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BIOKINETICS AND DOSIMETRIC STUDIES ABOUT ^{99M}TC(V)-DMSA DISTRIBUTION

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Abstract – Research for radiodiagnostic agents should considerate biological critical parameters which will give own contribution on the absorbed dose. The dimercaptosuccinic acid (DMSA) labeled with ^{99m}Tc(V) is a radiopharmaceutical which has well established role in medullar thyroid carcinoma and has been proposed in evaluation of bone metastasis. This work studied the biokinetics and dosimetry of ^{99m}Tc(V)-DMSA by animal model. The ^{99m}Tc(V)-DMSA was prepared from a (III)DMSA kit alkalized. Mice (n=5) received ^{99m}Tc(V)DMSA i.v., they were sacrificed (30 min, 1h, 5h and 12h), the organs excised and the activities measured by a gamma counter. The results were evaluated based on %activity/g and the absorbed dose was estimated (MIRDOSE 3.0 program) by extrapolation of data from animal to human scale. The results showed the majority of organs reached the top uptake at 30 min, the greatest kidney uptake was (4.81 ± 1.38)% activity/g, while the bone presented its highest uptake at 1h (5.49 ± 0.47)% activity/g, after 1h all the organs had activity exponential decrease. The biokinetic profile of ^{99m}Tc(V)-DMSA was well established, allowing quantifying of residence time, and the radiation dose estimates were made for this agent. About the absorbed dose, the preliminary results showed higher value to bone, being the soft tissue dose relatively low.

Key words: ^{99m}Tc(V)-DMSA, radiopharmaceutical, internal dosimetry, biodistribution.

INTRODUCTION

In diagnosis, radiopharmaceuticals have to present some characteristics in order to arise a high quality image at an able time and to offer a minimal dose to patient. The radionuclide half-life time, the photopeak energy, the kind of tissue considering and the metabolism will determinate the absorbed dose for each organ (7).

The absorbed dose (D) is defined as energy transfer per gram of any absorbing material, expressed as J/kg or gray (Gy - SI unit). In biokinetic models, D can be calculated from the cumulated activity and the S-value, a factor which depends on radiation type, the energy emitted per transformation, the mass of the target organ and the geometry of mathematical phantoms (4). In last way, D is related to potential risk which the patient is exposed.

Usually the first step to calculate D for human is extrapolate the dose from a animal model by different methods which can work metabolism, mass and time adaptations. Despite to the extrapolation methods are not consensus neither have strictly relation with human dose they are indispensable before clinical procedures (8).

Dose estimates have been calculated and published for several radiopharmaceuticals and are already available for all them used in the clinics, although new and unusual radiopharmaceuticals do not have their dosimetry available on classical tables.

Since 1985, a radiopharmaceutical based on dimercaptosuccinic acid (DMSA) has been used as tumor-seeking; in this case, the labeling is

Abbreviations: DMSA, dimercaptosuccinic acid; **ICRP,** International Commission on Radiological Protection; **MDP,** methylene diphosphonate.

made in the ^{99m}Tc 5+ oxidation state (V-DMSA) (11). The ^{99m}Tc(V)-DMSA has been studied in several clinical conditions for diagnosis in nuclear medicine. It has established role in medullar thyroid carcinoma and great perspectives in bone metastasis (1).

There are some commercial (V)DMSA kits available, but the (V)DMSA can be obtain successfully from a (III)DMSA kit by a specific method labeling frequently employed. The literature reports several works with (V)DMSA use but there is difficulty for find dosimetric data about it and there is not official report.

The aim of this work was as to study the ^{99m}Tc(V)-DMSA biokinetic, prepared from a (III)DMSA kit (IPEN), as well to estimate the absorbed dose in organs, by employing a animal model and extrapolation to human scale.

MATERIAL AND METHODS

Ethics Aspects

The follow procedures were submitted and approved by Animal Experimentation Ethics Committee of Biological Sciences Centre of Universidade Federal de Pernambuco, Brazil (CEEA-UFPE, of. 017/06), which strictly adheres to national laws relating to conduct of animal experimentation.

Animals

Mice (*Mus musculus* 'Swiss') were used for the biodistribution assays. The animals received a standard pelleted mouse diet and water *il libitium*, and were maintained under environmental conditions (26 ± 2 ºC, 12h of light/dark cicles). The assays were made with 70 days old mice (40g), both males and females.

Radiopharmaceuticals Prepare

DMSA lyophilized kits (containing 1mg of DMSA, 0.44mg of $SnCl_2.H_2O$, 0.7mg of ascorbic acid and 50mg of inositol) by Instituto de Pesquisas Energéticas e Nucleares – IPEN (Nuclear and Energetic Research Institute), São Paulo - Brazil, were used. The (V)DMSA was made from a DMSA kit by reconstituting with 3.5% $NaHCO_3$ (0.2mL), to elevate the pH to 8-9, followed by $^{99m}TcO_4$- addition (11,84 MBq - 320µCi), standing for 15 min. It was maintained at room temperature (26 ± 2ºC). Radiochemical by TLC and pH tests were performed.

Biodistribution Assay

The biodistribution assays were realized varying time (30min, 1h, 5h and 12h). To each assay, the groups (n=5) received 0.1mL of ^{99m}Tc-(V)DMSA which was administered IV (tail vein). After determinate time, the animals were anesthetized and sacrificed, the organs (blood, lung, liver, kidney, muscle and bone) were excised and the activity was measured by a NaI(Tl) well scintillation counter (Cobra II - Packard). Later the organs mass were weighed.

Biokinetic and Absorbed Dose Calculation

The biokinetic behavior was evaluated by the percentage of injected activity per gram of tissue (average ± SD) through the biodistribution time: the specific activity (Bq/g) was determinate for every organ at each biodistribution time, then, relating to total injected activity per each animal, the percentage of injected activity per gram of tissue was calculated. The differences between %activity/g at several times were analyzed by T-test Student.

In order to find a relation between the animal and human behavior, a methodology to extrapolate the data was used from quantifying tissue distribution. From the knowledge of % injected activity/g and the whole body weight, the data extrapolation from animal to human scale followed the equation 1 [8]. This method is considering as standard a 70kg adult male and the weight human organs are according to ICRP 53 [3].

$$\left(\frac{\%}{\text{organ}}\right)_{\text{human}} = \left[\left(\frac{\%}{g\ \text{organ}}\right)_{\text{animal}} \times \left(kg\ \text{TB weight}\right)_{\text{animal}}\right] \times \left(\frac{g\ \text{organ}}{kg\ \text{TB weight}}\right)_{\text{human}} \quad (1)$$

The result of equation 1 is the % activity per organ in human scale; these data were divided per 100 in order to obtain absolute values. Graphs were built from absolute values versus time whose equation integration arose the residence time related to each organ.

The absorbed doses per administered activity were estimated by a computational program (MIRDOSE 3.1) using the residence time information as input data.

RESULTS

The ^{99m}Tc-(V)DMSA had radiochemical purity was better than 95% and the pH of the compound ranged from 8 to 9. The biokinetic profile can be observed in the Figure 1.

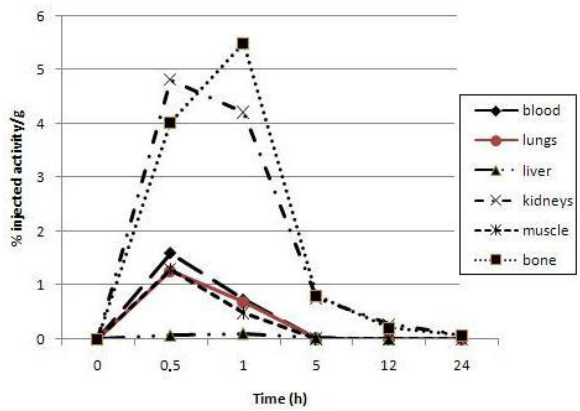

Figure 1. Biokinetic profile of ^{99m}Tc-(V)DMSA in mice (*Mus musculus* 'Swiss')

The results showed a fast distribution of the radiopharmaceutical within animal body, presenting a peak uptake at 30 min for majority organs, the point time of the highest radioactivity in the blood was not determined but it was before 30 min biodistribution.

The kidney is the organ that presents the highest level uptake at 30min (4.81±1.38)% activity/g. Only bone still increase the activity after 30min, the bone has the greatest uptake at 1h (5.49 ± 0.47)% activity/g and the liver also has it at 1h (0.473± 0.071)% activity/g. After

reach the top uptake, every organ decreased quickly the % activity/g, at 12h all the organs were nearly 0% uptake. Comparing the %activity/g in subsequent times, decreasing was significant ($p \leq 0.05$) in all organs until 12h biodistribution.

It is possible to observe blood, muscle, lungs and liver do not have specific uptake, neither the kidney, although kidney uptake was greater than the others. Only the bone have uptake more specific whereas the % activity/g increase until 1h biodistribution.

The residence time were calculated from activity extrapolated for every organ, except blood whose was not made dosimetry. The bone dose was obtained considering the trabecular and cortical mass, then, it was considered 50% of residence time for each one according to ICRP 53 [3].

The Table 1 shows the calculated residence time and the absorbed dose estimated.

Table 1. Residence times and absorbed doses per administered activity estimated by extrapolation from mice to human scale

Organ	Residence time (h)	D (mGy/MBq)
Liver	0.0228	4.34×10^{-4}
Lung	0.0209	4.79×10^{-4}
Kidney	0.0607	3.17×10^{-3}
Muscle	0.2512	2.80×10^{-4}
Red marrow	-	5.21×10^{-4}
Bone surfaces	0.1237	3.35×10^{-3}

Table 2. Biodistributions of (V)DMSA for mice by this work and of MDP for rabbits by Subramanian et al (1975) in 2 hours after the administration*

Organ	(V)-DMSA	MDP
Blood	0.56±0.65	0.193±0.093
Liver	0.44±0.16	0.124±0.038
Kidney	3.85±3.2	2.81±2.28
Muscle	0.43±0.71	0.03±0.01
Femur	4.05±0.66	8.81±1.39

*%activity/g (mean±SD)

DISCUSSION

Diagnostic methods in nuclear medicine are supposed to produce images from radiopharmaceuticals offering doses as low as possible (7). Thus, the dosimetry in radiopharmaceutic planning is fundamental to optimize the procedure, especially if the radionuclide is a long half-life one. The ^{99m}Tc is a short half-life radionuclide (6.02h) which generally offers low dose, but it depends on labeled molecule and kind of exam. Radionuclide and radiochemical impurities also can contribute with absorbed dose (4).

The ^{99m}Tc(V)-DMSA is a radiopharmaceutical that can be obtained from several methods, including preparation from a III-DMSA kit, being possible the presence of different isomers into the preparation (1). The results obtained in this work show that DMSA labeled with pentavalent ^{99m}Tc had a quick organic distribution and the % activity injected/g data are according to Yokoyama et al [11] at 1h. However, Yokoyama et al reported a higher uptake at all times until 24h biodistribution in comparison with the results in this work, especially for bone, but the animals used by them were younger (25g). A direct relation between the animal age and the (V)-DMSA uptake related to bone tissue has been reported, as younger is the animal as stronger will be the uptake(3).

The (V)DMSA presents a particular profile about bone uptake. The similarity between it uptake and the pharmaceutical standard for bone scans (MDP - methylene diphosphonate) uptake on bone metastasis is well reported, however, the (V)-DMSA does not fixe on normal human bone like MDP.

The uptake mechanism of (V)-DMSA has been studied. Bone sites with low pH, as bone grown or metastasis, uptakes more (V)-DMSA (3, 5) and it relation with phosphate ions uptake has been indicated (2). This fact can explain the high (V)DMSA uptake on young bone.

It is important remember this work used mice as animal model which belong specie characterized by incomplete bone maturation in the same way that rabbits (11), others species like rats have less bone uptake, as can be seen in the work of Wasburn et al (1995) (10). Subramanian et al reported firstly the MDP biodistribution for rabbits (9), in this case, we thought the (V)DMSA uptake could be compared with MDP uptake on bone tissue (tab.2). In spite of the little differences, the values are compatibles. The MDP have a proved affinity for bone (it fixation would be related to hydroxyapatite crystallization, new collagen or phosphatase enzymes) and it uptake happens on bone at general way (6). On the other hand, the (V)DMSA presents specific uptake on tumor sites and bone disorders where there is immature osseous stage, like the case of mice and rabbit maturation, and bone metastasis.

There are several methods to theoretical calculation and dose estimate, whereas many times the extrapolation from animal to human scale is not related strictly (8). In this method human dose was estimated by mass extrapolation using an equation reported as good approximation. A computational program, which compiles tissue S-value and others parameters, was used in order to calculate the final dose values.

In this case, it was found a low dose for soft tissue and higher dose for bone. The ^{99m}Tc(V)-DMSA dosimetry is not mentioned in the ICRP 53 (3) and human data related to (V)DMSA distribution were not find to compare the results of extrapolation method.

Table 3. Absorbed doses per administered activity (mGy/MBq) for (V)-DMSA estimated by extrapolation method (from mice to human scale) and for MDP calculated by MIRDOSE 3.1 program.

Organ	(V)-DMSA	MDP
Liver	4.34×10^{-4}	1.25×10^{-3}
Kidney	3.17×10^{-3}	7.28×10^{-3}
Red marrow	5.21×10^{-4}	5.54×10^{-3}
Bone surfaces	3.35×10^{-3}	3.86×10^{-2}

Thus, the (V)-DMSA data extrapolated to human scale were analyzed according to MDP human dosimetry also obtained by MIRDOSE. In table 3, we rewrite values of D of important organs in order to allow the comparison between (V)DMSA and MDP. The (V)DMSA values extrapolated were lesser than MDP doses.

Beyond the tumor affinity of (V)DMSA, several authors have suggested the (V)DMSA as auxiliary diagnosis for bone metastasis due to bigger contrast between metabolic disorders normal osseous (12).

In skeletal scans the (V)DMSA is used with lower activities than MDP, 15 and 20 MBq respectively, in addition the (V)DMSA would offers lower doses according to our result. But it is fundamental evaluate another aspect in the extrapolation method of dose: metabolic differences between species involved.

The first aspect here is the osseous metabolism, beyond mice have immature bone stage, in this work a femur sample was taken where the epiphyseal region could contribute more on uptake due to its role in growth, because it the real dose in human bone tissue is supposed to be lesser than extrapolation results.

On the other hand, this work did not measure the body while contribution on soft tissue dose, thus, the dose in this tissue is supposed to be underestimate. Anyway, the (V) DMSA absorbed dose is supposed to be a low one.

Other articles in this theme issue include references (13-20).

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