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DOSIMETRIC EVALUATION OF ANTI-CD20 LABELED WITH ^{188}Re

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ABSTRACT

Radioimmunotherapy has the potential to deliver lethal radiation energy directly to malignant cells via targeting of radioisotope-conjugated monoclonal antibodies (MAbs) to specific antigens. B-cell lymphoma is a particularly good candidate for radioimmunotherapy because the disease is inherently radiosensitive, malignant cells in the blood, bone marrow, spleen and lymphonodes are accessible, and MAbs have been developed to B-cell surface antigens that do not shed or modulate. Rituximab (RTX), the human IgG1-type chimeric form of the parent murine antibody ibritumomab, is specifically targeted against CD20, a surface antigen expressed by pre-B and mature human B lymphocytes. The use of rhenium-188 from a ^{188}W/^{188}Re generator system represents an attractive alternative radionuclide for therapy. ^{188}Re is produced from beta decay of the ^{188}W parent. In addition to the emission of high-energy electrons (E_β= 2118 keV), ^{188}Re also decays with emission of a gamma photon with an energy of 155 keV in 15% abundance. Besides the therapeutic usefulness of ^{188}Re, the emission of gamma photon is an added advantage since the biodistribution of ^{188}Re-labeled antibodies can be evaluated *in vivo* with a gamma camera. Also, rhenium has chemical properties similar to technetium. Thus, both can be conjugated to antibodies using similar chemistry methods. The objective of this work is to prove the usefulness of this radiopharmaceutical based on dosimetric studies, that are also required by the Brazilian Regulatory Agency (ANVISA).

1. INTRODUCTION

Radioimmunotherapy (RIT) is a new area in Nuclear Medicine that employs monoclonal antibodies (MAb) labeled with therapeutic radioisotopes (α or β^- emitters) in the treatment of cancer, in particular in the therapy of Non-Hodkin's lymphoma (NHL). The principle is to combine the mechanism of action of the "cold" antibody with the damage caused by radiation (1).

The anti-CD20 (Rituximab), is a specific chimeric monoclonal antibody directed against CD20 antigen surface on B lymphocytes, used in the treatment of non-Hodgkin lymphoma (NHL). The association with beta-emitters radionuclides have shown greater therapeutic efficacy (2, 3, 4).

The radiation emitted from the radiolabeled MAb has an important biological effect that helps to explain the higher efficiency of RIT compared to therapy using non-labeled antibody. The first target for radiation damage is DNA, but the radiation can also damage the membranes and organelles and initiate an apoptotic cascade in cells (5). It has been shown that irradiation of cancer cells with β^- emitters induces apoptosis and activates the apoptotic cascade in leukemia cells using mitochondrial pathways and receptor death (6). Taking into

account the fact the goal of RIT is irradiate and destroy the tumor, care must be taken that normal organs don't receive excessive radiation. The specific therapeutic activity to be injected into a patient is based on informations obtained from dosimetric studies.

Actually, two radiopharmaceuticals prepared with Anti-CD20 FDA have approval for treatment of NHL: ^{131}I-AntiCD20 (Bexar®) and ^{90}Y-AntiCD20 (Zevalin®) (7). Techniques for radiolabeling anti-CD20 have been developed with ^{188}Re (8, 9) to evaluate the clinical use of this radionuclide in particular. The radionuclides with properties more suitable for RIT are ^{188}Re, ^{90}Y e ^{131}I, while ^{177}Lu and ^{90}Y are used in therapy with peptides receptor (PRRT). The main physical characteristics of these beta emitters are listed in Table 1 (8).

Table 1. Therapeutical characteristics of the main radionuclides used for therapy.

Radionuclide	Half-life (h)	γ emission energy (MeV) [abundance (%)]	Medium energy of β^- particles (MeV)	Maximum energy of β^- particles (MeV)	Medium range of β^- particles in tissue (mm)	Maximum range of β^- particles in tissue (mm)
^{188}Re	16.9	0.155 [15]	0.764	2.12	3.1	10.4
^{90}Y	64.1	–	0.935	2.28	4.0	11.3
^{177}Lu	161	0.208 [6.1]	0.133	0.497	0.23	1.8
^{131}I	192	0.364 [82]	0.182	0.610	0.39	2.3

The choice of radionuclides depends on its physical characteristics as well as characteristics of the tumor, target receptor and ligant. The advantage of beta particle-emiting radionuclides is the high tumor radiation dose, while maintaining normal tissue toxicity within acceptable limits (10).

Radionuclides that decays by β^- emission are the most used for therapeutic applications in clinical practice, having an appropriate range in the tissue and low linear energy transfer (LET). Radionuclides that emits particles α have a limited range in tissue (50-80 μm) and a high LET (100 keV/μm) (11). The Auger electrons are emitted during the process of electrons capture and internal conversion, deposit large amounts of energy on subcelulares dimensions, resulting in destruction of tumor cells more efficiently (12).

The use of ^{188}Re, produced by the decay of ^{188}W ($T_{1/2}$ = 69 d), from a ^{188}W/^{188}Re generator system (Figure 1), has represented an alternative to RIT. In addition of β^- emission for therapy, ^{188}Re also decays by γ emission (155 keV), important in the evaluation of biodistribution studies *in vivo* using gamma-camaras (13, 14) and dosimetry before the treatment. In terms of chemical properties, Re is located below technetium in periodic table. Thus, both may be conjugated to antibodies using similar chemical methods. (15).

$$^{186}\mathrm{W} \xrightarrow{n,\gamma} {}^{187}\mathrm{W} \xrightarrow{n,\gamma} {}^{188}\mathrm{W} \xrightarrow{\beta^- (T_{1/2}=69\ \mathrm{d})} {}^{188}\mathrm{Re} \xrightarrow{\beta^- (T_{1/2}=16{,}9\ \mathrm{h})} {}^{188}\mathrm{Os}\,(\mathrm{Stable})$$

Figure 1. Production scheme for ^{188}W and ^{188}Re decay from the neutron irradiation of enriched tungsten-186.

Based on these informations, the present study aims to evaluate the effectiveness of ^{188}Re-anti-CD20 in relation to its dosimetry. Such dosimetric studies are performed from the study and development of animal models combined with mathematical simulations. This paper describes the preliminary results concerning the evaluation and understanding of the mathematical models that will modelate the animal body and the biodistribution studies of ^{188}Re-antiCD20.

2. EXPERIMENTAL

All work is being performed in the Radiopharmacy Directory at the Instituto de Pesquisas Energéticas e Nucleares (DIRF - IPEN / CNEN-SP).

2.1. Materials

2.1.1 Computational Routine

The code used in this work is the Visual Studio.NET and C++. The mathematical routines will be implement through the *Dynamically Linked Library – DLL (*16). Figure 2 describes the methodology proposed for this study.

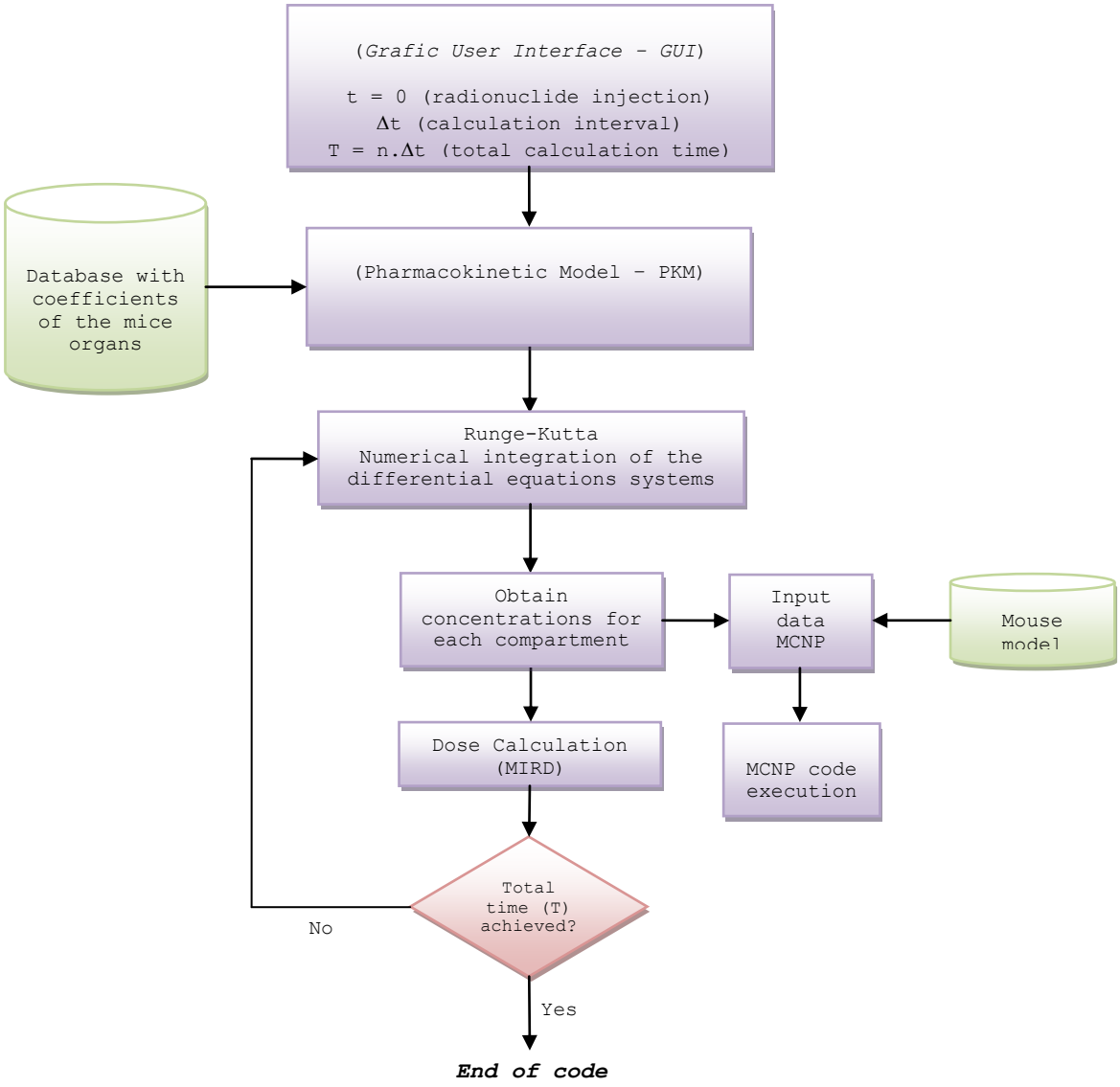


Figure 2. General flowchart, describing the methodology for the development of the code for studies of pharmacokinetics of ^{188}Re-anti-CD20 performed in an animal model.

The model is applied for each study radiopharmaceutical using biodistribution data obtained by DIRF research groups and literature. After the definition of the model, a simulation will performed using the Anti-CD20 monoclonal antibody labeled with ^{188}Re, evaluating tumor uptake, dosimetry in critical organs and biodistribution studies.

2.1.2 Code use

In this study we used the Monte Carlo n-Particles (MCNP) model 4C was used to simulate the radiation transport of the radionuclide ^{188}Re and for the determination of the dosimetry in critical organs. This first step consisted in determination of mouse geometry model by the code. This model was based on literature (17) and can see in Figure 3.

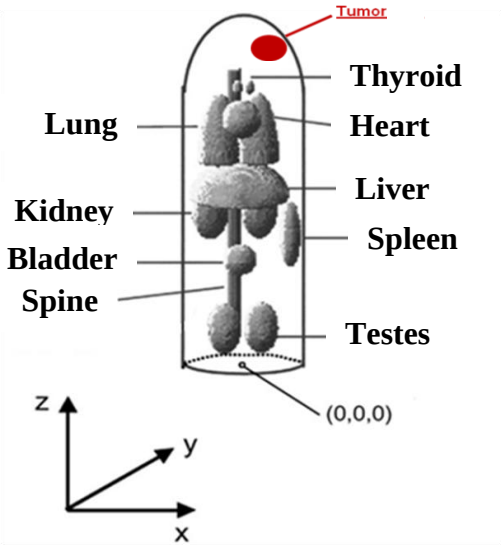


Figure 3. Mathematical model of mouse used in the determination of organ geometry applied in MCNP and tumor location.

The organs defined in the geometry by the code were: heart, thyroid, lungs, liver, spleen, kidneys, bladder, testicles, spine and the tumor. For the definition of the surfaces corresponding to each organ, ellipses (different dimensions) were used and a sphere for the tumor. Figures 4 and 5 show the distribution of organs and tumor in this simulation.

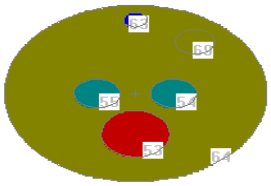
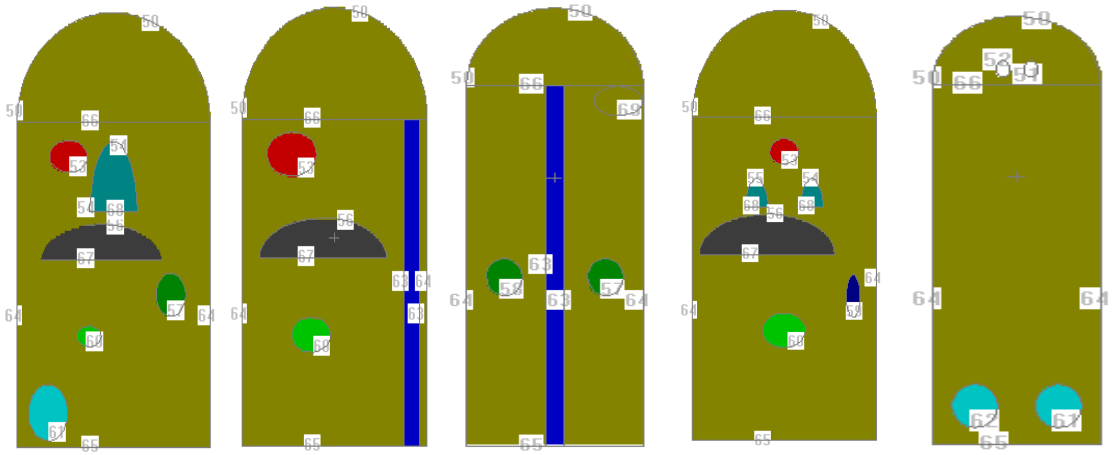


Figure 4. XY plane showing the location of tumor (69) between lung (54 and 55) and spine (63).



(1a) (2a) (1b) (2b) (3b)

Figure 5. Mouse geometry represented by YZ planes (1a and 2a) and XZ planes (1b, 2b and 3b). Organs viewed: thyroid (51 and 52), heart (53), lung (54 and 55), liver (56/67), kidneys (57 and 58), spleen (59), bladder (60), testes (61 and 62), spine (63) and the tumor (69).

To define the material that compose the organs and the tumor, water was used as a reference, the same material used for human phantoms simulations using the MCNP. In additional, each organ had its density determined according with the literature, as shown in Table 2.

Table 2. Density used in organs simulation.

Organ	Density ($g.cm^{-3}$)
Lung	0.3
Spine	1.4
Other organs and tumor	1.0

The source used in simulations was a spherical source of electrons, with the energy of 2.118 MeV (^{188}Re energy). For the process the source size was changed to evaluate the influence of the dosage in two cases: source fully distributed in the tumor to be treated (hypothetical case) and with a distribution after the injection for treatment (Dias, 2010). Each simulation was performed during 180 minutes:

a) Source uniformly distributed only in the tumor with radios of 0.1, 0.3 and 0.5cm – distribution 100%;
b) Source uniformly distributed throughout the tumor and source organs (heart, spleen, kidneys and bladder), with radios of 0.1, 0.3 e 0.5cm – distribution of 15%, 10%, 15% 15% e 45%, respectively.

The organ masses used for the absorbed dose calculation are shown in Table 3.

Table 3. Organ and tumor mass used to determinate the absorbed dose.

Organ	Mass (g)
Heart	0.12
Kidneys	0.28
Lung	0.15
Liver	0.89
Thyroids	0.02
Spleen	0.09
Bladder	0.03
Testes	0.25
Spine	0.19
Tumor	0.05

3. RESULTS AND DISCUSSION

Figures 6 and 7 show the absorbed dose in critical organs/tissues after the simulation of a spherical source of ^{188}Re with different diameters deposited only in the tumor (Figure 6) and in the tumor and organs (Figure 7) using MCNP.

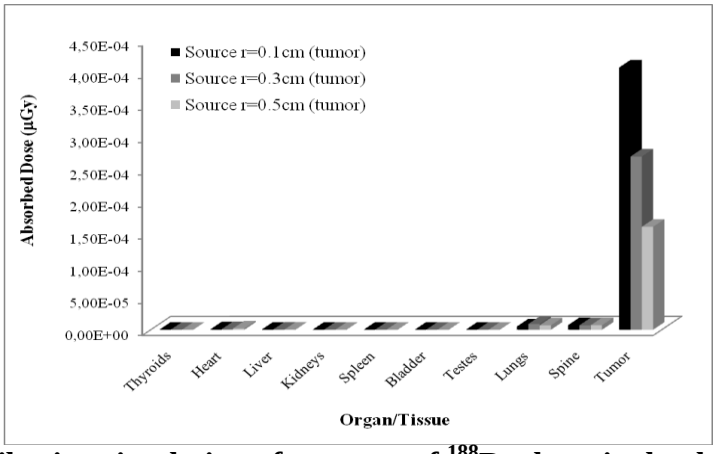
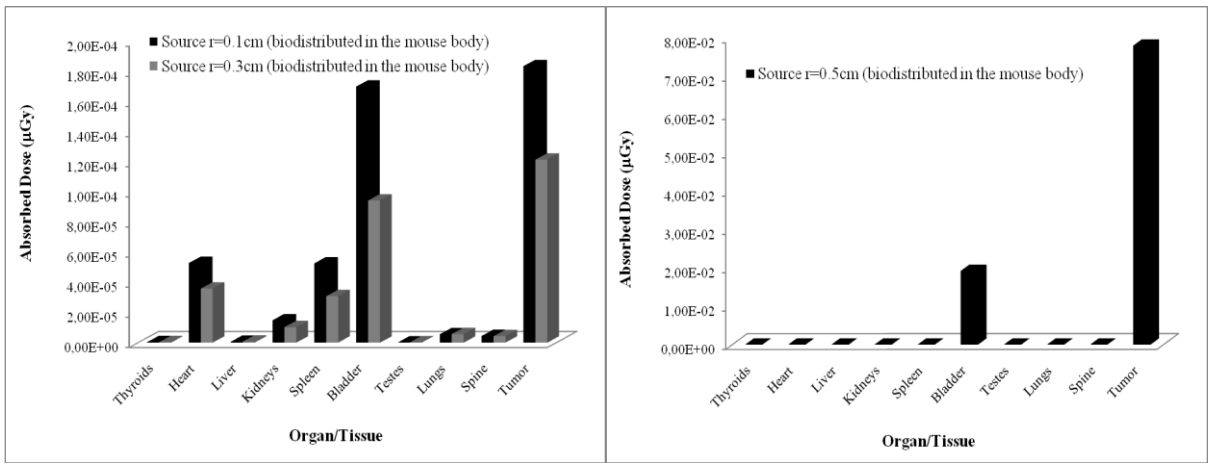


Figure 6. Biodistribution simulation of a source of ^{188}Re deposited only in the tumor and its influence in relation to other critical organs (hypotetical case).

It can see that in Figure 6 (hypotetical case study) that the absorbed dose is low to other organs and the tumor uptake is acceptable, even varying the radius of the tumor where the source is placed.



(7a) (7b)

Figure 7. Biodistribution simulation of a source of ^{188}Re deposited in the tumor and main organs of highest uptake and its influence in relation to other critical organs/tissues.

It can be seen that in the simulations with smaller diameters tumors (Figure 7a) the ^{188}Re-MAb uptake is uniform in more organs than larger diameters (Figure 7b). It is clean that with larger tumors the uptake of ^{188}Re-MAb is higher and the dose in the remaining organs/tissues is lower and the elimination is faster through the kidneys.

4. CONCLUSIONS

This work showed the initial proposed studies using MCNP and a mouse model for the simulation of biodistribution of hypothetical cases involving the uptake of ^{188}Re-antiCD20 applied to an animal model. This work will be extended to anti-CD20 labeled with other radionuclides (^{177}Lu, ^{90}Y, ^{99m}Tc and ^{131}I).

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