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2-ACETYLPYRIDINE N4-PHENYL- THIOSEMICARBAZONE AS A NEW TOOL FOR TUMOUR DIAGNOSIS

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ABSTRACT

The aim of this work was to determine *in vivo* biodistribution of radiolabelled 2-acetylpyridine N4 phenyl thiosemicarbazone (Ph) and to evaluate its applicability for tumour diagnosis. Ph was labelled with ^{125}I using lactoperoxidase method and radiochemical analysis was performed by chromatography. ^{125}I-Ph production was successful with 86 ± 9.2% of radiochemical purity and high specific activity (17.6 TBq /mmol). ^{125}I-Ph was used for biodistribution and pharmacokinetics studies on *Swiss* mice bearing Ehrlich solid tumour. ^{125}I-Ph presented a rapid blood clearance ($T_{1/2}$= 97.2 min.) and the kidneys were the main excretion pathway (CL= 0.01 mL/min). ^{125}I-Ph uptake was significant in tumour (2.5%ID/g) and tumour-to-normal tissue uptake was more than 20-fold higher depending on the organ. The uptake by the organs like heart, lungs, stomach and liver followed the blood perfusion. Our results suggest that ^{125}I-Ph possess indispensable characteristics for an efficient radiopharmaceutical for tumour diagnosis. The next step will be to evaluate the quality of tumour SPECT images provided by ^{131}I-Ph.

1. INTRODUCTION

Deaths from cancer in the world are projected to continue rising with an estimated 9 million people dying in 2015 and 11.4 million dying in 2030. The cause of this high mortality is the tumour drug resistance and mainly the absence of early diagnosis [1]. The development of alternative radiopharmaceuticals for tumour diagnosis is relevant in the attempt to improve prognosis and to increase the patient survival.

Thiosemicarbazones are a class of synthetic compounds that present a wide range of bioactivities such as antibacterial, antiviral, antiprotozoal and antitumor activity. Some authors described that thiosemicarbazones mechanism of action is due to their ability to

inhibit DNA biosynthesis, possible by blocking the enzyme ribonucleotide reductase, binding to the DNA nitrogen bases, blocking base replication or creation of lesions in DNA strands by oxidative rupture [2 - 4].

Moreover, thiosemicarbazones possess metal chelating property and some of them can bind to transferrin receptors (TfRs) in the cells [5]. Tumour cells have a TfR1 expression and Fe demand markedly increased, therefore, they are more susceptible to the effects of chelation when compared to normal cells [6, 7]. Considering these facts, it is important to evaluate the thiosemicarbazones specificity on tumours detection.

The aim of this work was to determine *in vivo* biodistribution of radiolabelled 2-acetylpyridine N4 phenyl thiosemicarbazone (Ph) and evaluate its applicability for tumour diagnosis.

2. MATERIALS AND METHODS

2.1. Reagents

All chemicals used were of analytical grade.

2.2. Animals

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

Adult female *Swiss* mice (25-30g body weight; ICB-UFMG, Belo Horizonte, Brazil) were used for Ehrlich tumour animal model. Ehrlich ascitic tumour (EAT), derived from a spontaneous murine mammary adenocarcinoma, was maintained in the ascitic form by passages in syngenic Swiss mice by weekly intraperitoneal transplantation of tumour cells. For the biodistribution and imaging studies, solid tumours were induced by injecting EAT cells (2.5×10^6/animal) subcutaneously into the left hind paw mice. Fifteen days after tumour cells inoculation, the animals were used in the experiments. All animals received water and food *ad libitum* under controlled environmental conditions.

2.3. Ph radioiodination

The radioiodination of Ph was done according to the method described by Santos *et al.* [8]. Briefly, Ph was reacted for two minutes with 3.7×10^6 Bq carrier-free $Na^{125}I$ in the presence of lactoperoxidase and H_2O_2 in 50mM phosphate buffer at pH 7.4. The reaction was stopped with phosphate buffer containing 1mg/mL BSA. Radiochemical analysis were performed using ascending chromatography in Whattman paper n° 1 and contaminants, mainly under the form of ^{125}I, were eliminated by anionic exchange chromatography on Dowex 1-X8.

2.4. Biodistribution in Ehrlich tumour-bearing mice

For biodistribution studies, ^{125}I-Ph (37KBq) was injected by intravenous via in the Ehrlich tumour-bearing mice. After different time intervals (10 minutes – 24 hours), the animals were

sacrificed and vital organs were removed, weighed and their respective radioactivity was measured in an automatic gamma spectrometer (1480 Wizard 3''– Wallac). The biodistribution was calculated as percentage uptake of injected dose per gram of organ (%ID/g).

2.5. Statistical analysis

Data were expressed as means ± S.D. Statistical significance of differences between means was determined by Student's t test. Differences were considered significant at the level $p<0.05$.

3. RESULTS AND DISCUSSION

In order to evaluate Ph biokinetics, as well as, its applicability for tumour diagnosis, this molecule was radiolabelled, using ^{125}I as radiotracer, and biodistribution assays were performed in Ehrlich tumour-bearing mice. ^{125}I-Ph production was successful with 86 ± 9.2% of radiochemical purity and high specific activity (17.6 TBq /mmol).

^{125}I-Ph was injected (*i.v.*) in *Swiss* mice bearing Ehrlich solid tumour that were euthanized at different times post-injection (10 minutes – 24hours). The radioactivity present in each mice organ was measured in a gamma counter and is given in Fig. 1 as %ID/g organ.

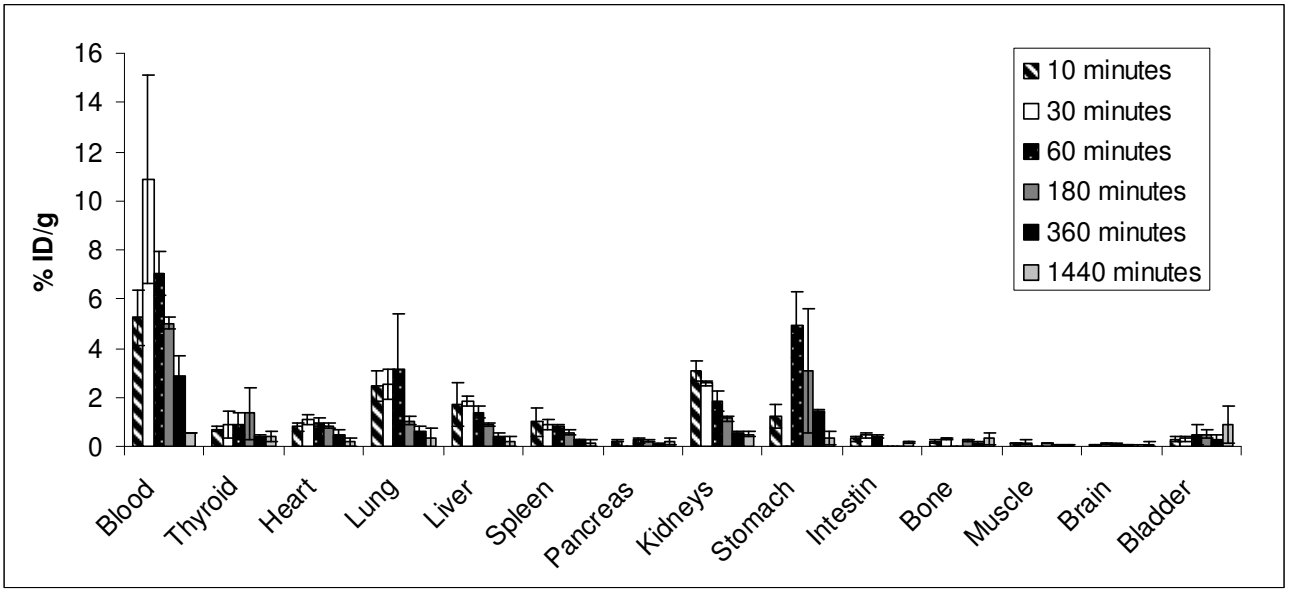

Figure 1. ^{125}I-Ph biodistribution profile in mice at different times after *i.v.* injection. Data were expressed as the percentage of total injected dose per tissue weight (%ID/g).

Biokinetics data from ^{125}I-Ph in mice tumour model showed rapid blood clearance (Fig. 2) and indicated the kidneys as the main excretion pathway. The uptake by the organs like heart,

lungs, stomach and liver followed the blood perfusion. Biokinetics data are expressed in Table 1.

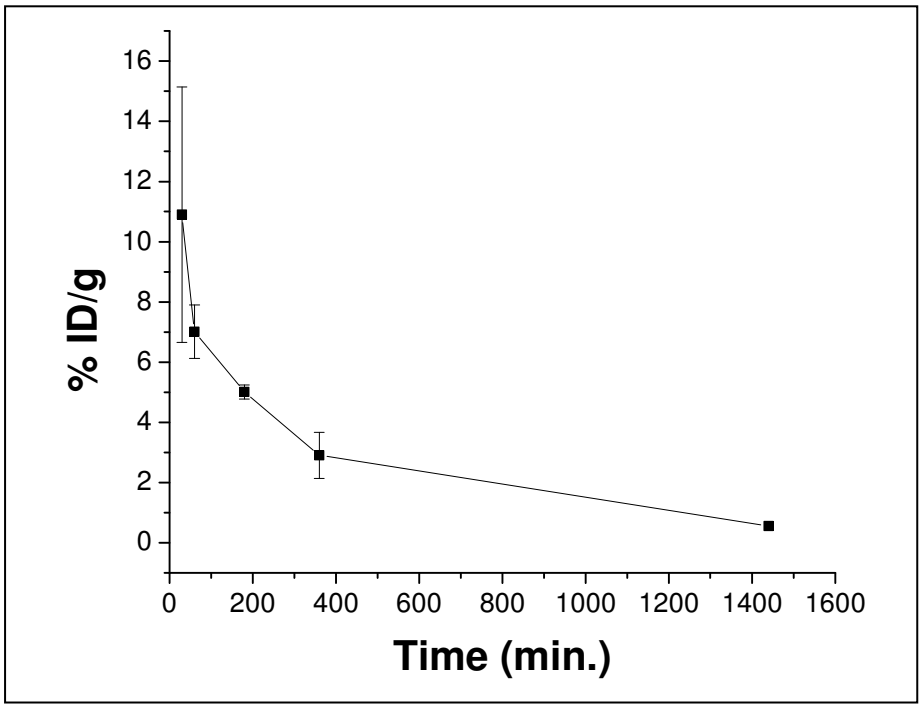

Figure 2. ^{125}I-Ph kinetics in blood. ^{125}I-Ph presented a rapid blood clearance.

Table 1: ^{125}I-Ph Biokinetics data
(Pharmacokinetic program: Murphy, R., Inaoe tonantzindra, Puebla, México 1991)

Biokinetic parameter	Values
$T_{1/2\ (fast\ phase)}$	97.2 min.
$T_{1/2\ (slow\ phase)}$	310.2 min.
Clearance	0.01 mL/min.
Distribution volume	5.33 mL

Figure 3 shows comparative data between tumour and normal paw ^{125}I-Ph uptake, after intravenous administration. Ten minutes after ^{125}I-Ph injection, tumour uptake was 1.2 ± 0.4 %ID/g reaching the highest concentration 180 minutes after the injection (2.5 ± 0.16 %ID/g). In the subsequent times, ^{125}I-Ph tumour uptake remained significantly higher than normal paw.

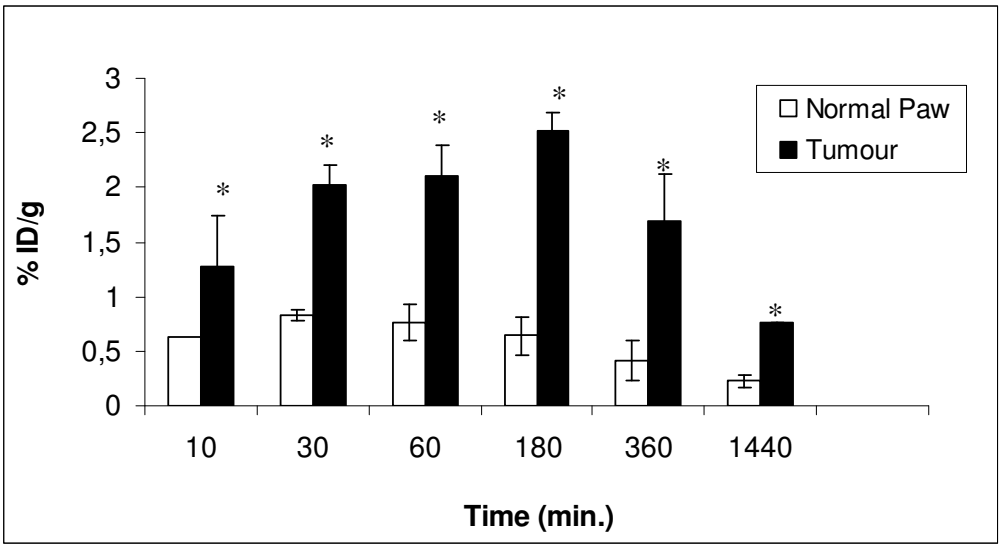

Figure 3. ^{125}I-Ph uptake after intravenous administration. Tumour uptake was significantly higher than normal paw in all evaluated times. *p<0.05.

Tumour-to-normal tissue ratios, for intravenous ^{125}I-Ph injection, were more than 20-fold higher depending on the organ. Three hours after the injection, tumour-to-muscle ratio was 21.2 and tumour-to-bone ratio was 10.5.

4. CONCLUSIONS

Ph was successfully labelled with ^{125}I and could specifically interact with breast tumour cells in vivo, suggesting that it possess indispensable characteristics for an efficient radiopharmaceutical for tumour diagnosis. The next step will be to evaluate the quality of tumour SPECT images provided by ^{131}I-Ph.

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