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SINGLE DOSE TOXICITY AND BIODISTRIBUTION STUDIES OF [^{18}F]FLUOROCHOLINE

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ABSTRACT

[^{18}F]Fluorocholine (^{18}FCH) is a valuable tool for non-invasive diagnosis using positron emission tomography (PET). This radiotracer has been proven to be highly effective in detecting recurrences and staging prostate cancer, diagnoses brain, breast, and esophageal tumors and also hepatocellular carcinoma. The higher uptake of fluorocholine by malignant tumors results from increased choline kinase activity due to accelerated cell multiplication and membrane formation. According to the Brazilian Health Surveillance Agency (ANVISA), radiopharmaceuticals have to be registered before commercialization. The aim of this work was to evaluate single dose toxicity and biodistribution of ^{18}FCH in mice, since preclinical safety studies are required for register. Experimental procedures were approved by the Ethics Committee on Animal Use (CEUA-IPEN/SP). Single dose toxicity and biodistribution studies were conducted in *Swiss* mice. No signs of toxicity were observed during clinical trial. No changes in the parameters which were examined, such as: body weight, food consumption, clinical pathology parameters or lesions microscopic were noted. Biodistribution results indicated high physiological tracer uptake in kidney, liver and heart 30 min after injection. Lower activities were recorded in other organs/tissues: pancreas, intestine, spleen, bone, bladder, muscle, brain and blood. Initial preclinical investigations showed no toxic effects of ^{18}FCH at investigated doses and a biodistribution profile very similar to other reports in literature. This information is essential to support future human trials.

1. INTRODUCTION

Nuclear medicine is a medical specialty that uses open sources of radionuclides for diagnostic and therapy purposes [1]. Positron emission tomography (PET) is a diagnostic technique that uses radiopharmaceuticals labeled with positron emitting isotopes [2]. This technique was

introduced as a noninvasive imaging modality for *in vivo* measurements of biochemical and physiological processes in normal and diseased tissues [3]. PET images are increasingly being used in the field of oncology, in early detection of several types of cancer, and for diagnosis, staging, and therapy response evaluation [4].

[^{18}F]Fluorocholine (^{18}FCH), an analog of choline, is a PET tracer developed in 2000 [5]. This radiotracer has been proven to be highly effective in detecting recurrences and staging prostate cancer, diagnosing brain, breast, and esophageal tumors and also hepatocellular carcinoma [6-12]. The higher uptake of fluorocholine by malignant tumors results from increased choline kinase activity due to accelerated cell multiplication and membrane formation [5, 13]. The technique using this radiopharmaceutical for PET imaging has been seen as a valuable tool for non-invasive diagnosis and an efficient tool for detection and localization of tumors, because the avid transport of the choline analogs into malignant tissues and their rapid distribution in the body [7].

Prostate cancer is the most common cancer and the second most common cause of cancer death in men in Brazil and USA [14, 15]. Choline presents a high affinity for malignant prostate tissue, the sensitivity of ^{18}FCH PET/CT to detect prostate cancer preoperatively is 73%, greater than that reported for ^{18}FDG PET/CT (31%). Also, the accuracy is greater with ^{18}FCH PET/CT (67%) than using ^{18}FDG (53%) [16]. ^{18}FCH is taken up by prostatic carcinoma as well as by distant metastases very fast, usually 5 min after the injection [17].

Currently, methods for ^{18}FCH production and quality control were developed at Radiopharmaceuticals Research and Production Facility of Nuclear Technology Development Center (CDTN/CNEN). This radiopharmaceuticals is not commercialized and Brazil yet and, according to the Brazilian Health Surveillance Agency (ANVISA) regulations, in this case, preclinical and clinical trials are required for register.

The aim of this work was to evaluate single dose toxicity and biodistribution of ^{18}FCH in *swiss* mice, since preclinical safety studies are required for register of new radiopharmaceuticals in Brazil. It should be emphasized that there is no national regulation or guideline which provides specific information for the preclinical safety testing of radiopharmaceuticals.

2. MATERIALS AND METHODS

[^{18}F]Fluorocholine was synthesized using a TRACERlab MX module (GE Healthcare) and reagent kits (ABX advanced biochemical compounds). The precursors dibromomethane and dimethylaminoethanol (DMAE) were acquired from Sigma-Aldrich.

Fluoromethylcholine bromide, the reference standard for [^{18}F]Fluorocholine, was purchased from ABX advanced biochemical compounds. Standard solutions were prepared by dissolving fluoromethylcholine bromide in NaCl 0.9%.

Experimental procedures were submitted and approved by the Ethics Committee on Animal Use (CEUA-IPEN/SP).

2.1. Single Dose Toxicity

Swiss healthy mice were obtained from CEBIO/UFMG (Belo Horizonte, MG). The animals, 24 male (7-8 weeks old) and 24 female (9-10 weeks), were housed in cages in temperature and humidity-controlled rooms with a 12h light/dark cycle. During the study period (14 days), mice were given *ad libitum* access to food and water. On the sacrifice day, animals were fasted for 6 hours.

For the single dose toxicity study, *swiss* mice were divided into four groups (6/group/sex) and each group received intravenously: (^{18}F)Fluorocholine after undergoing radioactive decay; Fluorocholine standard solution 2.64 x 10^{-4} mg (Standard 1); and Fluorocholine standard solution 5.29x10^{-3} mg (Standard 2). The control group received single dose of NaCl 0.9%.

Mice were monitored for clinical signs, mortality and changes in body weight for up to 14 days. After this period of observation, the animals were sacrificed with overdose of anesthetic pentobarbital (90 mg/kg animal weight). Blood and organs were collected for clinical analyses (hematology and clinical chemistry) and histopathology, respectively. Blood samples were obtained from the tail vein of mice and collected into tubes containing EDTA (hematology) or no anticoagulant (clinical chemistry).

The hematology parameters included: total leukocyte count (WBC), erythrocyte count (RBC), hemoglobin (HGB), hematocrit (HCT) and platelet count (PLT). The clinical chemistry analyses included: glucose (Gluc), cholesterol (Chol), triglycerides (Trig), serum alanine aminotransferase (ALT), gamma glutamyl transpeptidase (GGT), blood urea nitrogen (BUN), creatinine (Crea) and potassium (K). The heart, spleen, stomach, liver, small and large intestine, pancreas, lung and kidneys were removed and fixed in 10% neutral buffered formalin. The fixed tissues were trimmed, processed, and microtomed. The tissue sections were mounted on glass slides, stained with hematoxylin and eosin, and coverslipped for histopathological examination.

2.2. Biodistribution Test

Twenty healthy (5-6 weeks old) male *swiss* mice were obtained from SEB/FUNED (Belo Horizonte, MG). The animals were housed in cages in temperature and humidity-controlled rooms with a 12h light/dark cycle. In the days preceding the study, water *ad libitum* and free access to food were allowed. On the test day, animals were fasted for 6 hours.

Mice were injected intravenously via the tail vein with 100 µL (0.74-1.11 MBq) of ^{18}FCH. At 10, 20, 30 and 60 min after the intravenous injection, mice were sacrificed by cervical dislocation and their blood was obtained by cardiac puncture. Subsequently, tissues (heart, brain, pancreas, liver, kidney, bladder, spleen, intestine, stomach, muscle, bone and lungs) were quickly excide. All tissues were washed with normal saline, blotted dry and weighed; their decay-corrected radioactive content was then measured using an auto gamma counter (Wizard 1480, Perkin Elmer). Tissue localization at various intervals was expressed as percentage of injected dose per gram of tissue. Aliquots of the injection sample, containing

1% of the injected dose, were counted simultaneously at each time point to calculate radiopharmaceutical uptake in each organ.

2.3. Statistical analyses

Experimental data were submitted to analyses of variance, followed by multiple comparison tests. Results are expressed as mean ± SD and are considered statistically significant when $p<0.05$.

3. RESULTS

3.1. Single Dose Toxicity

The survival rate for all animals treated with Fluorocholine standard and (^{18}F)Fluorocholine after radioactive decay was 100%.

No clinical signs of toxicity were observed in any animals treated with saline, Fluorocholine standard and (^{18}F)Fluorocholine after radioactive decay.

No significant decreases in body weight were observed from animals from different groups.

No significant changes were noted in food and water consumption in the different groups.

There were no gross or microscopic histopathological lesions in the evaluated organs (kidney, liver, lung, spleen, heart, pancreas, stomach, small intestine and large intestine) of animals from different groups.

No statistically significant changes in biochemical parameters (glucose, cholesterol, triglycerides, creatinine, urea, potassium, alanine aminotransferase – ALT and gamma-GT) were observed in groups of animals treated with (^{18}F)Fluorocholine after radioactive decay or Fluorocholine standard (1 and 2) in relation to the control group. These results are presented in Table 1.

Table 1: Biochemical parameters obtained from males and females *swiss* mice after intravenous administration of ^{18}FCH after radioactive decay and Fluorocholine standard (1 and 2).

Parameters	Treatment			
	Control	^{18}FCH after radioactive decay	FCH standard 1	FCH standard 2
Glucose (mg/dL)	128.92 ± 26.53	138.17 ± 32.64	126.33 ± 46.59	161.92 ± 37.37
Cholesterol (mg/dL)	96.33 ± 29.00	91.08 ± 12.53	94.50 ± 33.35	103.25 ± 22.67
Tryglicerides (mg/dL)	127.15 ± 30.16	109.42 ± 11.77	131.36 ± 49.47	131.17 ± 30.42
ALT (U/L)	220.75 ± 77.48	243.50 ± 87.84	164.92 ± 54.50	201.17 ± 107.19
GGT (U/L)	5.17 ± 1.90	3.50 ± 1.93	3.50 ± 1.51	3.75 ± 1.60
Urea (mg/dL)	51.67 ± 6.72	50.92 ± 10.71	48.83 ± 12.38	56.17 ± 13.51
Creatinine (mg/dL)	0.32 ± 0.10	0.24 ± 0.07	0.24 ± 0.07	0.26 ± 0.07
Potassium (mEq/L)	1.92 ± 1.04	1.99 ± 0.98	1.39 ± 0.62	1.59 ± 0.98

Regarding the hematology test shown in Table 2, there was no statistically significant difference in the values of erythrocytes, hemoglobin, hematocrit, leukocytes and platelets of animals of different experimental groups.

Table 2: Hematology parameters obtained from males and females *swiss* mice after intravenous administration of ^{18}FCH after radioactive decay and Fluorocholine standard (1 and 2).

Parameters	Treatment			
	Control	^{18}FCH after radioactive decay	FCH standard 1	FCH standard 2
Erythrocytes (10^6/mm^3)	8.57 ± 1.45	9.28 ± 0.95	9.23 ± 1.10	9.34 ± 1.04
Hemoglobin (g/dL)	13.91 ± 2.31	14.74 ± 1.95	15.21 ± 2.17	14.83 ± 0.64
Hematocrit (%)	41.93 ± 6.06	43.83 ± 2.55	43.55 ± 2.30	44.87 ± 2.26
Leukocytes (10^3/mm^3)	9.45 ± 1.48	8.98 ± 1.79	8.72 ± 1.27	7.99 ±1.79
Platelets (10^3/mm^3)	249.67 ± 77.8	297.68 ± 88.19	295.34 ± 74.07	263.33 ± 65.75

3.2. Biodistribution Test

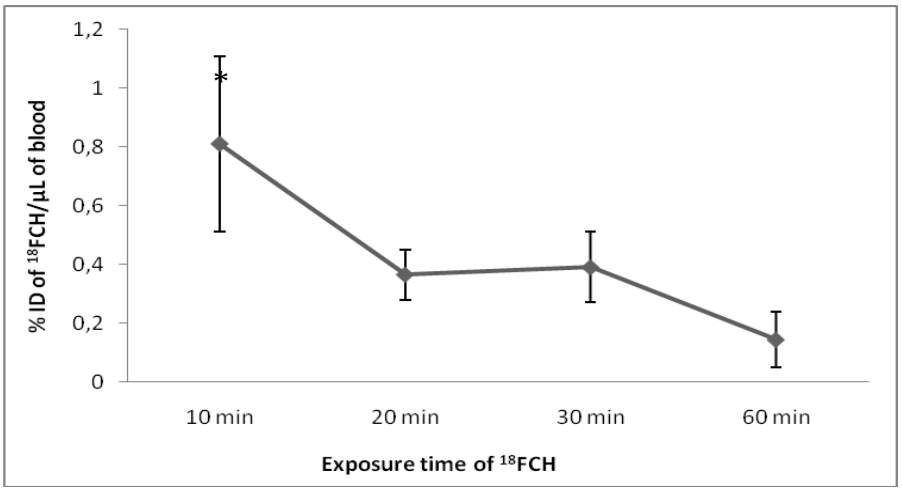
Biodistribution data for ^{18}FCH in selected organs are presented in Table 3. Data were expressed as percentage of injected dose per gram of tissue or per microliter of blood.

Table 3: Biodistribution of ^{18}FCH in the Swiss mice as a function of time

Organ/Tissue	10 min	20 min	30 min	60 min
Kidney	16.79 ± 2.02	15.25 ± 4.82	14.99 ± 3.49	10.90 ± 3.92
Liver	6.40 ± 0.56	6.05 ± 1.13	5.08 ± 0.89	4.60 ± 1.53
Heart	3.86 ± 1.95	4.18 ± 1.14	3.00 ± 1.30	2.69 ± 0.97
Intestine	3.61 ± 1.75	3.46 ± 0.69	2.16 ± 1.10	2.45 ± 0.84
Pancreas	3.33 ± 0.85	2.53 ± 1.32	3.35 ± 1.25	1.82 ± 1.20
Spleen	1.93 ± 0.34	2.21 ± 0.46	2.13 ± 0.99	1.58 ± 0.39
Bone	1.44 ± 0.31	1.68 ± 0.27	2.03 ± 0.66	1.56 ± 0.90
Bladder	1.05 ± 0.21	1.31 ± 0.46	1.43 ± 0.34	0.88 ± 0.54
Muscle	1.07 ± 0.17	0.79 ± 0.32	0.86 ± 0.13	0.38 ± 0.23
Brain	0.60 ± 0.26	0.38 ± 0.12	0.70 ± 0.37	0.30 ± 0.12
Blood	0.81 ± 0.29	0.37 ± 0.09	0.39 ± 0.12	0.14 ± 0.09

High physiological tracer uptake was observed at 30 min in kidney (14.99 ± 3.49%), liver (5.08 ± 0.89%) and heart (3.00 ± 1.30%). Lower activities were recorded in other organs/tissues: pancreas (3.35 ± 1.25%), intestine (2.16 ± 1.10%), spleen (2.13 ± 0.99%), bone (2.03 ± 0.66%), bladder (1.43 ± 0.34%), muscle (0.86 ± 0.13%), brain (0.70 ± 0.37%) and blood (0.39 ± 0.12%). ^{18}FCH biodistribution results present similar profile to that reported by Nejjari *et* al (2008).

No statistically significant difference between the percentage of injected dose of ^{18}FCH per gram of tissue at different time intervals was found for the evaluated organs, except for the muscles. In this case, a reduction in the percentage of injected dose per gram of tissue was observed by comparison of the initial and last evaluated interval, *i.e.* 10 and 60 min after injection, showing no accumulation of ^{18}FCH in the muscles. Additionally, a reduction in the percentage of injected dose of ^{18}FCH per microliter of blood was observed (Fig.1), indicating that ^{18}FCH is rapidly cleared from the circulation, as previously reported (DEGRADO et al., 2008).



(*) denote a statistically significant difference ($p < 0.05$) from the other groups.

Figure 1: ^{18}FCH biodistribution profile in the blood.

4. CONCLUSIONS

Preclinical investigations showed no toxic effects of [^{18}F]Fluorocholine produced at CDTN, at investigated doses, and a biodistribution profile very similar to other reports in literature. These data fulfill some of the ANVISA requirements for new radiopharmaceuticals register in Brazil and are essential to support future human trials. Efficacy studies in human xenograft tumor model are currently under progress.

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