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Dosimetry and characterization of a 25 MeV proton beam line for pre-clinical radiobiology research

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Abstract:

Purpose: With the increase of proton therapy centers, there is a growing need to make progress in preclinical proton radiation biology to give accessible data to medical physicists and practicing radiation oncologists.

Methods: A cyclotron usually producing radio-isotopes with a proton beam at an energy of about 25 MeV after acceleration, was used for radiobiology studies. Depleted silicon surface barrier detectors were used for the beam energy measurement. A complementary metal oxide semiconductor (CMOS) sensor and a plastic scintillator detector were used for fluence measurement, and compared to Geant4 and an in-house analytical dose modeling developed for this purpose. Also, from the energy measurement of each attenuated beam, the dose-averaged linear energy transfer (LET_d) was calculated with Geant4.

Results: The measured proton beam energy was 24.85 ± 0.14 MeV with an energy straggling of 127 ± 22 keV before scattering and extraction in air. The measured flatness was within ± 2.1 % over 9 mm. This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/mp.13512

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diameter. A wide range of LET_d is achievable: constant between the entrance and the exit of the cancer cell sample ranging from 2.2 keV/ μ m to 8 keV/ μ m, beyond 20 keV/ μ m, and an average of 2-5 keV/ μ m in a SOBP calculated for an example of a 6 mm-thick xenograft tumor.

Conclusion: The dosimetry and the characterization of a 25 MeV proton beam line for pre-clinical radiobiology research was performed by measurements and modeling, demonstrating the feasibility of delivering a proton beam for preclinical *in vivo* and *in vitro* studies with LET_d of clinical interest.

Key words: proton beam, preclinical studies, small animal irradiation, *in vitro* irradiation

I. INTRODUCTION

Despite the high potential of proton therapy (PT), the clinical evidence supporting the broad use of protons is still under debate.^{1,2} Reducing the dose to normal tissues and organs surrounding the target areas is the key feature of protons versus photons irradiation.³ However, does the advantage of proton dosimetry be reflected in significant gains for patients? Translation of this absorbed dose reduction into clinically relevant benefit has still not been consistently demonstrated.⁴ However, a semi-personalized approach can be used based on the linear energy transfer (LET) to reoptimize the treatment plan for intensity modulated PT.⁵ This method demonstrated a safer treatment by mitigating a potentially increased risk of side effects resulting from elevated relative biological effectiveness of proton beams near the end of the range.⁵ Recently, studies focused on predicting patient-specific dosimetric benefits of PT⁶ and dose escalation. For example, Liu et al. demonstrated that proton radiation dose escalation improved local control but also increased toxicity.⁷ However, the lack of identifying biomarkers for conclusive patients outcome, limits the transfer of PT into personalized medicine.

Small-animal proton irradiations are required to contribute to overcome these issues. Most of experiments are performed with clinical energy protons (between 70 and 250 MeV) to mimic for example space radiation conditions,⁸ with whole body and/or whole brain irradiations. Although a clinical proton beam energy seems relevant for radiobiological studies, animals or cell samples are usually irradiated in the plateau of the Bragg curve with a LET of about 1.5 keV/ μ m, which is a typical LET found in patients normal tissues (beam entrance). However, Grassberger et al. demonstrated that single fields with various range in different patients lead to high LET_d areas around 8–12 keV/ μ m into pristine peaks (depending on beam energy) and of about 8 keV/ μ m for passive scattering spread-out Bragg peak (SOBP) fields.⁹ In intensity modulated proton therapy treatment plans, LET_d values within the target (e.g. brain tumor) range from \approx 1.5 to 4 keV/ μ m, depending on the optimization dose delivery technique used.⁹ Thus, radiobiologists focus on a wide range of LET to study different scenarios happening in patients. For instance, Ogata et al. irradiated human fibrosarcoma cells at 5 points along a 190 MeV proton beam with its Bragg peak modulated to 6 cm showing that proton irradiation suppresses metastatic potential.¹⁰ This common irradiation method is usually performed to obtain a broad range of LET despite the large uncertainties of LET values mostly near the Bragg peak. In a recent study, Prezado and colleagues demonstrated that 100 MeV proton minibeam, corresponding to an estimated LET of about 5 keV/ μ m at the entrance and 15 keV/ μ m in the Bragg peak, minimized normal tissue damage in the rat brain compared to broad beam.¹¹ In addition, several low energy proton platforms (of about 3 MeV corresponding to a LET \approx

12 keV/ μm at the entrance of the depth-dose curve) allow cell samples irradiation¹² ranging from the single-cell scale^{13, 14} to *in vivo* skin irradiation.¹⁵

Currently, proton preclinical platforms ($E \leq 50$ MeV) are burgeoning, offering the technical precision required to deliver precise millimeter-sized beams to localized regions within rodents.^{16–18} Small-animal radiotherapy research platforms mimicking accurately human radiotherapy conditions are needed to reproduce pathologies observed in patients (improvement of animal models) and better understand the therapeutic possibilities and toxicities of PT. Several studies already improved the beam delivery and dose calculation for focal irradiation in the rat brain with gamma-rays¹⁹ and image-guided X-rays.^{20, 21} Also, recent improvement in preclinical radiotherapy research have stimulated the development of dedicated X-rays irradiators for small animals such as mice and rats.^{22–24}

This article presents the development of a proton beam platform dedicated to *in vivo* and *in vitro* radiobiological studies, using the 25 MeV cyclotron CYRCé (IPHC) as a proton source. A dedicated treatment planning system (analytical calculation) is used to deliver the absorbed dose to small animals and cells,²⁵ and the beam energy and fluence are characterized. Based on the energy measurement and calculation, and a beam energy degrader wheel, leading to a broad range of LET_d, this platform is demonstrating the feasibility of radiobiological studies of clinical interest.

II. MATERIALS AND METHODS

II.A. Experimental set-up

The experimental set-up is illustrated in Figure 1. The proton beam is extracted from the CYRCé cyclotron (TR24, ACSI, Canada), used initially for the production of radio-isotopes, with an extracted beam energy of about 25 MeV after acceleration. To reach the beam stability (in a range of a corresponding dose rate of 2Gy/ min) with an intensity fluctuation less than 1% over 1 to 10 minutes irradiation duration, the beam current was set to obtain 200 fA to 30 pA at sample position. The irradiation duration was controlled with a beam kicker located between the source and the entrance of the cyclotron acceleration cavity (30 keV ion beam) to allow a fast beam shut off. The output beam passes through a switching magnet followed by a beam pipe where the protons are centered with a 5 mm collimator. A homogeneous broad beam is obtained with a movable Al-scattering foil (200 μm thick) set behind the 5 mm collimator 240 cm upstream from the end of the beam line, where an exit window is made of 50 μm thick aluminum. An energy degrader is located three centimeters downstream this exit window (Fig. 1). The energy degrader consists on Al thicknesses ranging from 147 μm to 2761 μm , allowing LET variation and SOBP delivery (see section II.E). The final transverse shape of the proton beam is managed by a 10 mm thick aluminum collimator located after the degrader.

In addition to being mechanically centered inside the vacuum pipe, the proton beam is horizontally centered (no vertical control centering) using the switching magnet before each irradiation procedure. The beam centering is controlled by a beam profiler, which is made of 0.5 mm-thick tungsten blade placed on a (X, Y) automated movable system (travel range of 41 mm, precision < 1 nm). The profiler, connected to a Keithley picoammeter (6487, Tektronix), measures the beam current by collecting proton charges from beam interacting with the tungsten blade. The beam is scanned in steps of 0.5 mm and lead to a 1D Gaussian profile. To control the beam current, a movable Faraday cup is set at the end of the beam line.

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The extracted proton beam energy and the energy straggling in air was measured behind the collimator. For this measurement, four stacked depleted Silicon Surface Barrier Detectors (SSBD) (ORTEC B-018-150 and A-019-150) were used (Fig. 2), with a sensitive depth of 252 μm , 1007 μm , 2022 μm and 3000 μm respectively.

Each SSBD was firstly calibrated in vacuum chamber with a tri-alpha source of ^{239}Pu , ^{241}Am , ^{244}Cm , emitting particles of 5.805, 5.486 and 5.155 MeV respectively. Since the energy of the beam to be measured is of the order of 25 MeV, using only this low energy calibration would lead to an important error for large energy deposits. An iterative self-calibration method was then used.

For this measurement, the maximum energy achievable by the cyclotron has been fixed. The energy loss and the residual energy in each detector were then measured for different beam energies using the energy degrader whose aluminum thicknesses are known (9 positions was used to vary the beam energy between approximately 25 to 10 MeV). The energy loss in the degrader and therefore the energy deposited in each detection stage depend on the beam initial energy.

Then, a self-calibration method is performed consisting in the variation of the theoretical energy of the initial beam around its theoretical energy (25 MeV) and calculating for each position of the degrader the energy loss in the Al foil as well as the energy then expected in each detection stage. It was then compared to its theoretical value given by an analytical algorithm (see section II.E), which calculates the outgoing proton energy using the PSTAR stopping power database. The real energy of the measured beam is then obtained when for all 4 detectors the calculated points are aligned with the experimental data as well as with the points measured with the 3-alpha source.

From this method, the initial energy of the proton beam was found $E_0 = (24.85 \pm 0.14)$ MeV with an energy straggling $\sigma_E = (127 \pm 22)$ keV. The energy uncertainty was obtained by means of residuals on the calibration curve, and the energy straggling uncertainty was obtained by unfolding the silicon detectors resolution and accounting for the impact of the proton beam scattered by the two 200 μm -thick and 50 μm -thick Al foils (most significant contributions). The energy straggling assessment was previously described by Vanstalle et al.²⁵

Finally, the platform offers both *in vitro* and *in vivo* irradiations. For example, tumor xenograft located in mice hind paws can be irradiated directly in contact with the collimator, using an in-house immobilization bed (Fig. 3a). To date, cells can be irradiated into 24, 48 and 96 well plates (depending on the collimator size used) in a dedicated automated (X, Y) sample holder (Fig. 3b). For instance, the diameter of the bottom well (where cells grow) of a 48 well plate is 9.8 mm (Multiwell TC plates, Corning Life Sciences), with a center-to-center distance of 12.9 mm. Therefore with a 10 mm diameter collimator and a precise automated displacement (X, Y translation tables precise at 100 μm), we can obtain uniform irradiation over the cell layer.

II.B. Fluence measurements

It is noteworthy that no standard protocol is available for preclinical proton beam commissioning with energies below 30 MeV. Indeed, due to a sub-centimeter penetration depth in water, ionization chambers cannot be easily used in a standard water phantom, and the measurement of the percentage depth-dose remain challenging due to the proton energy that is about 3 MeV in the Bragg peak, corresponding to approximately 160 μm range in water.

A plastic scintillator detector and a CMOS sensor used for fluence measurements were positioned behind a 10 mm diameter collimator (40 mm downstream of the exit window) and exposed to the proton beam perpendicularly incident. Briefly, a water-equivalent plastic scintillator detector of $25 \times 25 \times 10 \text{ mm}^3$ (BC-420, Saint-Gobain, Newbury, OH, USA) was placed downstream of the collimators to count the number of protons (related to beam current) at the irradiation position and compared to the beam current measured by the Faraday cup. It worth to mentioned that these to measurements cannot be performed simultaneously. When the correlation between the plastic scintillator and the cup is obtained, the plastic scintillator is removed and the beam flux is set only with the Faraday cup.²⁶ The CMOS sensor MIMOSA28 was characterized in a previous study²⁶ for fluence measurement (total number of particles collected per cm^2) with the set-up described in section II.A. The CMOS chip has a sensitive area of about $2 \times 2 \text{ cm}^2$ and consists of an array of 928 lines and 960 columns of pixels with 20.7 microns pitch. The total thickness of the sensor is 50 μm , including the sensitive epitaxial layer of 14 μm -thick silicon, with a spatial resolution below 10 μm .²⁷ The detection efficiency of this sensor is 99.5% for the relevant range of proton energies in our beam, since it was optimized for minimum ionizing particles in high energy particle experiments.²⁸⁻³⁰

II.C. Geant4 simulation

To compare calculation with the measured beam profiles, Monte Carlo simulations were performed with Geant4 10.03,³¹ using the binary light ion cascade (BIC) to model proton interactions.³² The BIC model, called *G4BinaryLightIonReaction*, is an extension of the binary cascade model. The pre-defined physics list was *QGSP_BIC*, modeling proton interactions along its path, including the Au scattering foil, the Al collimator and extraction foil (interface vacuum-air) at the end of the beam line, and secondary particles. Also, for LET calculations simulating *biological experiment conditions*, the water equivalent thickness of the petri dish and the energy straggling were taken into account. In this model, the participating particles, i.e. primary particles or particles generated during the cascade process, are described by means of Gaussian wave functions. Also, a range cut of 1 mm and a step size of 0.1 mm were applied, which is sufficiently small as demonstrated in several studies.³³⁻³⁵

II.D. Dose-averaged LET calculation

The LET is usually averaged over a target volume and an energy spectrum of a specified type of charged particles. In this study, we used the dose-averaged LET (LET_d),^{9, 36} including both the dose and the original definition of the LET. LET_d distributions are generated by scoring each energy deposition in the medium. At each energy deposition associated with a particle energy loss (dE), the length of the particle step (dx) was obtained. All values were scored voxel by voxel (v) and as dose-to-tissue to calculate the LET_d in MeV/g cm^2 :

$$\text{LET}_d(v) = \frac{\sum_{events} dE \cdot (dE/dx) \frac{1}{\rho}}{\sum_{events} dE} \quad (1)$$

The electronic stopping power (dE/dx) is used in the calculation (PSTAR database), which implies that LET is calculated without the production of delta electrons. Also, the nuclear stopping power is neglected and ρ is the mass density of the current medium (here, water) where proton energy is deposited.

II.E. Calculation of the absorbed dose

An analytical algorithm, referred to as PSD function, was developed to define the weight of each Bragg peak composing the SOBP delivery in small animal tumors.²⁵ It is noteworthy that the PSD function is valid for low energy protons (below 50 MeV) and applicable for various geometry and size of tumors, but in this study, an example of a 6 mm thick xenograft tumors will be considered. It consists in a calculation based on Bortfeld et al.³⁷ using the proton range extrapolated from the PSTAR database.³⁸ The PSD function implements the range straggling σ_R , and the influence of aluminum thicknesses (energy degrader) on the transmitted proton beam. Indeed, proton beam fluence decreases due to nuclear reactions between incident protons and the target. Also, to reproduce the beam propagation in the target, a multiple scattering calculation was used. Using the PSD function, the irradiation duration to deliver the right number of protons for each proton beam energy to cell samples and composing the SOBP is set to deliver the proper dose into the target volume.

III. RESULTS

III.A. Beam characterization

The 2D beam fluence was assessed with the CMOS sensor located behind the 10 mm collimator with a fluence fixed at approximately $6.7 \times 10^7 \text{ cm}^{-2}$ (corresponding to $\approx 25 \text{ cGy}$) in the plateau region of the beam path (Fig. 4, left panel) at maximum energy (24.85 MeV). After extraction in air the beam energy entering the CMOS sensor was $23.52 \pm 0.15 \text{ MeV}$ (30 mm from the exit window). The beam lateral profiles were assessed with the CMOS sensor and compared to Geant4 calculation and the PSD function (Fig. 4, right panel). The lateral profiles were flat in the central region, then fall off rapidly in the penumbral region. Spatial heterogeneities obtained with the CMOS sensor were: $\pm 1.8\%$ on the X-axis (cyan line) and $\pm 2.1\%$ on the Y-axis (cyan dots) over a circular region of 9 mm diameter. A slight increase on the right edge of the Y profile can be observed and is due to a misalignment of the beam since no vertical control centering is available to date. The full width at half maximum of lateral profiles were in agreement within a distance of 0.1 mm between the CMOS sensor, Geant4 simulations and the PSD function, demonstrating the validity of the in-house lateral scattering calculation of the proton beam.

In routine irradiation procedures, the beam current in air I_{air} (expressed in Ampere) is calculated from the beam current measured by the Faraday cup. Then, the irradiation time t_{irr} (precision $< 190 \mu\text{s}$) can be determined according to Eq. 1¹² to obtain a theoretical absorbed dose D into the scintillator detector (corresponding to biological samples position). This is done by accounting for the LET_d of 23.52 MeV protons in water ($2.2 \text{ keV}/\mu\text{m}$) in the region of the plateau of the Bragg curve:

$$t_{irr} = \frac{D}{1.6 \times 10^{-9} \times LET_d \times \left[\frac{I_{air} \times \frac{1}{A}}{qp} \right]} \quad (2)$$

where, q_p is the proton charge, and A is the area of the collimator (cm^2). Overall, the relative uncertainty of the absorbed dose can be assessed based on (2):

$$\frac{\Delta D}{D} = \sqrt{\left(\frac{\Delta t_{irr}}{t_{irr}}\right)^2 + \left(\frac{\Delta LET_d}{LET_d}\right)^2 + \left(\frac{\Delta I_{air}}{I_{air}}\right)^2 + \left(\frac{\Delta A}{A}\right)^2} \quad (3)$$

with, $\Delta t_{irr}/t_{irr}$ negligible, $\Delta A/A$ negligible since the collimator size is (2 ± 0.01) cm, $\Delta LET/LET < \pm 5\%$ (given by PSTAR database) and $\Delta I_{air}/I_{air} < \pm 1\%$ (the plastic scintillator measurement set-up processes up to 700 000 protons per second). Therefore, the relative uncertainty on the absorbed dose is of about $\pm 5\%$ with a beam uniformity of $\pm 2\%$ over 2 cm diameter irradiation.

III.B. Experimental verification of the fluence based on the analytical algorithm: a specific example

The PSD function was used to calculate the SOBP corresponding to an absorbed dose of 1 Gy in a 6 mm xenograft tumor volume with energy modulation (18 Al-thicknesses) (Fig. 5, left panel).

On the right panel of Figure 5, the difference in number of protons between measurements and the PSD function at the entrance of the water-equivalent depth (corresponding to the Bragg peak with the lowest weight) represents a deviation $< 0.1\%$ over the total absorbed dose, which is negligible considering its contribution in the SOBP calculation (low weighting factor).

III.C. Dose-averaged LET Range

The proton dose-averaged LET range versus the penetration depth in water was calculated with Geant4 (Fig. 6, left panel) for different proton energies. For cell irradiation (zoom-in region in Fig. 6), growing on a 2 mm thick petri dish (or flask), the proton beam energy can be degraded ranging from 23 MeV ($LET_d = 2.8$ keV/ μm) to 15 MeV ($LET_d \approx 7$ keV/ μm), obtaining a constant LET_d (within 5%) across the cell nucleus (10 μm) growing in a monolayer culture. In addition, for *in vivo* experiments with for example a xenograft tumor of 6 mm (as described in section III.B) irradiated with a total absorbed dose of 1 Gy, the LET_d is about 2-5 keV/ μm with a maximum of 40 keV/ μm in the pristine Bragg peak composing the SOBP (Fig. 6, right panel).

Discussion

With the increase of proton therapy centers there is a growing need to make progress in proton radiation biology to give accessible data to practicing radiation oncologists. The measurements and modeling results presented here demonstrate the feasibility of delivering a proton beam for preclinical *in vivo* and *in vitro* studies with LET_d of clinical interest. With the low energy straggling and lateral scattering of the beam, this proton preclinical platform offers the technical precision required to deliver a uniform beam to localized regions within a small animal and *in vitro* studies. The nominal energy of the beam is $E_0 = (24.85 \pm 0.14)$ MeV with an energy straggling $\sigma_E = (127 \pm 22)$ keV, the beam flatness is better than $\pm 2.1\%$, and the absorbed dose is estimated better than $\pm 5\%$. Moreover, using the degrader wheel, the energy is well known and controlled, guaranteeing robust LET_d values. Also, this system produces an SOBP at a maximum depth that is useful for small animal tumor models within 6 mm depth.

Because of a lack of biological input parameters, the ability to predict relative biological effectiveness (RBE) for all tissues in treatment planning is limited, and thus the use of a generic RBE of 1.1 was recommended.³⁹ However, in the Bragg peak area, the average proton energy decreases rapidly, leading to an increase of the LET values that influences (amongst others parameters such as dose, fractionation, tissue, and biological endpoint) the RBE of protons. Consequently, there is RBE-based heterogeneity throughout the target and adjacent normal structures.⁴⁰ As discussed by Unkelbach et al.,⁵ this is an issue for proton therapy planning, especially for intensity modulated proton therapy, which may deliver highly inhomogeneous LET distributions, even for homogeneous physical dose distributions. This may result in LET hot spots in critical structures within or near the target volume, with LET values higher than those observed for passive scattering for instance. Although it might be difficult to incorporate all parameters influencing RBE, biologically motivated treatment planning could at least be guided by LET_d, as suggested by Grassberger et al.⁹ Thus, it might be feasible to incorporate physics-related RBE effects using a single physics parameter, the LET_d. With a constant LET_d values between the entrance and the exit of the cell sample ranging from 2.2 keV/μm to 8 keV/μm, higher LET_d beyond 20 keV/μm, and an average LET_d of 2-3 keV/μm in a SOBP delivered to a xenograft tumor (for example), LET_d-dependent biological effects may be observable with this proton beam platform. A final challenge will be to experimentally determine the LET_d for the range of energies instead of using only Monte Carlo and PSTAR database. With carefully designed *in vivo* and *in vitro* experiments it may be possible to explore a wide range of LET_d response and implement these radiobiological results in clinical treatment planning system.

Several proton irradiation platform were specifically designed for radiobiology research. For example, Greubel et al. adapted a tandem 14 MV van de Graaff accelerator to accelerate protons up to 25 MeV. It has been used to study the response of a head-and-neck squamous cell carcinoma cell line to proton irradiation using a flank xenograft model¹⁶ and the response of skin⁴¹ to proton microbeams with high dose rates. A limitation of these studies, as well as our, is the lack of the percentage depth-dose measurement. We measured the lateral fluence of the beam with the CMOS sensor²⁶ compared to Geant4 and PSD function calculation, demonstrating an excellent homogeneity and flatness, however, the integral depth dose measurement in water of the 10 mm collimated proton beam at 23.5 MeV would have been required to fully characterize the proton beam and the validity of the in-house treatment plan.²⁵ The percentage depth-dose will be measured in a future study. Nevertheless, the nominal proton beam energy was appropriately assessed as well as each energy downstream Al-thicknesses attenuator.

Conclusions

The fluence and energy characterization, the in-house and Geant4 modeling demonstrate the feasibility of using an existing cyclotron system to deliver a proton beam that is suitable for radiobiology studies with the possibility to explore a wide range of dose-averaged LET.

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Figure captions:

Fig. 1. Schematic view of the horizontal beam line, including the energy degrader and an adaptable collimator. The adaptable collimator was set at 5 mm and 10 mm diameter for energy and fluence measurement respectively.

Fig. 2. Proton beam energy measurement set-up.

Fig. 3. End station of the beam line for radiobiological studies: a mouse immobilization system (a), and an automated cell sample holder (b).

Fig. 4. Fluence-maps measured with the CMOS sensor (left panel) and comparison of the fluence lateral profiles between the CMOS sensor, Geant4 and the PSD function.

Fig. 5. Specific example of a 6 mm xenograft tumor. On the left, PSD calculation of a SOBP delivering a total absorbed dose of 1 Gy. On the right panel, the number of protons delivered in a 6 mm water equivalent depth for each Bragg peak composing the SOBP.

Fig. 6. Distributions of LET_d as a function of depth from 15 MeV to 23 MeV protons (left) and a LET_d in 6 mm water equivalent depth (example of a xenograft tumor) (right) calculated with Geant4. The water equivalent thickness of the petri dish and the energy straggling were taken into account for the estimation of LET.





