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[^{67}Ga]GALLIUM-COMPLEX WITH 2-ACETYLPYRIDINE N4-ORTHO FLUOR PHENYL THIOSEMICARBAZONE AS A RADIOTRACER FOR BRAIN TUMOR DIAGNOSIS

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ABSTRACT

The aim of this work was to develop a ^{67}Ga-based SPECT imaging agent derived from 2-acetylpyridine N4-ortho fluor phenyl- thiosemicarbazone (PhoF). For this purpose, PhoF was radiolabeled using ^{67}Ga as radiotracer, and after quality control analysis its biodistribution and SPECT imaging were evaluated on Swiss mice and Nude mice bearing glioblastoma multiforme tumor (U87-MG). The labelling of PhoF with $^{67}GaCl_3$ was performed in methanol for 30 minutes at room temperature. Radiochemical analyses were done by HPLC with radioactivity detection. ^{67}Ga- PhoF was successful produced with 97.5 ± 0.6% of radiochemical purity and high specific activity (1.0 TBq /mmol). ^{67}Ga- PhoF showed to be a stable compound keeping its stability, when stored at 2-4ºC. In biodistribution studies, ^{67}Ga- PhoF displayed not only a significant tumor uptake, but also rapid blood clearance ($T_{1/2\ fast\ phase}$= 3.7 min. and $T_{1/2\ slow\ phase}$= 127.2 min.) and low accumulations in non target tissues, resulting in high target-to-non target ratios. Scintigraphic images of ^{67}Ga- PhoF in nude mice bearing U87-MG tumor showed a significant activity in tumor (~7% of total activity) and tumor-to-normal tissue ratio was more than 10-fold higher depending on the organ. Our results suggest that ^{67}Ga-PhoF possess indispensable characteristics for a good radiopharmaceutical for brain tumor diagnosis.

1. INTRODUCTION

Cancer high mortality is mainly due to the absence of early diagnosis tools [1]. In this field, the development of new radiopharmaceuticals for tumor diagnosis is an important issue in improving patients' prognosis and quality of life.

Thiosemicarbazones are a class of synthetic compounds that have been focus of interest because of their wide range of bioactivities, such as antibacterial, antifungal and antiprotozoal activity [2, 3, 4]. This class of compounds is also known to have potent antitumoral activity, as previously reported [5, 6, 7].

Thiosemicarbazones possess metal chelating property and could form metal complexes. In a recent work we demonstrated that gallium complexation enhanced thiosemicarbazones antitumoral activity [8]. In addition, the chelating property of these compounds allows their labelling with radiometals for imaging purpose. Radioactive copper complexes with bis(thiosemicarbazone) have been investigated as perfusion and hypoxia-imaging agents and showed to be stable, lipophilic and cell-permeable [9].

As well as copper, gallium radioisotopes possess physical properties and availability that make them interesting nuclides for radiopharmaceutical research. Besides its therapeutic utility, for over a half century, gallium-67 has been used as a diagnostic agent for cancer imaging by single photon emission tomography (SPECT) [10].

In the present work, we aimed to develop a ^{67}Ga-based SPECT imaging agent derived from 2-acetylpyridine N4-ortho fluor phenyl- thiosemicarbazone (PhoF).

2. MATERIALS AND METHODS

2.1. Reagents

All chemicals used were of analytical grade. Matrigel high concentration was from BD Biosciences (USA); sodium chloride and trifluoroacetic acid were from Sigma-Aldrich Co. (USA); methanol and acetonitrile were from Merck Chemical Co. (Germany).

2.2. Animals

The animal experiments were conducted in accordance with the UFMG Ethics Committee on Animal Experimentation (Protocol number: 107/2008).

Adult male *Swiss* mice (4 to 6 weeks old, 25-30 g weight) and nude mice (4 to 8 weeks old, 15-20 g weight) (provided by animal facility of IPEN/CNEN, São Paulo, Brazil) were used for biodistribution and imaging studies. All animals received water and food *ad libitum* under controlled environmental conditions.

Male nude mice were inoculated, subcutaneously, with 5×10^6 human *glioblastoma multiforme* cells (U87) suspended in 0.1 mL phosphate buffered saline/ Matrigel (2:1 v/v). Fifteen days after tumor cells inoculation, the animals were used in the experiments.

2.3. Radiolabeling

The solution of [^{67}Ga]$GaCl_3$ (37 MBq, 1 mCi) was heated to dryness at 90 °C. 2-acetylpyridine N4-ortho fluor phenyl- thiosemicarbazone (PhoF) in absolute methanol (10 µg ~ 34 nmoles) was added to the gallium-containing vial and vortexed at room temperature for 30 minutes. The radiolabeled molecule was diluted in normal saline and checked for radiochemical purity.

2.3. Radiochemical purity

Radiochemical purity was evaluated by high performance liquid chromatography (HPLC – Shimadzu, Japan) equipped with radioactivity detection (Shell) using RP C18 (Waters 4 x 150 mm, 15 µm, USA), flow rate of 1 mL/min a linear gradient of 10 – 80 % v/v 0.1% TFA/acetonitrile and 0.1% TFA/ H_2O for 20 minutes. 67GaCl and the unlabeled molecule (UV detection on 270nm) were also analysed.

The stability of the radiolabeled molecule was checked, with the same HPLC system, after 24 hours of incubation at 2-8 ^oC.

2.4. Biodistribution in *Swiss* mice

For biodistribution studies, ^{67}Ga- PhoF (1.85 MBq/0.1 mL 0.9 % NaCl, 0.5 µg of PhoF) was injected by intravenous via in *Swiss* mice. After different time intervals (60, 180 and 1440 minutes), the blood was collected, the animals were sacrificed and vital organs were removed, weighed and their respective radioactivity was measured in an automatic gamma counter. The biodistribution was calculated as percentage uptake of injected activity per gram of organ (%IA/g). Pharmacokinetics studies were performed by measuring ^{67}Ga-PhoF in blood (60 µL) collected from the orbital plexus vein 2.5, 10, 30, 60, 180 and 1440 minutes post injection.

2.5. Scintigraphic studies

Imaging studies were performed in normal male *Swiss* mice (30, 60, 180 and 1440 minutes post 3.7 Mbq *i.v.* injection) and *Nude* mice bearing U87 tumor xenografts (60 and 180 minutes post 3.7Mbq *i.v.* injection). The mice were anesthetized, placed under a gamma camara (Mediso Imaging System, Hungria) and the images were acquired for 300000 counts in a 512 x 512 x 8 matrix, applying a low energy high resolution (LEHR) collimator.

2.6. Statistical analysis

Data were expressed as means ± S.D. Statistical significance was determined by Student's t test and One-way ANOVA for paired data. Differences were considered significant at the level $p<0.05$.

3. RESULTS AND DISCUSSION

In order to evaluate PhoF pharmacokinetics, as well as its applicability for tumor diagnosis, this molecule was radiolabeled, using ^{67}Ga as radiotracer, and biodistribution and imaging assays were performed in both healthy *Swiss* and *Nude* mice bearing U87 tumor. ^{67}Ga- PhoF production was successful with 97.5 ± 0.6% of radiochemical purity and high specific activity (1.07 TBq /mmol) (Table 1). ^{67}Ga- PhoF showed to be a stable compound 2 – 4 ºC. HPLC analyses showed the presence of two different radiolabeled species. This fact probably occurred because the interaction of one or two molecules of PhoF with one isotope of ^{67}Ga.

It is important to emphasize that PhoF was easily labeled with ^{67}Ga. Few minutes after incubation of this molecule with radioisotope, it was observed the formation of a yellow compound (data not shown), characteristic of Ga and thiosemicarbazones complexation [11]. This fact show that PhoF is an excellent chelating molecule for ^{67}Ga radiolabeling and this property must be studied more.

Table 1. Retention time in HPLC (min) of $^{67}GaCl_3$ and labeled and unlabeled PhoF

Molecule	Retention time (min)*
$^{67}GaCl_3$	2.58 ± 0.02 3.64 ± 0.03
Unlabelled PhoF	10.00 ± 0.10
^{67}Ga- PhoF	10.00 ± 0.10 11.00 ± 0.02 14.0 ± 0.2

^{67}Ga-PhoF was injected (*i.v.*) in *Swiss* mice that were euthanized at different times post-injection (60, 180 and 1440 minutes). The radioactivity present in each organ was measured in a gamma counter and is showed in Fig. 1 as percentage of total injected activity per gram organ (%IA/g). ^{67}Ga-PhoF showed fast blood clearance (Fig. 2), performed mainly by renal pathway. The uptake by heart, lungs, pancreas, spleen, stomach, intestine, muscle and brain followed the blood perfusion. However, it was observed a significant accumulation of the labeled compound in the liver and this may be a critical organ for dosimetry. Biokinetics data are expressed in Table 2.

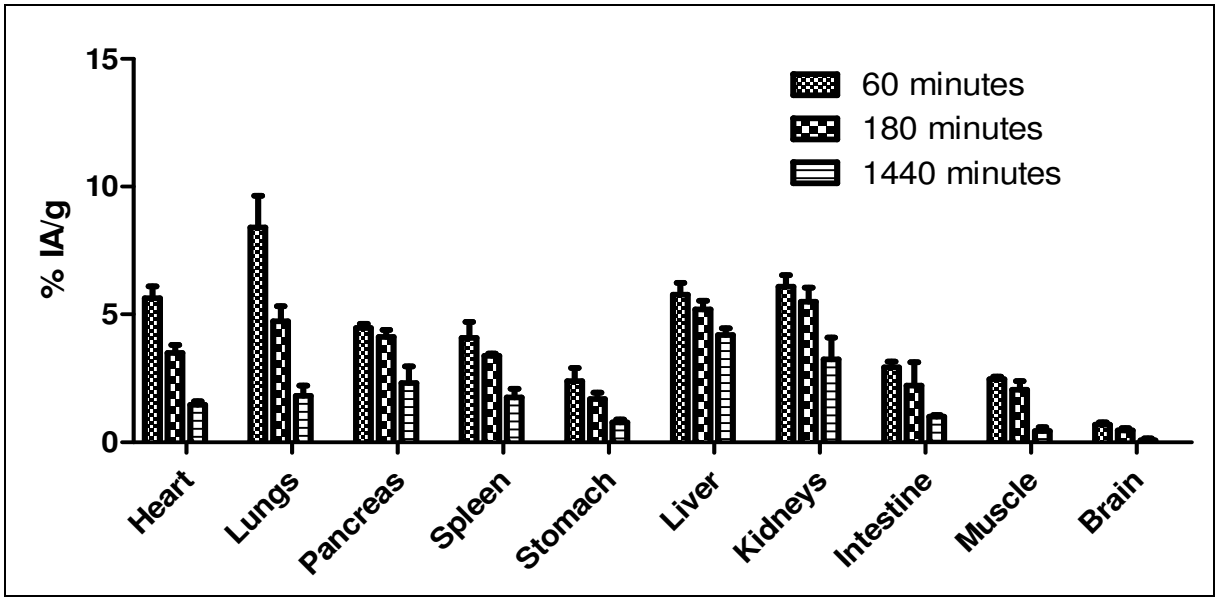

Figure 1. ^{67}Ga-PhoF biodistribution profile in healthy *Swiss* mice at different times after *i.v*. injection. Data were expressed as the percentage of total injected activity per tissue weight (%IA/g) (n = 3).

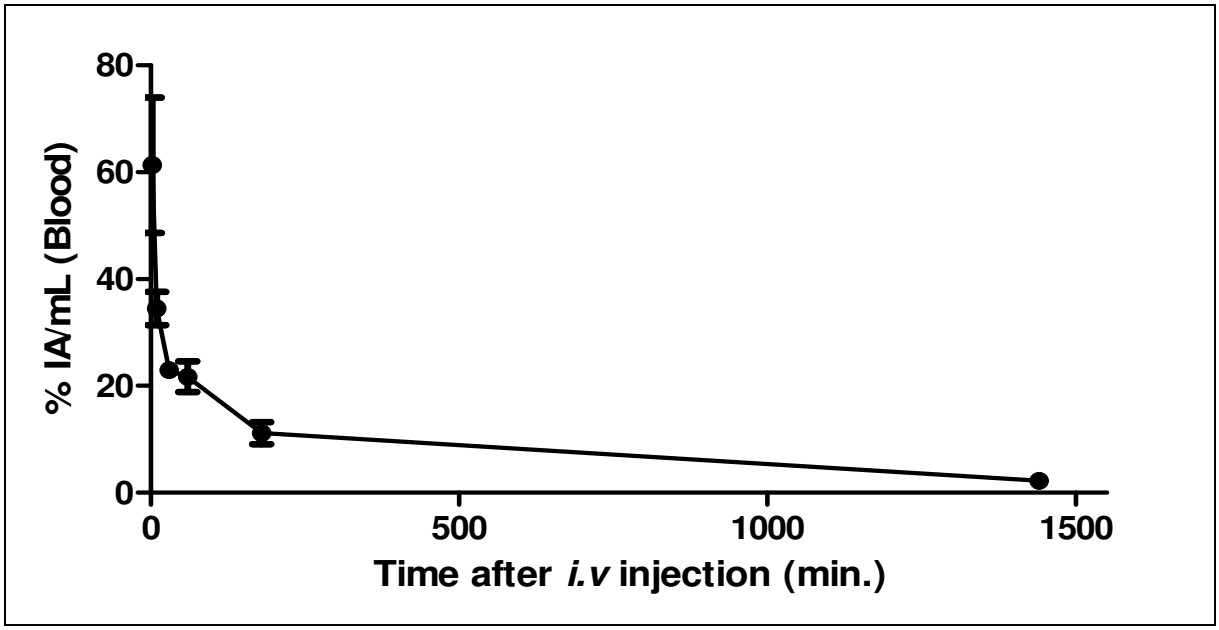

Figure 2. ^{67}Ga-PhoF kinetics in blood. ^{67}Ga-PhoF presented a fast blood clearance (n = 3).

Table 2. ^{67}Ga-PhoF Biokinetics data

Biokinetic parameter	Values
$T_{1/2\ (fast\ phase)}$	3.7 min.
$T_{1/2\ (slow\ phase)}$	127.2 min.

Scintigraphic images of ^{67}Ga-PhoF in healthy *Swiss* mice (Fig. 3) confirmed the results of biodistribution assay, showing liver accumulation and kidneys excretion in the first times analysed (30 and 60 minutes) and liver accumulation in the latest times (180 and 1440 minutes). The images in *Nude* mice bearing U87 tumor showed important tumor uptake (Fig. 4). The region of interest (ROI) was calculated as percentage of tumor region radioactivity compared to whole body radioactivity and was 6.9% and 8.4% at 60 and 180 minutes p.i., respectively.

Greater tumor-to-normal tissues ratios were obtained 180 minutes after the injection and were higher than 10-fold higher depending on the organ. Three hours after the injection, tumor-to-muscle ratio was 3.8.

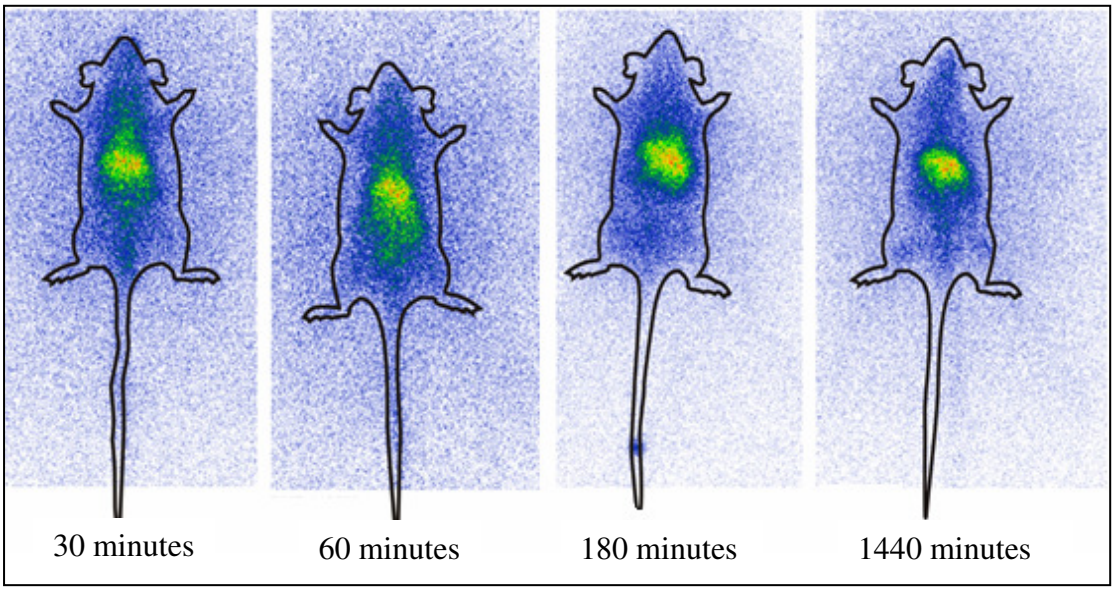

Figure 3. Scintigraphic images of ^{67}Ga-PhoF in normal Swiss mice 30, 60, 180 and 1440 minutes post intravenous injection.

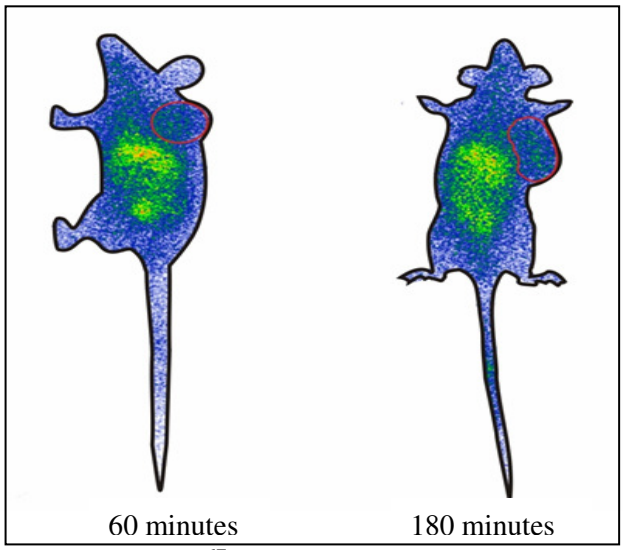

Figure 4. Scintigraphic images of ^{67}Ga-PhoF in *Nude* mice bearing U87 tumor 30 and 180 minutes post intravenous injection. Circles indicate tumor localization.

4. CONCLUSIONS

In this work we successfully labeled, for the first time, PhoF thiosemicarbazone with ^{67}Ga. Besides being an excellent chelate molecule, PhoF could also interact with brain tumor cells *in vivo*, allowing tumor imaging. These results suggest that ^{67}Ga-PhoF possesses indispensable characteristics for an efficient radiopharmaceutical for tumor diagnosis. However, dosimetric studies will be done to confirm this hypothesis.

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