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SMALL ANIMAL IGRT SPECIAL FEATURE: FULL PAPER

Murine vs human tissue compositions: implications of using human tissue compositions for photon energy absorption in mice

¹LOTTE EJR SCHYNS, MSc, ^{1,2}DANIËLLE BP EEKERS, MD, ¹BRENT VAN DER HEYDEN, MSc, ¹ISABEL P ALMEIDA, MSc, ¹ANA VANIQUI, BSc and ¹FRANK VERHAEGEN, PhD

¹Department of Radiation Oncology (MAASTRO), GROW-School for Oncology and Developmental Biology, Maastricht University Medical Centre, Maastricht, The Netherlands

²Proton Therapy Department, South-East Netherlands (ZON-PTC), Maastricht, The Netherlands

Address correspondence to: Prof Dr Frank Verhaegen

E-mail: frank.verhaegen@maastro.nl

Objective: To investigate the implications of using human tissue compositions for dose calculations in mice for kilovoltage photon beams.

Methods: Dual energy CT (DECT) images of 9 female mice were used to extract the effective atomic number Z_{eff} and the relative electron density ρ_e for each voxel in the images. To investigate the influence of the tissue compositions on the absorbed radiation dose for a typical kilovoltage photon beam, mass energy-absorption coefficients Q_{en}/ρ were calculated for 10 different tissues in each mouse.

Results Differences between human and murine tissue compositions can lead to errors around 7.5 % for soft tissues and 20.1 % for bone tissues in Q_{en}/ρ values for kilovoltage photon beams. When considering the spread within tissues, these errors can increase up to 17.5 % for

soft tissues and 53.9 % for bone tissues within only a single standard deviation away from the mean tissue value.

Conclusion: This study illustrates the need for murine reference tissue data. However, assigning only a single mean reference value to an entire tissue can still lead to large errors in dose calculations given the large spread within tissues of Q_{en}/ρ values found in this study. Therefore, new methods such as DECT and spectral CT imaging need to be explored, which can be important next steps in improving tissue assignment for dose calculations in small animal radiotherapy.

Advances in knowledge: This is the first study that investigates the implications of using human tissue compositions for dose calculations in mice for kilovoltage photon beams.

INTRODUCTION

Dedicated small animal treatment planning systems such as SmART-ATP can accurately calculate dose distributions in both tumors and surrounding healthy tissues for kilovoltage photon beams used in image-guided precision irradiation platforms.¹⁻³ However, to accurately calculate a dose distribution in a medium using first-principles methods (e.g. Monte Carlo simulations), the elemental composition of the medium should be taken into account, since dose deposition is influenced by the atomic number Z . In the field of small animal radiotherapy, it is particularly important to use correct tissue compositions due to the low energies of the photons in the treatment beam.⁴ For low energy photons, photoelectric absorption is the dominant interaction. The atomic cross-section for photoelectric absorption depends strongly on Z ($\propto Z^{4-5}$) and thus, in the case of tissues, the elemental composition.⁵ The Z dependence of the atomic cross-section for photoelectric

absorption results in a Z dependence of the absorbed radiation dose ($\propto Z^{3-4}$).

It is common practice to use human tissue compositions for dose calculations in mice, since detailed murine tissue compositions are not available in the literature. Very few studies have been published that present quantitative data on the elemental compositions of murine tissues. Most of these studies focus on trace elements,⁶⁻⁹ which only make up a small part of the total tissue composition. In addition to elemental compositions, mass densities ρ are another crucial factor in dose calculations. These mass densities are usually assigned using a cone beam CT (CBCT) image that is acquired before the treatment. For this method, a predetermined calibration curve is used to convert CT numbers to mass densities. Another method is to assign mass densities using the human reference data. The well-established reference data for human tissue compositions and

mass densities are listed in several reports by the International Commission on Radiation Units and Measurements (ICRU) and the International Commission on Radiological Protection (ICRP).^{10–12}

In this study, the dosimetric implications of using human tissue compositions for dose calculations in mice are investigated using dual energy CT (DECT) imaging, which is a novel imaging modality in the field of small animal radiotherapy that allows for *in vivo* extraction of the effective atomic number Z_{eff} and the relative electron density ρ_e . Both Z_{eff} and ρ_e can be resolved for each voxel, which also allows for an assessment of the spread in tissue composition and density within tissues.

Z_{eff} is defined as $Z_{\text{eff}} = \sqrt[n]{\sum_i \frac{\omega_i Z_i}{A_i} Z_i^n / \sum_i \frac{\omega_i Z_i}{A_i}}$ and ρ_e is defined as $\rho_e = \rho \sum_i \frac{\omega_i Z_i}{A_i} / \rho_{e,\text{water}}$. ω and A represent the elemental weight fraction and mass number, respectively, for element i . ρ is the mass density, $\rho_{e,\text{water}}$ is the electron density of water (≈ 0.555 mole electrons/cm³) and the exponent n is defined as 3.3, which was determined to be optimal in a previous study.¹³

To investigate the influence of tissue compositions on the absorbed radiation dose for a typical kilovoltage photon beam, the mass energy-absorption coefficient μ_{en}/ρ was calculated for 10 different tissues in a series of 9 mice. μ_{en}/ρ can be multiplied by the photon energy fluence to calculate the dosimetric quantity collision kerma, which is closely related to absorbed radiation dose. Assuming an approximately linear relationship between μ_{en}/ρ and the absorbed radiation dose for kilovoltage

photon beams (*i.e.* the potential photon fluence perturbation by the different media is ignored), differences in μ_{en}/ρ are proportional to differences in absorbed radiation dose.

METHODS AND MATERIALS

Figure 1 shows the workflow of the methods used in this study. First, CBCT images were acquired with two different X-ray spectra, which were then registered before applying the DECT methods to obtain Z_{eff} and ρ_e images. These images were used to calculate the means and standard deviations for both Z_{eff} and ρ_e for 10 different tissues in each mouse. A noise correction was applied to the standard deviations which were then used to calculate the ± 1 standard deviation ranges of Z_{eff} and ρ_e to represent the spread in tissue composition and density, respectively. The mean tissue composition and its spread were then used to calculate the mean μ_{en}/ρ and its spread. The following sections explain these steps in greater detail.

Image acquisition

The X-RAD 225Cx system (Precision X-ray, North Branford, CT) was used to image 9 female Naval Medical Research Institute nude mice.¹⁴ For each mouse, two CBCT images were acquired with two different peak voltages: 50 and 90 kVp. This combination was determined to be optimal in terms of Z_{eff} and ρ_e errors, as shown in a previous study.¹⁵ The acquired images were reconstructed using the Feldkamp backprojection algorithm that is implemented in an in-house developed software platform for

Figure 1. Workflow of the methods used in this study. The top half shows how the elemental compositions are calculated from the 50 and 90 kVp cone beam CT images and the bottom half shows how the mass-energy absorption coefficients are calculated from these compositions.

Table 1. DECT imaging protocols

Parameter	Low energy CT	High energy CT
Peak voltage (kVp)	50	90
Exposure (mAs)	670.8	249.6
Frame rate (s ⁻¹)	10	5
Panel mode	Mid Gain 1 × 1	Low Gain 1 × 1
Imaging dose (cGy)	30	30
Number of voxels	1024 × 1024 × 1024	
Voxel dimensions (μm ³)	100 × 100 × 100	

preclinical CBCT image reconstruction, built using the open source software RTK (Creatis, Lyon, France).^{16,17} The DECT imaging protocols are summarized in Table 1.

Image registration

Since the 50 and 90 kVp CBCT images were acquired consecutively, image registration was required to correct for motion of the animal between the two scans. A multiresolution rigid registration was applied first, followed by a multi resolution deformable registration. The open source software Elastix (Image Sciences Institute, Utrecht, Netherlands) was used to determine the deformation fields and the open source software Transformix (Image Sciences Institute, Utrecht, Netherlands) was used to apply them.¹⁸ The 50 kVp CBCT images were selected to be the fixed images and the 90 kVp CBCT images were selected to be the moving images in the image registration process. The image registration parameters are listed in appendix A (table A1, Supplementary Material 1).

Contouring

A total of 10 tissues were contoured by a radiation oncologist using the clinical treatment planning software Eclipse (Varian Medical Systems, Palo Alto, CA). The contoured tissues were brain, cortical bone, eye, femur, heart, kidney, liver, marrow, muscle and rib. The cortical bone and marrow tissues were contoured in the femur and the muscle tissue was contoured in the gluteal region. One rib was contoured for each mouse. On average, this was the 10.3th rib when counting from the head towards the tail. Separate contours were created for each tissue in each mouse. All tissues were contoured on the 50 kVp CBCT images, since these images provided better soft tissue contrast than the 90 kVp CBCT images.

Image processing

The tissue contours were converted into bitmasks using the poly2mask function in MATLAB R2016b (MathWorks, Natick, MA). An erosion with a spherical structuring element (radius 2 pixels) was applied to the bitmasks for all tissues in all mice to remove boundary voxels that might be affected by partial volume effects. The bitmasks were applied to the Z_{eff} and ρ_e images to obtain Z_{eff} and ρ_e values for each voxel in the contoured tissue. Mean values and standard deviations were calculated for the Z_{eff} and ρ_e voxels in each bitmask of 10 tissues in all of the 9 mice.

Calibrations

The procedure to calibrate for Z_{eff} and ρ_e was adopted from a previous study.¹⁵ A mouse-sized phantom with 12 cylindrical inserts (SmART Scientific Solutions, Maastricht, Netherlands) was used to establish a calibration curve for both Z_{eff} and ρ_e . Z_{eff} images were calculated using the tissue substitute method described by Landry et al and ρ_e images were calculated using a method described by Saito.^{13,19} Equation 1 was used to fit Z_{eff} and equation 2 was used to fit ρ_e . In equation 1, μ is the mean linear attenuation coefficient for each phantom insert calculated from the measured CT numbers, Z_{eff} is the reference effective atomic number calculated from the elemental compositions provided by the manufacturer and A, B, C, D, E, F, n and m are fit coefficients. In equation 2, HU is the mean CT number for each phantom insert, ρ_e is the reference relative electron density calculated from the mass densities and elemental compositions provided by the manufacturer and a, b and α are fit parameters.

$$\mu_{ratio} = \frac{\mu_{LowEnergy}}{\mu_{HighEnergy}} = \frac{A + B \cdot Z_{eff}^{n-1} + C \cdot Z_{eff}^{m-1}}{D + E \cdot Z_{eff}^{n-1} + F \cdot Z_{eff}^{m-1}} \quad (1)$$

$$\rho_e = a \cdot \frac{(1 + \alpha) \cdot HU_{HighEnergy} - \alpha \cdot HU_{LowEnergy}}{1000} + b \quad (2)$$

Z_{eff} and ρ_e were combined to obtain elemental weight fractions using a method described by Hünemohr et al.²⁰ In this method, weight fractions for the i th element were derived by a linear fit of Z_{eff} , ρ_e and an interaction of both. The weight fractions for the individual elements were combined to obtain tissue compositions. Negative weight fractions were replaced by zero and the summed weight fractions were normalized to unity. In contrast to the fitting procedure for Z_{eff} and ρ_e (which was applied to the phantom tissues), the fitting procedure for the elemental weight fractions was applied to the 71 human reference tissues listed by Schneider et al.²¹ However, the lung tissue was excluded from the fitting procedure due to its aberrant values. A calibration curve (equation 3) was fitted through the 70 remaining points. A separate fit was performed for the soft tissues ($Z_{eff} < 8.2$) and the bone tissues ($Z_{eff} \geq 8.2$). In this equation, a, b, c and d are fit parameters. The index i iterates over the six most abundant elements in the human body, which are hydrogen (H), carbon (C), nitrogen (N), oxygen (O), phosphorus (P) and calcium (Ca).

$$\omega_i = a_i \cdot \rho_e + b_i \cdot Z_{eff} + c_i \cdot \rho_e Z_{eff} + d_i \quad (3)$$

Standard deviations

The standard deviations in Z_{eff} and ρ_e represent the spread in tissue composition and density. However, the measured spread in Z_{eff} and ρ_e is not solely caused by the heterogeneity of the tissues. When acquiring measurements of homogeneous materials such as phantom inserts, a small spread in Z_{eff} and ρ_e can be observed, which is mainly caused by imaging noise in the CBCT images. Since this study focuses on the actual heterogeneity of the tissues (σ_{tissue}), the contribution of noise to the spread in Z_{eff} and ρ_e needs to be removed first. To this end, data from a previous study were used in which the same imaging protocols were used and thus the same noise levels

can be expected.¹⁵ From these data, standard deviations for eight homogeneous validation phantom inserts were obtained (σ_{noise}). The validation phantom in the previous study contained 3 out of 12 empty insert positions and a Teflon insert which yielded aberrant results. The empty insert positions and the Teflon insert were left out. The standard deviations were then quadratically subtracted from the standard deviations that were obtained in the present study ($\sigma_{measured}$) using equation 4. In this equation, the mean standard deviation σ_{noise} equals 0.30 for Z_{eff} and 0.034 for ρ_e .

$$\sigma_{tissue} = \sqrt{\sigma_{measured}^2 - \sigma_{noise}^2} \quad (4)$$

Mass energy-absorption coefficients

To investigate the influence of tissue compositions on the absorbed radiation dose, μ_{en}/ρ was calculated using the NIST tables of photon mass attenuation coefficients and mass energy-absorption coefficients.²² The 225 kVp spectrum for the X-RAD 225Cx system including 0.3 mm copper filtration was calculated using SpekCalc.^{23–25} This spectrum, which is assumed to be invariant throughout the mouse, has a mean photon energy of 86.1 keV and a half value layer of 0.975 mm copper or 11.6 mm aluminum. μ_{en}/ρ values for the 225 kVp spectrum were calculated for the individual elements (H, C, N, O, P and Ca), which were then multiplied by the elemental weight

Figure 2. Distribution of the effective atomic number. Top: mean ± 1 standard deviation ranges for the mice with the most extreme mean values for each tissue. Black and gray error bars indicate the corrected and uncorrected standard deviations, respectively. Bottom: comparison of the spread in effective atomic number (indicated by the ± 1 standard deviation ranges) between mice and within tissues.

Figure 3. Distribution of the relative electron density. Top: mean ± 1 standard deviation ranges for the mice with the most extreme mean values for each tissue. Black and gray error bars indicate the corrected and uncorrected standard deviations, respectively. Bottom: comparison of the spread in relative electron density (indicated by the ± 1 standard deviation ranges) between mice and within tissues.

fractions and summed to obtain μ_{en}/ρ for the different tissue compositions.

Reference data

The human reference data used in this study were taken from ICRU and ICRP reports.^{10–12} In addition, two publications from the 1980's by Woodard and White, which formed the basis for these ICRU and ICRP reports, were used in this study.^{26,27} Two entries for marrow tissues are listed in the reference data: one for yellow marrow and one for red marrow. For this study, the assumption from Schneider et al that marrow consists of a 1:1 mixture of yellow and red marrow was adopted.²¹ The reference data that are listed for the femur applies to the total bone, which

is a mixture of bone and marrow. For the rib tissues, two entries are listed in the reference data: one for the 2nd and 6th rib and one for the 10th rib. The reference data for the 10th rib was used.

RESULTS

The distributions of Z_{eff} and ρ_e voxels in the examined murine tissues are shown in Figures 2 and 3, respectively. The crosses and diamonds represent the extracted mean values and the circles represent the reference values. The corrected standard deviations are indicated by the black error bars and the uncorrected standard deviations are indicated by the gray error bars. The black and gray error bars overlap for most of the bone tissues. The spread (indicated by the ± 1 standard deviation ranges) within

Figure 4. Distribution of the mass-energy absorption coefficients. The crosses represent the mean values (± 1 standard deviation ranges) extracted using the dual energy CT method. The circles represent the mass-energy absorption coefficients calculated from the reference data.

tissues is considerably larger than the spread of mean values between mice. This can be observed in both Figure 2 for Z_{eff} and Figure 3 for ρ_e . For Z_{eff} , a good agreement ($<2.5\%$ difference) was found between the extracted mean values and the reference values for brain, eye, heart, kidney, liver and muscle. Larger differences ($>5.0\%$) were found for cortical bone, femur, rib and especially marrow (17.4%). The difference between extracted mean Z_{eff} and reference Z_{eff} was found to be positive for some tissues and negative for other tissues. A more one-sided difference was found for ρ_e . All tissues examined in this study, with the exception of cortical bone and rib, have a higher extracted mean ρ_e than reference ρ_e . On average, the ρ_e difference equals 6.8%. The largest errors were found for cortical bone (16.6%), marrow (12.6%) and rib (18.7%). A large spread in both Z_{eff} and ρ_e can be observed between the different voxels of each tissue examined in this study. On average, the ± 1 standard deviation ranges were found to be 1.5 for Z_{eff} and 0.2 for ρ_e .

Figure 4 shows the mean μ_{en}/ρ values and their mean spread for the tissues examined in this study. For the soft tissues, μ_{en}/ρ was found to be larger for the extracted data than for the reference data. The opposite was found for the bone tissues. An average difference of 11.3% was found between the extracted data and the reference data, more specifically 7.5% for soft tissues (4.0% excluding marrow) and 20.1% for bone tissues. When considering the spread within tissues, errors ranging from -5.5% (eye) to $+17.5\%$ (marrow) were found for the soft tissues and errors ranging from -53.9% to $+38.0\%$ (both femur) were found for bone tissues. Both error ranges apply to the ± 1 standard deviation interval, which only makes up 68% of the voxels in the case of a normal distribution.

DISCUSSION

This work shows that differences between human and murine tissue compositions can lead to errors around 7.5% for soft

tissues and 20.1% for bone tissues in μ_{en}/ρ values. Assuming an approximately linear relationship between μ_{en}/ρ and the absorbed radiation dose for kilovoltage photon beams (*i.e.* the potential photon fluence perturbation by the different media is ignored), differences in μ_{en}/ρ are proportional to differences in absorbed radiation dose. Therefore, errors around 7.5% for soft tissues and 20.1% for bone tissues can be expected in dose calculations for kilovoltage photon beams. When considering the spread within tissues, these errors can increase up to 17.5% for soft tissues and 53.9% for bone tissues within only a single standard deviation away from the mean tissue value. Even larger errors can be expected for softer X-ray spectra (*i.e.* when using lower tube voltages or thinner filters for the treatment beam) in which μ_{en}/ρ differs even more between different tissues.² Although an overall good agreement was found between the extracted and reference Z_{eff} , the strong dependence of the atomic cross-section for photoelectric absorption on Z leads to large differences in μ_{en}/ρ and thus in the absorbed radiation dose. The one-sided difference that was found between the extracted (murine) and reference (human) ρ_e could lead to errors in methods that rely on the reference data for densities. For example, errors in CT number predictions could occur when using the stoichiometric method described by Schneider et al.²⁸ When assigning densities using the human reference data, the differences in ρ_e could also lead to errors in dose calculations.

The accuracy of the extracted Z_{eff} and ρ_e is influenced by a number of different factors. One of these factors is imaging noise. In this study, the extracted standard deviations are corrected for the contribution of noise based on measurements in a phantom with homogeneous tissue-equivalent inserts. Another factor is beam hardening, which could be a possible explanation for the large difference found between the extracted and reference values for marrow. The marrow tissue is surrounded by dense cortical bone tissue, which could substantially harden the X-ray spectrum and

thus influence the CT numbers from which Z_{eff} and ρ_e are calculated. Other imaging artifacts, such as scatter, partial volume effects and motion of the animal could also compromise the accuracy of CT numbers. More research is required to investigate the influence of these factors on Z_{eff} and ρ_e derived from DECT.

The discrepancies for the marrow tissue could also be caused by the fact that the assumption that marrow consists of a 1:1 mixture of yellow and red marrow is not accurate for mice. For the femur, any discrepancies between the measured and reference values could be caused by a difference in bone/marrow ratio between mice and humans. Rib tissue discrepancies could be caused by the fact that the different ribs in the thorax contain different amounts of cartilage. This could also explain the large difference between the mouse with the lowest value and the mouse with the highest value in Figures 2 and 3. Aside from being different to human tissues, murine tissues might also vary between different strains, genders and ages among others.

Uncertainties in tissue compositions lead to an additional uncertainty in dose calculations. As a first step to investigate the magnitude of this uncertainty, μ_{en}/ρ was calculated for 10 different murine tissues and compared to μ_{en}/ρ values that were calculated from the human reference data. To investigate the accumulated dose difference to an actual tumor, dose calculations are required to account for differences in absorption and

attenuation of the treatment beam which are caused by differences in the elemental compositions and densities of the tissues through which the beam passes.

CONCLUSION

This study illustrates the need for murine reference tissue data. However, a large collection of data would be required to include all different strains, genders and ages among others. Moreover, assigning only a single mean reference value to an entire tissue can still lead to large errors in dose calculations given the large spread within tissues of μ_{en}/ρ values found in this study. Therefore, new methods such as DECT and spectral CT imaging need to be explored, which can be important next steps in improving tissue assignment for dose calculations in small animal radiotherapy.²⁹

COMPETING INTERESTS

FV is a founder of SmART Scientific Solutions (Maastricht, Netherlands), which works with the company Precision X-ray (North Branford, CT) on commercial development of small animal treatment planning software.

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