is limited (32-34). In a retrospective study, Chung et al examined cancer incidence in patients receiving mixed proton/photon (protons being the predominant dose component) vs. photons (32). Although longer follow-up is needed (32, 33), second cancer incidence (combined in-field out-of-field) did not significantly differ between the photon vs proton treated cohort over the ~ 6 year median follow-up period. In a recently published 2014 study (34), Sethi et al examined in-field and out-of-field cancer incidence in proton vs. photon treated patients with retinoblastoma. In-field cancer was significantly higher in photon treated patients. With an ~ 7 year median follow-up, the incidence of out-of-field cancer did not significantly differ in the proton vs. photon treated patients.

The present study pertains to the lifetime risk of cancer in young-adult mice, which were 10-16 weeks of age at the time of exposure. As pertains to humans, relative to the risk from exposure during young adult-hood, cancer risk increases with decreasing age at exposure, and more moderately decreases with increasing age at exposure (16). Due to their

significantly higher relative risk per Gy and shorter latency, leukemia and solid tumors risk are separately reported. Risk estimates obtained in this study pertain to female mice and are compared to the risk in females in the human population. Radiation induced solid cancer risk is approximately 50% lower in males than in females in humans, and appears to be similarly lower in male mice (9, 19).

CONCLUSION

Exposure of mice to neutrons generated by 600 Gy of a 165 MeV SOBP proton beam at 21.5 cm lateral to the edge of the mid SOBP, results in a 40% increase in deaths from solid cancer plus lymphocytic leukemia. For solid cancer, the observed 22% increased risk is of marginal statistical significance. The results indicate that the lifetime risk of out-of-field cancer from neutrons generated by a passively scattered SOBP beam, administered over 6 weeks, is 6 to 10 times lower than current risk estimates.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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SUMMARY

This study evaluates life shortening and the risk of cancer in mice following 6 weeks fractionated dose exposure to clinical proton beam generated neutrons. The results indicate that the risk of out-of-field neutron-induced solid cancer from a passively scattered SOBPs proton beam is 6-10 times lower than current risk estimates.

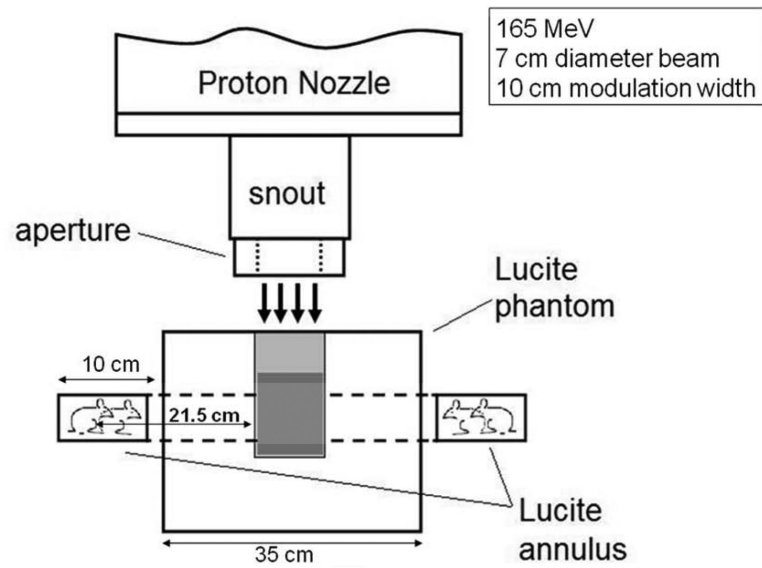


Fig. 1. Conscious active mice in Lucite annuli were exposed to neutrons at a distance of 16.5-26.5 cm lateral to the edge of the mid SOBP.

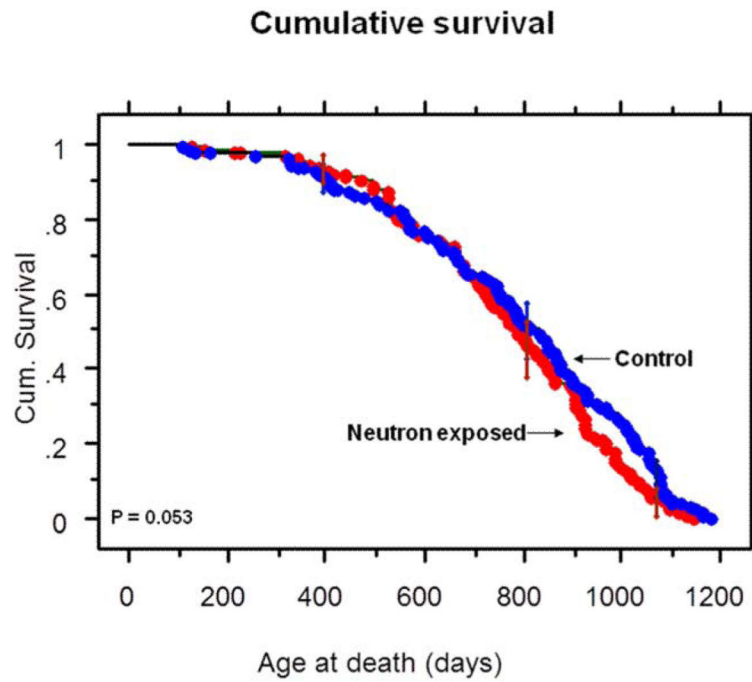


Fig. 2. Lifetime cumulative survival of control and neutron exposed mice. Intentionally sacrificed non-moribund and abruptly dying mice were censored. N = 139 control and 138 neutron exposed mice. Bars are 95% confidence intervals for the control cohort of animals at the 10%, 50% and 90% cumulative survival level, and for the exposed mice at the same age as the control mice. Log-rank P = 0.053.

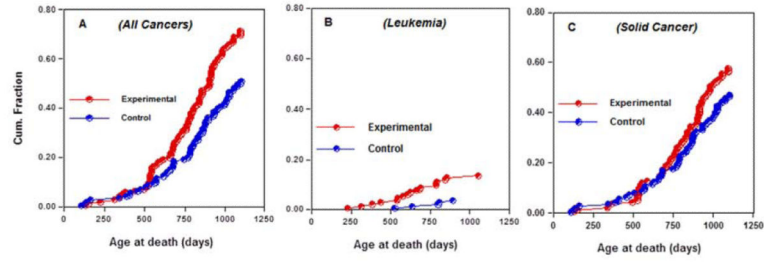


Fig. 3. Cumulative deaths as a fraction of all deaths over the lifetime of the mice. Panel A: death from cancer of all types ($P = 0.0006$); Panel B: deaths due to lymphocytic leukemia ($P = 0.004$); Panel C: death from solid cancer ($P = 0.09$).