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Improved tumour response prediction with equivalent uniform dose in pre-clinical study using direct intratumoural infusion of liposome-encapsulated ^{186}Re radionuclides

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Abstract

Crucial to all cancer therapy modalities, is a strong correlation between treatment and effect. Predictability of therapy success/failure allows for the optimization of treatment protocol and aids in the decision of whether additional treatment is necessary to prevent tumour progression. This work evaluated the relationship between cancer treatment and effect for intratumoural infusions of liposome-encapsulated ^{186}Re to head and neck squamous cell carcinoma xenografts of nude rats. Absorbed dose calculations using a dose point kernel convolution technique showed significant intratumoural dose heterogeneity due to the short range of the beta-particle emissions. The use of three separate tumour infusion locations improved dose homogeneity compared to a single infusion location as a result of a more uniform radioactivity distribution. An improved dose-response correlation was obtained when using EUD calculations based on a generic set of radiobiological parameters ($R^2 = 0.84$) than when using average tumour absorbed dose ($R^2 = 0.22$). Varying radiobiological parameter values over ranges commonly used for all types of tumours showed little effect on EUD calculations which suggests that individualized parameter use is of little significance as long as the intratumoural dose heterogeneity is taken into consideration in the dose-response relationship. The improved predictability achieved when using EUD calculations for this cancer therapy modality may be useful for treatment planning and evaluation.

1. Introduction

The value of any cancer therapy modality is largely determined by predictability; that is, the relationship between treatment and effect. This has hindered the clinical use of many anti-cancer agents with unpredictable treatment responses resulting from extensive variation in inter-patient pharmacokinetics and intratumoural uptake patterns. With cancer therapy using radionuclides, poor predictability can lead to delivery of insufficient absorbed dose for achieving tumour eradication or significant levels of absorbed dose delivered to healthy organs or tissues of the body causing complications as well as an increased risk for developing radiation-induced cancers. To alleviate this problem, use of tracer kinetics for treatment planning has been employed with the assumed condition of similar pharmacokinetics at therapeutic dosage levels (Bischof Delaloye 2000). Other techniques disregard a predictable dose-response relationship and simply use methods of improving radionuclide localization to escalate tumour absorbed dose levels while sparing normal tissues (Milenic and Brechbiel 2004, French *et al* 2010), basing the treatment on the broadly

accepted theory of tumour control being a probabilistic event with higher radiation dose resulting in improved tumour control.

Passive or active targeting and intralesional delivery techniques have been used to increase radiopharmaceutical uptake to tumours in a more predictable manner while decreasing systemic toxicity issues. The improved localization allows for absorbed dose escalation resulting from increased normal tissue sparing. In a Phase I clinical trial, intralesional infusions of colloidal ^{32}P for nonresectable pancreatic cancer delivered ablative average absorbed dose levels to tumours up to 17,000 Gy (Order *et al* 1996), a level unachievable with current systemic delivery approaches to solid tumour therapy as well as external beam radiotherapy modalities due to risk of healthy organ/tissue complications. This advantage is achieved through excellent target localization and retention in addition to the focal dose delivery patterns from the mm range of beta-particle emissions from the therapeutic radionuclides. However, this short range radiation can also result in significant intratumoural absorbed dose heterogeneity, possibly leading to therapy failure. Simple absorbed dose escalation may be inefficient in achieving adequate tumour absorbed dose coverage for tumour control. (O'Donoghue 1999, Prideaux *et al* 2007, Kalogianni *et al* 2007, Amro *et al* 2010, Hrycushko *et al* 2011b).

The method of radiation delivery evaluated in this work uses direct intratumoural infusions for improved localization and liposome-encapsulation for sustained intratumoural retention. Liposomes are nano-scale biodegradable lipid vesicles shown to be effective and safe drug-delivery vehicles for the treatment of human diseases (Gabizon 2007). A potential benefit of this form of radiation delivery is the radiobiological consequences of transitioning from a high dose-rate to low dose-rate over the course of treatment with the initial high dose-rate providing for intense tumour cell killing which is advantageous for gross tumour debulking. This work evaluates the dose-response relationship for intratumourally infused ^{186}Re -liposome to head and neck squamous cell carcinoma (HNSCC) xenografts in nude rats using a commonly used radiobiological index and average tumour dose calculations. The results of this work may be helpful for predicting therapy outcome with this radiation delivery technique which is necessary if implemented in a clinical setting.

2. Methods

The animal study in this work was performed to evaluate the improved therapy effect when using infused ^{186}Re -liposomes following neoadjuvant bevacizumab therapy as well as comparing the treatment effect when using multiple versus single intratumoural infusion locations as predicted by a previous $^{99\text{m}}\text{Tc}$ -liposome imaging study (Hrycushko *et al* 2011b). This paper reports on the tumour dose-response relationship by correlating intratumoural dosimetric and radiobiological index calculations from in vivo small animal micro-SPECT/CT images with tumour volume changes and is not meant to compare and contrast treatment groups.

2.1 HNSCC xenografts in nude rats

Animal experiments were conducted in accordance with the NIH Animal Use Guidelines and were approved by the University of Texas Health Science Center at San Antonio (UTHSCSA) Institutional Animal Care Committee. During all animal handling procedures, animals were anesthetized with 1-3% of isoflurane (Vedco, St. Joseph, MO) in 100% oxygen using an anesthesia inhalation machine (Bickford, Wales Center, NY).

A human HNSCC xenograft model was used in nude rats as previously described (Bao *et al* 2006). Male *rnu/rnu* athymic nude rats (Harlan, Indianapolis, IN) at age 4-5 weeks (75-100g) were inoculated subcutaneously with 5×10^6 SCC-4 tumour cells (ATCC,

Manassas, VA) in 0.2 ml of saline on the dorsum at the level of the scapulae. This is a head and neck SCC cell line of human tongue cancer and has previously been characterized with hematoxylin-eosin (HE) staining. Tumour size was obtained daily by measuring the length (l), width (w), and thickness (d) of each tumour with a caliper up to 43 days post-treatment. Tumour volumes (V) were calculated using an ellipsoid volume formula, $V = (\pi/6)lwd$ (Tomayko and Reynolds 1989).

2.2 Liposome preparation and radionuclide encapsulation

Liposome preparation and characterization followed a previously described protocol (French *et al* 2010). Neutrally charged liposomes were comprised of distearoyl-phosphatidylcholine (DSPC) (Avanti Polar Lipids, Alabaster, AL) and cholesterol (Calbiochem, San Diego, CA) (molar ratio of 55:45). Following sequential extrusion through polycarbonate filters with different pore sizes from 2 μ m to 100 nm at 55°C (Lipex Extruder, Northern Lipids, Vancouver, Canada), resulting liposomes had 60 mM (36.5 mg/ml) total lipid concentration with a medium of 150 mM sucrose, 200 mM glutathione (GSH) and 300 mM ammonium sulfate (pH 5.1). Repeated ultracentrifugation (41,000 rpm; Ti 50.2 rotor) (Beckman, Fullerton, CA) for 50 minutes as well as washing with 300 mM ammonium sulfate containing 75 mM sucrose (pH 5.1) in sterile water was used to remove un-encapsulated glutathione. Final liposome pellets were re-suspended in 300 mM ammonium sulfate (pH 5.1) containing 300 mM sucrose in sterile water at a total lipid concentration of 60 mM and stored at 4°C until needed. Liposome particle diameter was measured with a 488-nm DLS laser light scattering instrument equipped with a DynaPro Dynamic Light Scattering system (Wyatt Technology, Santa Barbara, CA) and was found to be 108.0 ± 26.4 nm (Mean $\pm$ SD). Endotoxin levels were assayed with limulus amoebocyte lysate (LAL) Pyrotell (Associates of Cape Cod Inc., E. Falmouth, MA) and were less than 12.5 EU/ml. There were no bacteria or fungus growth during the 14-day culture.

Labeling of liposomes with ^{186}Re -radionuclides consisted of ^{186}Re -N,N-bis(2-mercaptoethyl)-N',N'-diethyl-ethylenediamine (BMEDA) preparation and post-loading of ^{186}Re -BMEDA into liposome nanoparticles as previously described (Wang *et al* 2008). In brief, the preparation of ^{186}Re -liposomes included two steps: 1) the preparation of ^{186}Re -BMEDA mediated by the reduction of ^{186}Re -perrhenate with stannous chloride and glucoheptonate (GH); and 2) post-loading of ^{186}Re -BMEDA into the liposomes followed by purification of ^{186}Re -liposomes with Sephadex G-25 column chromatography eluted with PBS (pH 7.4) buffer. The labeling efficiency of ^{186}Re -liposomes (as a percentage of ^{186}Re radioactivity associated with liposomes) was 77.8%.

2.3 Treatment groups

Each of the 13 nude rats (average tumour volume = 1.75 ± 0.57 cm³) in the treated groups was infused with a total volume of ^{186}Re -liposomes equal to 45% of the individual tumour volumes (5.0 mCi/cm³ tumour). The three treated groups were distinguished as follows: 1) single location intratumoural infusion ($n = 4$) with the cannula kept in place for three consecutive infusions at a rate of 0.5 ml/min in 20 minute intervals (a volume equal to 15% of the tumour volume with each infusion); 2) three separate intratumoural infusion locations ($n = 4$) with each cannula location uniformly distributed throughout the tumour and an infusion volume equal to 15% of the tumour volume infused at 0.5 ml/min for each cannula location; and 3) two neoadjuvant bevacizumab treatments (i.p. injection; 1 mg per injection) in two-day intervals followed by the same ^{186}Re -liposome treatment protocol as in group 2 ($n = 5$).

2.4 Image acquisition and analysis

Static planar gamma camera images were acquired using a micro-SPECT/CT scanner (XSPECT, GammaMedica, Northridge, CA) using a parallel hole collimator with the rat in the prone position immediately following infusion, 2 h, 4 h, 20 h, 44 h, and 140 h for attaining clearance kinetics. A standard ^{186}Re -source with known activity was positioned adjacent to the rat within the field of view (FOV) for image quantification. Regions of interest (ROIs) were drawn around tumours and the standard using Mango imaging software (Research Imaging Institute, UTHSCSA, San Antonio, TX) to quantify radioactivity within each ROI, and first order exponential curves were fit to determine clearance rates for individual rats. Between 20 h and 44 h planar imaging, animals were imaged with 1-mm pinhole collimators using the micro-SPECT/CT scanner to determine intratumoural activity distribution. SPECT images were acquired with the radius of rotation (ROR) set as small as possible while keeping each tumour within the FOV during the entire image acquisition. Image acquisitions included 64 projections at 30 s per projection for tomographic image reconstruction. CT images were acquired (75 kVp, 370 mA, 256 projections) with tumours maintained at the same position for SPECT/CT image co-registration.

Tumours were segmented from CT images of the co-registered SPECT / CT images using Mango imaging software and imported to Matlab software (ver. 7.4.0.287 [R2007a], Mathworks, Natick, MA) in matrix format with voxel sizes of $0.35 \times 0.35 \times 0.35 \text{ mm}^3$. The effective clearance rate, calculated from the curve fitting from planar imaging, was assumed to be the same for every voxel and was used to calculate initial ^{186}Re activity in each voxel. It is important to note that this technique, as previously described and used as an alternative to multiple SPECT images, has the implicit assumption that the relative spatial distribution measured during the SPECT/CT image acquisition remains constant (Sgouros *et al* 2003, Prideaux *et al* 2007). Different regions within the tumour may have variations in clearance rates, effects of which have previously been investigated for uptake of systemically administered radionuclides; however, intratumoural variation in clearance rates is unknown for direct intratumoural infusion of liposome encapsulated radionuclides (Sgouros *et al* 2004). The assumption of low intratumoural variation seems appropriate due to the very slow clearance following the disappearance of the delivery convection force following infusion and lipid nanoparticles, being larger than small molecular radionuclide compounds, have a lower capacity for intratumoural diffusion. Cumulative activity was then calculated for individual voxels to be used with intratumoural absorbed dose calculations.

2.5 Dosimetry and radiobiological modeling

Table 1 provides a list of symbol definitions and applicable values used for dosimetric and radiobiological modeling. The dose point kernel (DPK) convolution technique, which is based on convolving a radionuclide DPK with a cumulative activity distribution, was used to calculate intratumoural dose distributions (Hrycushko *et al* 2011a, Bao *et al* 2005, Giap *et al* 1995). The EGSnrc Monte Carlo simulation user code EDKnrc was used to generate beta and photon DPKs for ^{186}Re within a water medium (Kawrakow and Rogers 2000, Rogers *et al* 2003). The DPKs were input into matrix format in Matlab with the same voxel dimensions as that of the SPECT/CT images, and the convolution of the DPK matrix with the tumour cumulative activity matrix was calculated using Fourier transform (FT), multiplication ($\cdot$), followed by inverse Fourier transform (FT^{-1}) (Hrycushko *et al* 2011a, Bao *et al* 2005):

$$D(i, j, k) = \text{FT}^{-1} \left\{ \text{FT} [K(i, j, k)] \cdot \text{FT} [\tilde{A}(i, j, k)] \right\} \quad (1)$$

where i, j , and k are voxel indices. The calculated absorbed dose distributions were used to assess intratumoural absorbed dose heterogeneity with the use of dose-volume histograms (DVHs).

Effective uniform dose (EUD) was used to assess the radiobiological effects and predict therapy response from the nonuniform intratumoural dose distributions. In this case, EUD is defined as the uniform value of biologically effective dose (BED) which would produce the same surviving fraction as the nonuniform distribution seen from the animal study (O'Donoghue 1999). Assuming uniform tumour clonogen density distribution, the EUD is determined from the linear-quadratic model and was calculated for each tumour using:

$$\text{EUD} = \frac{-\ln \left[\frac{1}{N} \sum_{i=1}^N \exp(-\alpha \cdot \text{BED}_i) \right]}{\alpha} \quad (2)$$

For the protracted radiation delivery provided by the liposome-encapsulated radionuclides, voxel BED values are calculated using (Butler *et al* 2009):

$$\text{BED}_i = D_{\text{Teff},i} \times \text{RE}_{\text{Teff},i} - \frac{\gamma \cdot T_{\text{eff},i}}{\alpha} \quad (3)$$

$$T_{\text{eff},i} = \frac{1}{\lambda_{\text{ET}}} \ln \left(\frac{\alpha \cdot \dot{D}_{0,i}}{\gamma} \right) \quad (4)$$

$$D_{\text{Teff},i} = D_i [1 - \exp(-\lambda_{\text{ET}} \cdot T_{\text{eff},i})] \quad (5)$$

$$\text{RE}_{\text{Teff},i} = 1 + \left(\frac{\beta}{\alpha} \right) \left[\left(\frac{2\dot{D}_{0,i} \cdot \lambda_{\text{ET}}}{(\mu - \lambda_{\text{ET}}) [1 - \exp(-\lambda_{\text{ET}} \cdot T_{\text{eff},i})]} \right) \right] \times \left(\frac{1 - \exp(-2\lambda_{\text{ET}} \cdot T_{\text{eff},i})}{2\lambda_{\text{ET}}} - \frac{1 - \exp[-(\mu + \lambda_{\text{ET}}) T_{\text{eff},i}]}{\mu + \lambda_{\text{ET}}} \right) \quad (6)$$

3. Results

3.1 Absorbed dose calculations

Intratumoural absorbed dose calculations were performed using the DPK convolution technique as described in the methods section. This section shows the considerable amount of intratumoural dose heterogeneity with this method of radiation delivery and issues associated with the relationship between average tumour dose and tumour control.

3.1.1 Average tumour absorbed dose—Table 2 shows the results of absorbed dose and EUD calculations for individual rats of each therapy group. Rats receiving a single tumour infusion location have more intratumoural dose heterogeneity as evident from the higher relative standard deviations of absorbed dose (standard deviation/average absorbed dose). The average relative standard deviation of absorbed dose was 1.04 ± 0.23 for the single infusion location group, 0.90 ± 0.08 for the multiple infusion location group, and 0.65 ± 0.12 for the group receiving the neoadjuvant bevacizumab protocol in addition to multiple

infusion locations. The use of multiple tumour infusion locations improved intratumoural dose distribution homogeneity. It is also clear that considerable levels of average tumour doses were delivered for each rat for low levels of infused activity; however, calculated EUD values are much lower due to regions of the tumour receiving inadequate dose. This distinction has previously been seen (Prideaux *et al* 2007, Hrycushko *et al* 2011b). Table 2 shows therapy response as calculated by relative volume change; that is, ratio of final day 43 tumour volume to initial day 0 tumour volume. Figure 1 was used to assess the validity in using average tumour dose to predict tumour response with this therapy modality. In this figure, anything below the 'no change' line had a reduction in tumour volume resulting from the therapy, while any data points above this line had an increase in tumour volume by the end of the therapy evaluation period. There was a poor correlation between average tumour absorbed dose and therapy outcome when fitting to an exponential model ($R^2 = 0.22$). High average absorbed dose did not necessarily predict improved tumour control while average dose levels on the lower end did not necessarily correspond with reduced treatment efficacy.

3.1.2 Intratumoural dose heterogeneity—Intratumoural absorbed dose distributions are represented in average DVHs for the three therapy groups in figure 2. The DVHs show the percentage of tumour volume on the y-axis receiving at least the absorbed dose represented on the x-axis. Figure 2a clearly shows the large intratumoural dose heterogeneities which are characteristic of radionuclide therapy modalities. The single tumour infusion location group had better tumour coverage along the high dose region due to a more focused radioactivity distribution from localized infusions occurring at the same place, while the groups receiving three tumour infusion locations had better tumour coverage at lower dose levels resulting from more homogeneous intratumoural radioactivity distributions. Normalization of EUD values to average tumour dose may be used as a measure of absorbed dose heterogeneity. ANOVA analysis showed both multiple infusion location groups had significantly improved intratumoural dose uniformity compared with a single infusion location ($P < 0.05$). Of the two groups with three separate tumour infusion locations, the group receiving the neoadjuvant bevacizumab treatment protocol had improved tumour dose coverage due to the enhanced radioactivity retention following infusion. Figure 2b shows the same results using a semilogarithmic plot to better illustrate the low dose region. The use of a single tumour infusion location results in a large tumour volume receiving minimal absorbed dose levels. This is reflected in the lower EUD values for this group compared to the groups receiving three infusion locations, as seen in table 1, and substantiates that reported by Niemierko (1997) and Tome and Fowler (2002) on cold spots having a great impact on EUD and tumour control.

3.2 Equivalent uniform dose calculations

This section evaluates the relationship between calculated EUD and therapy outcome using the radiobiological parameters of table 1. The radiobiological parameters used for modeling purposes come from widely used values of published works, as the exact parameter values for the HNSCC xenografts used are currently unknown. Accordingly, to understand how these parameters may effect the relationship between treatment and therapy outcome, the influence of each parameter to calculated EUD values has been evaluated.

3.2.1 EUD versus tumour response—In a similar manner to the methods used for section 3.1.1, figure 3 assesses the validity in using EUD to predict tumour response for this therapy modality. An excellent correlation was seen between EUD and therapy outcome in contrast to that observed when using average tumour dose in figure 1. Fitting resulted in an R^2 value of 0.84 for figure 3 in contrast to an R^2 value of 0.22 for figure 1 when using an exponential model.

The above EUD calculations used standard radiobiological parameters for head and neck cancers. Since the radiobiological parameters of the tumour model used in this work are currently undetermined, the following sections describe the sensitivity of the calculated EUD values by varying each parameter.

3.2.2 Variation in α/β ratio—In a similar approach taken by Ebert (2000), the sensitivity of EUD to α/β was evaluated; in this case, for the actual intratumoural activity distribution from the technique of radiation delivery used in this animal study having much higher standard deviations in absorbed dose. Average group EUD values were calculated by varying values of α/β over that commonly seen for tumours for different values of α , as shown in figure 4. Similar to that discussed by Ebert (2000), EUD values vary little with changes in α/β with an average percent difference of 2.5% and a maximum percent difference of 3.2%. A much larger difference was seen by varying values of α , with lower calculated EUD values seen with increasing α .

3.2.3 Variation in cell proliferation rate—The sensitivity in EUD versus tumour cell proliferation rate was evaluated by keeping the radiobiological parameters used in section 3.2.1 constant and varying the potential doubling time (T_{pot}) for each rat as seen in figure 5. T_{pot} indicates the cell production rate of the tumour as the estimated doubling time of the tumour cell population without cell loss. The legend of the figure shows the therapy outcome in parentheses. It is interesting that the EUD values seem to increase rapidly and then level off with further increasing of T_{pot} values ($T_{pot} = 0.693/\gamma$) for rats which had favorable therapy outcomes as seen from reduction in tumour size. Rats of which had poor therapy outcomes have decreasing calculated EUD values as T_{pot} is increased with the radiobiological model used in (2-6). These trends lead to improved relationships between tumour control and calculated EUD values at higher T_{pot} values, suggesting minimal tumour cell proliferation during treatment.

3.2.4 Variation in cell repair rate—The sensitivity in EUD versus tumour repair rate was evaluated by keeping the radiobiological parameters used in section 3.2.1 constant and varying the sublethal repair half-time ($T_{rep} = 0.693/\mu$) values for each rat as seen in figure 6. As before, the legend of the figure shows the therapy outcome in parentheses. Calculated EUD values have little variation with increasing T_{rep} for rats which had a favorable therapy outcome, with the slight increase in EUD with T_{rep} for those rats with the most favorable therapy outcomes. Similar to that seen with increasing T_{pot} values, calculated EUD values decrease with increasing T_{rep} for rats with unfavorable therapy outcome with the radiobiological models use in (2-6). These trends suggest little tumour cell repair during treatment.

4. Discussion

Radionuclide therapy modalities are able to deliver ablative tumour absorbed doses unachievable with commonly used radiation delivery techniques due to extreme focal treatment ranges on the millimeter to centimeter scale. However, limited success has been seen in a clinical setting and it has been speculated that intratumoural absorbed dose non-uniformity plays a significant role in treatment success or failure (O'Donoghue 1999, Kalogianni *et al* 2007, Amro *et al* 2010). The aim of this study was two-fold: 1) To look at the intratumoural absorbed dose heterogeneity following different methods of direct intratumoural infusion of ^{186}Re -liposomes to HNSCC xenografts of nude rats; and 2) To evaluate the relationships of average tumour dose and EUD with therapy effect.

This work showed that using multiple tumour infusion locations improves intratumoural dose homogeneity. This, as well as the use of beta-emitting radionuclides with higher energy

emissions has previously been predicted to improve dose homogeneity and therapy outcome (Hrycushko *et al* 2011b). Using multiple tumour infusion locations is much more efficient in terms of radionuclide activity usage in achieving tumour control compared with simple absorbed dose escalation by increasing specific activity. As a result, normal tissue/organ toxicity issues are less of a concern.

A strong correlation was seen between an EUD model and therapy outcome while a relationship between average tumour dose and therapy outcome remained elusive for this radiation delivery technique. This conclusion is under the assumption that tumour shrinkage during the time frame considered with this animal study correlates with tumour control. This is important to consider for all radionuclide therapy modalities as many radioimmunotherapy or radionuclide therapy guidelines, for simplicity, assume uniform activity uptake and assess an average tumour doses (Macey *et al* 2001). Correlations between average dose and tumour shrinkage have previously been investigated for non-Hodkin's lymphoma patients treated with ^{131}I -tositumomab therapy and agree with that observed in this work. Amro *et al* (2010) showed poor correlation ($R^2 = 0.09$) with a marginally significant ($P = 0.03$) increase in tumour shrinkage with higher tumour doses. Sgouros *et al* (2003) showed a slight increase in tumour response with improved uniformity ($r = 0.37$; $P = 0.06$), yet no correlation between tumour response and average dose, maximum dose, or minimum dose ($P = 0.25$ to $P > 0.5$). Amro *et al* (2010) did show a strong correlation between EUD values and tumour shrinkage ($R^2 = 0.77$) which also agrees with the results of this work.

It is important to consider the voxel size and image resolution when assessing intratumoural dose distributions and using EUD calculations to predict tumour response. The micro-SPECT/CT scanner used in this work with pinhole imaging magnification has excellent sub-millimeter resolution currently unattainable for clinical SPECT/CT systems which may achieve resolutions on the order of several millimeters (Patton *et al* 2009). Poor resolution combined with the short beta-particle penetration of ^{186}Re emissions [$E_{\text{avg}} = 0.329$ MeV and CSDA range = 0.483 g/cm 2 (Syme *et al* 2003)] will smear the activity and absorbed dose uniformly over larger voxel sizes and increase the calculated EUD value. Consequently, this may detract from the correlation between calculated EUD and therapy outcome.

The exact radiobiological parameters of the tumour model used in this work were unknown, as is commonly seen in a clinical setting. Generally, standard published values for the type of tumour in question are used for radiobiological modeling; however, newer techniques using maximum-likelihood estimation for parameter determination and compatibility testing seems beneficial in building strong dose-response relationships for tumours and normal tissues in a clinical setting (Mavroidis *et al* 2010). This work assessed EUD sensitivity to changes in each radiobiological parameter similar to previous work by Ebert (2000). Little variation in calculated EUD values seem to indicate that actual implementation of an EUD model to take into account intratumoural dose heterogeneity is much more important for predicting tumour response than considering the accuracy of individualizing radiobiological parameters for each rat as long as radiobiological parameters are kept consistent. The trends for EUD versus T_{pot} and T_{rep} indicates little tumour cell proliferation and repair during this treatment modality. This is in agreement with that stated by Dale and Jones for short treatment times (1998); short treatment times may delay cell cycle progression due to enhanced radiosensitivity at the G2/M checkpoint.

5. Conclusions

This work evaluated the relationship between cancer treatment and effect for intratumoural infusion of ^{186}Re -liposomes to HNSCC xenografts in nude rats. An improved dose-response

was shown when using an EUD model compared with the commonly used average tumour dose. The results indicated the importance of taking the intratumoural dose heterogeneity into consideration for treatment planning and evaluation.

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Figure 1.

Ratio of day 43 tumour volume to that of day 0 tumour volume versus average tumour absorbed dose for individual rats. ● represents rats receiving one tumour infusion location of ^{186}Re -liposomes, ▲ represents rats receiving three tumour infusion locations of ^{186}Re -liposomes, and ■ represents rats receiving neoadjuvant bevacizumab treatment in addition to three tumour infusion locations of ^{186}Re -liposomes.

Figure 2. Average DVHs for the three therapy groups. (a) Linear plot used to show significant tumour absorbed dose heterogeneity; (b) Semilogarithmic plot used to better illustrate low dose regions.

Figure 3.

Ratio of day 43 tumour volume to that of day 0 tumour volume versus EUD for individual rats. ● represents rats receiving one tumour infusion location of ^{186}Re -liposomes, ▲ represents rats receiving three tumour infusion locations of ^{186}Re -liposomes, and ■ represents rats receiving neoadjuvant bevacizumab treatment in addition to three tumour infusion locations of ^{186}Re -liposomes. The dotted line represents an exponential decay fit ($R^2 = 0.84$) to all data points.

Figure 4.

Variation in average group EUD values with α/β for different α values. ◆ represents rats receiving one tumour infusion location of ^{186}Re -liposomes, ■ represents rats receiving three tumour infusion locations of ^{186}Re -liposomes, ▲ represents rats receiving neoadjuvant bevacizumab treatment in addition to three tumour infusion locations of ^{186}Re -liposomes, and × represents α/β and α values for each group used for EUD calculations in figure 3.

Figure 5.

Variation in individual rat EUD values with T_{pot} . Numbers in parenthesis of legend represent relative tumour volume changes from table 1. Solid lines represent rats receiving one tumour infusion location of ^{186}Re -liposomes, dotted lines represent rats receiving three tumour infusion locations of ^{186}Re -liposomes, and dashed lines represent rats receiving neoadjuvant bevacizumab treatment in addition to three tumour infusion locations of ^{186}Re -liposomes, and $\times$ represents T_{pot} values for each rat used for EUD calculations in figure 3.

Figure 6.

Variation in individual rat EUD values with T_{rep} . Numbers in parenthesis of legend represent relative tumour volume changes from table 1. Solid lines represent rats receiving one tumour infusion location of ^{186}Re -liposomes, dotted lines represent rats receiving three tumour infusion locations of ^{186}Re -liposomes, and dashed lines represent rats receiving neoadjuvant bevacizumab treatment in addition to three tumour infusion locations of ^{186}Re -liposomes, and $\times$ represents T_{rep} values for each group used for EUD calculations in figure 3.

Table 1
Symbol explanations and values used for simulations

Symbol	Description	Value (if applicable)
$D(i,j,k)$	Voxel dose	N/A
K	DPK matrix	N/A
$\bar{A}$	Cumulative activity matrix	N/A
α/β	Ratio describing irreparable and reparable mechanisms	15 Gy ^a
α	Relates to initial slope of linear-quadratic model	0.35 Gy ⁻¹ ^b
γ	Repopulation time constant	0.14 days ⁻¹ ^c
$T_{eff,i}$	Effective treatment time	N/A
$D_{Teff,i}$	Dose delivered in effective treatment time	N/A
$RE_{Teff,i}$	Relative effectiveness per unit dose	N/A
λ_{ET}	Effective decay constant from tumour	N/A
μ	Repair time constant	5.55 days ⁻¹ ^b
$D_{0,i}$	Initial dose rate of voxel i	N/A
N	Total number of tumour voxels	N/A

^aData from Maciejewski *et al* 1989

^bData from Dale and Jones 1998

^cData from Fowler 2001

Table 2
Resulting absorbed dose calculations, EUD calculations, and therapy outcomes for individual rats receiving one tumour infusion location of ^{186}Re -liposomes (group 1), three tumour infusion locations of ^{186}Re -liposomes (group 2), and neoadjuvant bevacizumab treatment in addition to three tumour infusion locations of ^{186}Re -liposomes (group 3)

Group	Inj Act (mCi)	Avg Dose (Gy)	St Dev (Gy)	Max Dose (Gy)	Min Dose (Gy)	EUD (Gy)	Relative volume change
1	14.66	981.64	719.43	4645.57	0.38	12.39	0.56
1	7.87	497.03	583.50	4320.30	0.16	6.10	4.85
1	5.36	597.10	747.86	3637.59	0.10	6.15	6.77
1	8.58	741.79	755.93	3577.74	0.14	6.93	1.72
2	13.23	392.28	363.48	2287.12	0.42	8.21	0.75
2	8.94	830.10	675.60	3968.47	0.25	14.07	0.22
2	10.37	439.75	439.78	2656.64	0.11	6.90	5.44
2	8.58	556.57	478.14	2789.19	0.22	8.37	0.57
3	12.16	725.21	466.99	2688.29	0.29	11.21	0.33
3	11.44	520.69	438.18	2672.19	0.10	8.36	1.63
3	10.73	660.85	403.56	2874.11	1.70	22.41	0.21
3	4.65	645.61	339.13	1900.65	1.26	26.99	0.17
3	5.36	883.03	539.29	3404.80	0.56	15.75	0.15