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COMPARTMENTAL ANALYSIS TO PREDICT BIODISTRIBUTION IN RADIOPHARMACEUTICAL DESIGN STUDIES

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ABSTRACT

The use of compartmental analysis allows the mathematical separation of tissues and organs to determinate the concentration of activity in each fraction of interest. Although the radiochemical purity must observe Pharmacopeia specification (values upper 95%), very lower contains of free radionuclides could contribute significantly as dose in the neighborhood organs and make tumor up take studies not viable in case of radiopharmaceutical on the basis of labeled peptides. Animal studies with a product of Lutetium-177 labeled Bombesin derivative (^{177}Lu-BBNP) developed in IPEN-CNEN/SP and free Lutetium-177 developed in CNEA/EZEIZA was used to show how subtract free ^{177}Lu contribution over ^{177}Lu-BBNP to estimate the radio-pharmaceutical potential as diagnosis or therapy agent. The first approach of the studies included the knowledge of chemical kinetics and mimetism of the Lutetium and the possible targets of the diagnosis/therapy to choose the possible models to apply over the sampling standard methods used in experimental works. A model with only one physical compartment (whole body) and one chemical compartment (177Lu-BBNP) generated with the compartmental analysis protocol ANACOMP showed high differences between experimental and theoretical values over 2.5 hours, in spite of the concentration of activity had been in a good statistics rang of measurement. The values used in this work were residence time from three different kinds of study with free 177Lu: whole body, average excretion and maximum excretion as a chemical compartment. Activity concentration values as time function in measurements of total whole body and activity measurement in samples of blood with projection to total circulating blood volume with^{177}Lu-BBNP. Considering the two sources of data in the same modeling a better consistence was obtained. The next step was the statistic treatment of biodistribution and dosimetry in mice (Balb C) considering three chemical fractions of the designed radiopharmaceutical: ^{177}Lu-BBNP, free ^{177}Lu, and total radiopharmaceutical (free^{177}Lu+^{177}Lu-BBNP). Using a mamillar models with six compartments and Human anatomic data from ICRP Report 89, these studies were also performed in Nude mice following a tumor model. The selected parameters were very critical, considering the blood flux in each body region and tissue. Over flow due ANACOMP feeding with high variable data retarded a lot the resolution of the equations system, but it is a valorous resource if considered the further application in medical use. The results showed a potential use of the ^{177}Lu-BBNP as therapeutic agent because of the good rate between a 0.56 grams tumor and the bone marrow doses. The ^{177}Lu-BBNP use as imagining agent could be better investigated if more experimental sampling times have been used in the first 4 hours of experimentation since peripheral organs presented quite the same uptake and retention values.

1. INTRODUCTION

Drugs design spends large amounts of resources before demonstrate safety and effectiveness. The rigid rules to approval radiopharmaceuticals and the reduced statistics studies carry out during the investigations act as barriers to replacement of established medical routines in favor of the use of the peptide receptor radionuclide therapy (PRPT).

The use of other researches data has been one of the mainly recommended actions allowing the 3Rs concepts to reduce de number of experimental procedures involving animal and improve results.

In the same way, the compartmental analyses support the radiopharmaceuticals design permitting the mathematical separation of tissues and organs to determinate the concentration of activity in each fraction of interest and pointing toward inconsistency in biodistribution and dosimetry studies. Additionally, by compartmental analysis is possible to considerer different chemicals species and predict metabolites. Although the radiochemical purity must observe Pharmacopeias specification (values upper 95%), very lower contains of free radionuclides could contribute significantly as dose in the neighborhood organs and make tumor up take studies not viable in case of radiopharmaceutical on the basis of labeled peptides.

2. METODOLOGY

The $^{177}LuCl_3$ (Lutetium free - ^{177}Lufree) ^{177}Lu labeled Bombesin derivative peptide (^{177}LuBBNP) biokinectics and dosimetry were calculated using the ANACOMP code as previously described. [1, 2]

Data used in the present work were: original data from Jimenez ^{177}Lufree studies in murine species Balb C mice (~25g) and from ^{177}LuBBNP studies in Balb C mice (4-5weeks old, 20-25g weight) and in Nude mice, inoculated in dorsal region with cells PC -human prostate carcinoma model PC Nude (~17g weight). [3,4]

Animal anatomic data was compared using Pearson's Correlation Coefficient (PCC), Eq. 1, between related experiments showed in the Table 1 before mathematical treatment.

$$r = \frac{\sum (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{[\sum (x_i - \bar{x})^2][\sum (y_i - \bar{y})^2]}} \qquad (1)$$

Where, *r é Covariance between the sample mass: organ (x_i) and total body (y_i) and the average mass: organ ($\bar{x}$) and total body ($\bar{y}$) presented by other researchers divided for the product of standard deviations.*

Table 1. Organs and tissues weight correlation from different studies.

Tissue/Organ	JIMÉNEZ	STABIN	MILLER	LARSON	PUJATTI	
					Balb C	PC Nude
Whole body	25	27	25	35	25	16.8
Blood	0.409	N.A.	N.A.	N.A.	1.3	1.30
Liver	1.609	0.78	1.05	2.699	1.078	0.948
Spleen	0.108	0.022	0.09	0.133	0.09	0.062
Kidneys	0.533	0.334	0.265	0.422	0.37	0.276
Stomach	0.487	0.298	0.175	0.618	0.20	0.366
Small intestine	3.152	0.952	1.21	3.034	2.75	1.91
Lung	0.204	0125	0.15	0.155	0.26	0.156
Femur	0.113	N.A.	0.11	0.08	0.09	0.047
Bone marrow (Femur)	N.A.	N.A.	0.0009	0.061	N.A.	N.A.
Pancreas	N.A.	N.A.	N.A.	N.A.	0.1875	0.158
Tumor	N.A.	N.A.	N.A.	N.A.	N.A.	0.526
PCC	0.951±0.006	0.949±0.004	0.945±0.005	0.938±0.004	0.951±0.005	0.9438±0.006

N.A. *Not available*. Adapted from Jimenez, Y. V., 2007 [3]

2.2. ANACOMP code data feeding

ANACOMP code is able to calculate kinetics and dosimetric parameter. In the calculations the code was fed with original data from studies carry out by Pujatti and Jimenez[3] to all calculations.

Data considered from Lutetium free studies was: the average residence time in the whole body, $\tau_{wb}=177$ *hours* and the residence time in Balb C mice blood, $\tau_{blood}=133$ *hours* calculated using ANACOMP from excretion and whole body direct measurements and calculated maximum excretion supported by Table 2 data. [3]

Table 2. ^{177}Lu free residual activity in Balb C mice.

A(t)/Ao									
Time (h)	2	24	48	120	144	168	192	216	336
Average excretion	0.902	0.785	N.A.	N.A	N.A	0.367	N.A.	N.D.	0.083
Maximun excretion	0.867	0.734	N.A.	N.A.	N.A.	0.311	N.A.	N.D..	0.071
Whole body	0.966	0.792	0.627	0.428	0.318	0.281	0.272	0.264	0.114

N.A. *Not available.* Jimenez, Y. V., 2007 [3]

Data considered from ^{177}Lu BBNP measurements were the residual injected activity (%) by animal as time function presented in the Table 3 and the biodistribution of ^{177}LuBBNP in Balb C and PC Nude mice presented in the Table 4.

Table 3. ^{177}Lu BBNP residual activity in Balb C mice.

Average Residual Activity by Animal (%).								
Time (h)	0	0.5	1	2.5	4	24	96	120
	100	46.9±9.9	38.7±4.1	14.4±1.34	12.95±1.44	8.59±0.75	5.07±0.79	3.8±0.49

After modeling the ^{177}Lu BBNP biokinectics using a model with one physical compartment and one chemical compartment as illustrated in the Figure 1A, a theoretical curve was growing by the inclusion of ^{177}Lufree data from Table 2 in a two chemical compartment model as illustrated in the Figure 1B.

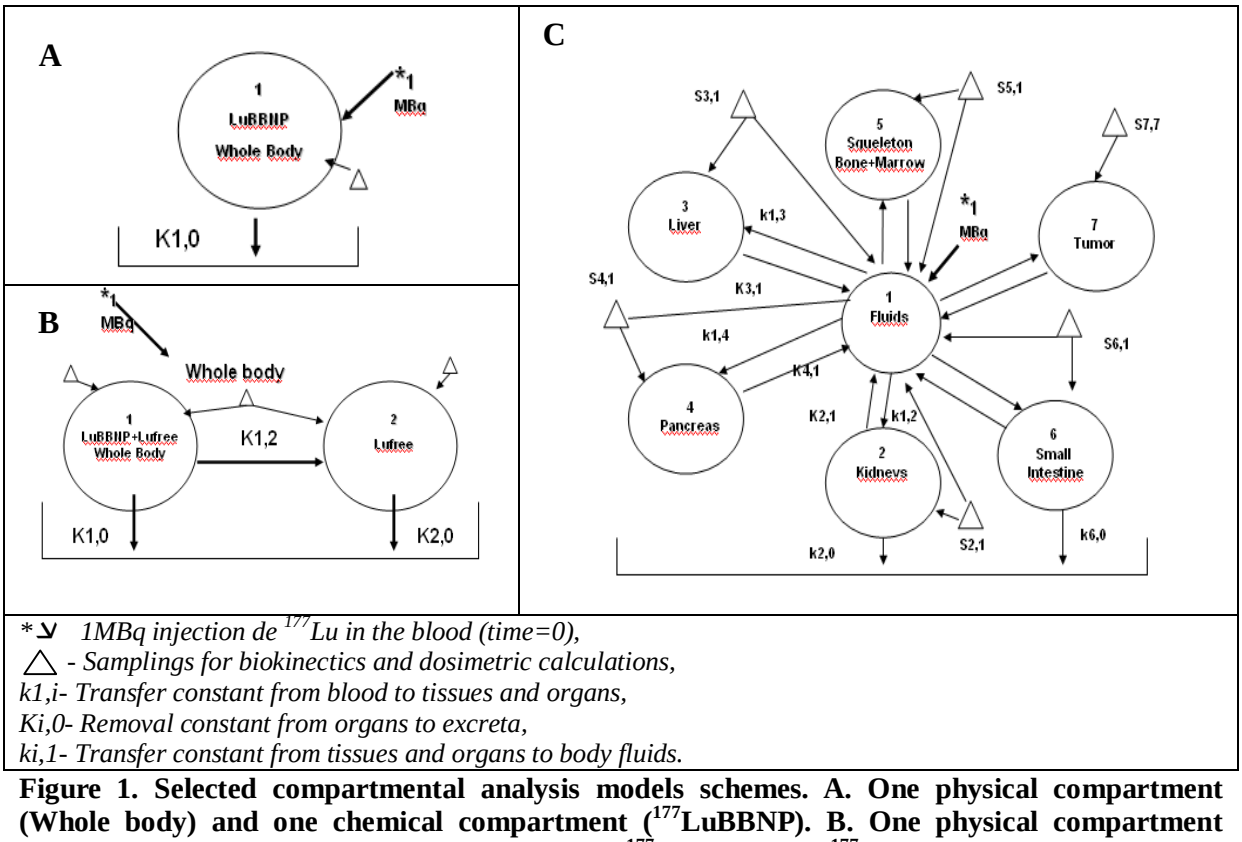
The improved concordance between theoretical calculated and experimental data as shown in the Table 5 and made possible the use of a more complex model using six or seven compartment as illustrated in the Figure 1C and Human anatomic data from ICRP Report 89. [54]

The projection of dose between murine and man was made using the time-mass scale considering $B^{1/4}$.The model used was de PC Nude.

Table 4. ^{177}Lu BBNP Biodistribution in mice.

Time (h)		1	4	24
Tissue/Organ	Weight/Volume (g or ml)	Residual Activity (%)	Residual Activity (%)	Residual Activity (%)
Blood	1.65* 1.30**	0.32* 0.45**	0.13* 0.09**	0.14* 0.004**
Pâncreas	0.19* 0.16**	0.014* 0.28**	0.008* 0.005**	0.011* 0.002**
Kidneys	0.32* 0.28**	0.75* 1.0**	1.00* 0.74**	0.60* 0.23**
Small Intestine	2.54* 1.91**	0.32* 0.55**	0.93* 0.45**	0.29* 0.30**
Squeleton*** Bone Marrow	2.95* 2.06** 0.47* 0.047**	0.64* 1.01** 0.022* 0.014**	1.65* 0.73** 0.016* 0.025**	1.36* 0.18** 0.005* 0.039**
Tumor**	0.53	0.23	0.083	0.02

Balb C mice* ****PC Nude mice.* * *Projected on basis of femur measurements.*



**⩛ 1MBq injection de ^{177}Lu in the blood (time=0),*
△ - Samplings for biokinectics and dosimetric calculations,
k1,i- Transfer constant from blood to tissues and organs,
Ki,0- Removal constant from organs to excreta,
ki,1- Transfer constant from tissues and organs to body fluids.

Figure 1. Selected compartmental analysis models schemes. A. One physical compartment (Whole body) and one chemical compartment (^{177}LuBBNP). B. One physical compartment (Whole body) and two chemical compartments (^{177}LuBBNP and ^{177}Lufree). C. Six compartment model to Balb C and seven compartment model to PC Nude.

The validation of ANACOMP results of absorbed dose in organs to compare with tumor dose used de Equations 2-5. [7]

$$N = \int_0^{\infty} A_0 e^{-\lambda t} = 1.443.A_0.T_{\frac{1}{2}Phys} \quad (2)$$

$$\tilde{A}_s = \int_0^{\infty} A_s(t)dt \quad (3)$$

$$\tilde{D} = \frac{N\sum y_i E_i \phi wr}{m_t} \quad (4)$$

$$D_{\infty} = FG.\overline{E}_{p+r}.\tilde{D}.T_{\frac{1}{2}Eff} \quad (5)$$

Where,

N *Total number of disintegration for the injected activity,*

A_0 *Initial injected activity (MBq),*

$\tilde{A}_s$ *Cumulative activity integrated time from 0 to infinity (MBq-hr),*

$\tilde{D}$ *Absorbed dose in the organ/tissue target,*

D_{∞} *Absorbed dose in the tumo,*

$T_{\frac{1}{2}Phys}$ *Physic half-time,*

$T_{\frac{1}{2}Eff}$ *....Effective half-time,*

$\overline{E}$ $\sum y_i E_i \phi w_r$ *.Dose factor considering radionuclide disintegration energies,*

FG*.....Energy rate constant eV-J,*

E_{p+r}*....Radionuclide Beta particle average energy,*

m_t*.....Organ/tissue mass.*

3. RESULTS AND DISCUSSION

The concordance between theoretical, calculated and experimental data is shown in the Table 5.

Table 5. Comparison between theoretical and experimental results obtained by ANACOMP B: One physical compartment (Whole body) and two chemical compartments (^{177}LuBBNP and ^{177}Lufree).

Time (h)	Compartment 1 ^{177}LuBBNP	Compartment 2 ^{177}Lufree	Experimental data ^{177}LuBBNP (total ^{177}Lu)		Experimental data ^{177}LuBBNP (^{177}Lufree fraction)	
	Theoretical	Theoretical	Observed	Calculated	Observed	Calculated
0	99.643	0.357	99.643	99.643	0.356	0
0.5	34.868	3.439	46.900	42.709	N.D.	N.D.
1.0	12.202	4.517	38.720	24.231	N.D.	N.D.
2.5	0.523	5.064	14.414	15.359	N.D.	N.D.
4.0	0.022	5.079	12.956	14.630	N.D.	N.D.
24	0	4.958	8.585	9.795	N.D.	N.D.
96	0	4.545	5.072	2.316	5.072	6.493
120	0	4.4145	3.796	1.432	3.796	4.015

N.D *Not determined.*

3.1 Equations from the model

The Equations 6-18 generated by ANACOMP supported by BBNP studies are presented below.

Balb C mice:

Blood (6)

$$f_1 = 2.65\, e^{-5.59\,t} - 12.37\, e^{-4.64\,t} + 0.167\, e^{-1.4\,t} + (6.61\,E-03)\, e^{-9.9\,E-04\,t}$$

Kidneys (7)

$$f_2 = 15.4\, e^{-5.59\,t} + 70\, e^{-4.64\,t} + 0.875\, e^{-1.41\,t} + (3.35\,E-05)\, e^{-9.9\,E-04\,t}$$

Liver (8)

$$f_3 = 2.44\, e^{-5.59\,t} - 0.137\, e^{-4.64\,t} - 6.12\, e^{-1.41\,t} + 0.184\, e^{-9.9\,E-04\,t}$$

Pancreas (9)

$$f_4 = -2.57\, e^{-5.59\,t} + 2.62\, e^{-4.64\,t} + (6.87\,E-02)\, e^{-1.41\,t} + (2.01\,E-07)\, e^{-9.9\,E-04\,t}$$

Esqueleton (10)

$$f_5 = -0.11\, e^{-5.59\,t} - 0.63\, e^{-4.64\,t} - (2.81\,E-02)\, e^{-1.41\,t} - 0.016\, e^{-9.9\,E-04\,t} + 0.85\, e^{-9.9\,E-15\,t}$$

Small intestine (11)

$$f_6 = -0.315\, e^{-5.59\,t} - 1.9\, e^{-4.64\,t} + 2.38\, e^{-1.41\,t} + (2.26\,E-06)\, e^{-9.9\,E-04\,t}$$

PC Nude mice:

Blood (12

$$f_1 = (1.09E-03)e^{-1.64t} + (1.57E-03)e^{-0.14t} + (7.68E-04)e^{-0.06t} + (5.94E-06)e^{-9.9E-05t}$$

Kidneys (13)

$$f_2 = 56.75e^{-5.04t} + (1.53E-03)e^{-1.64t} + (2.15E-03)e^{-0.14t} + (1.05E-03)e^{-0.06t} + (8.09E-06)^{-9.9E-05t}$$

Liver (14)

$$f_3 = -0.159e^{-5.04t} - (1.47E-05)e^{-1.64} + (4.07E-04)e^{-0.14t} + 0.17e^{-0.06t} + (8.09E-06)e^{-9.9E-05t}$$

Pancreas (15)

$$f_4 = -(5.59E-03)e^{-5.04t} + (5.83E-03)e^{-1.64t} + (5.37E-07)e^{-0.14t} + (2.48E-07)e^{-0.06t}$$

Bone and Bone marrow (16

$$f_{5,1} = -(0.71E-03)e^{-5.04t} - (6.39E-05)e^{-1.64t} - (1.08E-03)e^{-0.14t} - (1.31E-03)e^{-0.06t} + 0.76e^{-9.9E-05t} + 1.64e^{-1.0E-03t}$$

$$f_{5,2} = 11.55e^{-5.04t} + (3.31E-04)e^{-1.64t} + (4.74E-04)e^{-0.14t} + (2.36E-04)e^{-0.06t} + (1.79E-06)e^{-9.9E-05t}$$

Small itestine (17)

$$f_6 = -(1.85E-04)e^{-5.04t} - (2.84E-07)e^{-0.14t} + (3.44E-04)e^{-0.06t} + 1.63e^{-9.9D^{-5}t} + (1.96E-04)e^{-1.0E-03t}$$

Tumor (18)

$$f_7 = -0.13\,e^{-5.04\,t} - (1.26\,E-05)\,e^{-1.64\,t} - (0.14\,E-04)\,e^{-0.14\,t} + (1.61\,E-04)\,e^{-0.06\,t} + 7.39\,e^{-9.9\,E-05\,t}$$

3.2. Biokinetics and dosimetry

ANACOMP code overflow retard the resolution of the equations systems, but allow the calculation of effective half-time, residence time and absorbed dose to Balb C and PC Nude mice and to Standard Man of 73.3kg as presented in the Table 6.

Table 6. The effective half-time, residence time and absorbed dose calculated by ANACOMP to Balb C and PC Nude mice and to Standard Man.

Parameter	T1/2 (h)		T (h)		Absorbed Dose (mGy/MBq)		
Specie	Balb C	PC Nude	Balb C	PC Nude	Balb C	PC Nude	Human*
Whole body	3.11E-01	6.18E-03	4.49E-01	4.49E-01	5,38E+00	8E-01	7.7E-01
Blood	3.94E-02	6.18E-04	5.68E-02	8.92E-04	N.D.	N.D	N.D.
Kidneys	3.59E-02	1.49E-02	2.29E-02	2.16E-02	4,66E+01	5.76E+01	5.27E+01
Liver	6.93E+02	1.22E+01	1E+02	1.76E+01	5.6E+02	3.16E+01	38.69E+01
Pancreas	4.68E-02	4.22E-01	6.74E-02	6.1E-01	1,38E+00	2.75E+00	9.3E-01
Bone	6.93E+04	6.93E+04	1E+05	1E+04	6,67E+00	1.2E-01	5.55E+01
Small intestine	1.59E-02	6.89E-02	2.29E-02	9.94E-02	2,61E-01	1.5E-01	8E-01
Tumor	N.D	1.39E+01	N.D	2E+01	N.D.	2.3E-03 (0.56g)	N.D
Bone marrow	6.93E+00	6E-03	8.79E-02	8.63E-03	N.D	1.2E-01	9.99E+01
Blood clearence	9.90E-02	N.D	1.43E-02	N.D	N.D	N.D	N.D

N.D. *Not determined.* **Calculated on basis of PC Nude data.*

Results showed uptake differences between the two murine species greater than expected with consequent effect on calculated dose.

4. CONCLUSIONS

The selected parameters were very critical, considering the blood flux in each body region and tissue. Over flow due ANACOMP feeding with high variable data retarded a lot the resolution of the equations system, but it is a valorous resource if considered the further application in medical use.

The results showed a potential use of the ^{177}Lu-BBNP as therapeutic agent because of the good rate between a 0.56g tumor and the bone marrow doses. Considering the mice-man

scale, the values predict to man can be more favorable, since the rate health tissues/tumor peptide derivative radiopharmaceuticals concentrations generally improves with the tumor weight due the over expression of BB_1, BB_2 and BB_3 receptors in endocrine tumors. [7, 8]

Nude mice tumor model used PC3 implanted in the sub dermal flank to better definition of tumor shape and mass during the dissection and to reduce of the neighborhood organs overlapping contribution during imaging studies. Consequently, the ^{177}Lu-BBNP use as imagining agent could be better investigated if more experimental sampling times have been used in the first 4 hours of experimentation since peripheral organs presented quite the same uptake and retention values of absorbed dose per mass in times currently used to clinical patient image studies.

The design of radiopharmaceuticals needs more severe criteria before using animal-man scales. Although the animals used in the BBNP experiments were from the same subgenus (*Mus*), the significant differences in the calculated kinetics parameters shown that the most current way to extrapolate dose from laboratory animals to man, the mass-time scale is an oversimplification.

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