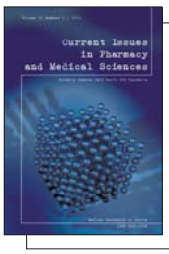

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Current Issues in Pharmacy and Medical Sciences

Formerly ANNALES UNIVERSITATIS MARIAE CURIE-SKLODOWSKA, SECTIO DDD, PHARMACIA
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Radiolabeling and biodistribution of new acetylcholinesterase inhibitor – 6-Hydrazino-*N*-[5-(2,3-dihydro-1*H*-cyclopenta[*b*] quinolin-9-ylamino)pentyl]nicotinamide hydrochloride

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ABSTRACT

Nowadays research concerning new strategies for earlier diagnosis is among the most active areas in Alzheimer's science. Brain imaging techniques are among the most promising areas of research focused on early detection of Alzheimer's disease (AD). In the present study we describe radiolabeling of a novel acetylcholinesterase inhibitor 6-hydrazino-*N*-[5-(2,3-dihydro-1*H*-cyclopenta[*b*]quinolin-9-ylamino)pentyl]nicotinamide (**5C-5**) with 99m-Technetium (^{99m}Tc). Biodistribution study of compound **5C-5** radiolabeled with ^{99m}Tc after intravenous administration to rats was also performed. According to biodistribution study ^{99m}Tc-radioactivity was mainly localized in the liver, partly also in the kidney, lung and gastrointestinal tract. Furthermore, compound under study revealed comparatively rapid blood radioactivity clearance.

INTRODUCTION

Radiopharmaceuticals are the fundamental tool of the diagnosis of modern medicine. Actually clinical practice of nuclear medicine focuses around the use of systemically administered gamma- or positron-emitting radiopharmaceuticals as diagnostic tools for imaging the human body and physiological functions of organs. Using special cameras designed to detect gamma photons leaving the patient we can observe regional radiotracer distribution and its kinetics. On the other hand, radiopharmaceuticals are used for the treatment of many illnesses [14]. An example of such utilization of radiopharmaceuticals is bone pain palliation therapy, which is a cost-effective systemic therapy to relieve pain in skeletal metastases [6, 7].

Alzheimer's disease (AD), the most common form of dementia, is a progressive neurodegenerative disorder of the brain characterized by memory impairment, cognitive deterioration, and functional decline spreading in a hidden way. The progression of AD is gradual and statistically patient lives 8-10 years after the beginning of symptoms. [9] The pathological, distinctive hallmarks of AD are β-amyloid plaques, neurofibrillary tangles, and reactive gliosis. Furthermore, among postmortem brains of patients suffering from AD changes in certain neurotransmission pathways are observed. One of the most significant neurotransmitter alterations found in brain of AD patients is a loss of the cholinergic neurotransmission; mainly the levels of choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) are affected. [15] Therefore, these two enzymes have become important markers for the diagnosis of AD.

In the last two decades, there has been an incredible interest in the development and utilization of brain imaging techniques for early detection, treatment and prevention of AD. Imaging techniques have proven to significantly influence the scientific study of AD, and what is more, they are expected to play growing roles in search of effective AD-modifying therapies [10]. Especially two techniques: positron emission tomography (PET) and single photon emission computed tomography (SPECT) which enable *in vivo* mapping of AChE are clinically advantageous for diagnosis of AD. Two radioligand approaches: radiolabeled substrates and inhibitors of AChE have been applied in *in vivo* studies of AChE.

There have been conducted several studies concerning development and evaluation of radiolabeled AChE inhibi-

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tors for the in vivo mapping of AChE. Among them are: N-[^{11}C]methyltacrine and [^{11}C]physostigmine, however, because these radioligands are characterized by a low selectivity of AChE over BChE and moderate binding properties to AChE, have led to nonspecific binding in the brain regions [8, 12]. Donepezil, drug approved for the treatment of AD with an excellent efficacy, has been also radiolabeled with ^{11}C isotope. Unfortunately, in vivo visualization of AChE by ^{11}C-labeled donepezil showed no specific binding to the enzyme, and as a consequence donepezil does not appear to be a good ligand for the visualization of AChE by PET [16].

Recently several radioligands showing a uniform regional distribution such as 3-[1-(4- [^{18}F]fluorobenzyl) piperidin-4-yl]-1-(1-methyl-1H-indol-3-yl)propan-1-one, its 3-[^{18}F]fluoromethylbenzyl derivative [3], and 6-[^{11}C]methoxy-3-[2-[1-(phenylmethyl)-4-piperidinyl] ethyl]-1,2-benzisoxazole ([^{11}C]MPPB) [2] have been synthesized.

The purpose of this study was to radiolabel 6-hydrazino-N-[5-(2,3-dihydro-1H-cyclopenta[b]quinolin-9-ylamino)pentyl]nicotinamide (5C-5) with technetium (^{99m}Tc). Furthermore, in order to estimate the potential of ^{99m}Tc-5C-5 as a potential diagnostic marker in AD, biodistribution study of radioactivity after intravenous administration to rats was conducted.

MATERIALS AND METHODS

Spectrophotometric Experiments. The absorption spectrum was obtained by scanning the sample between 200 and 350 nm with a Perkin Elmer spectrophotometer. The experiment was started with a solution of pure water at room temperature. The stability of compound **5C-5** was assessed by monitoring the variability of the spectrum at regular intervals (15 min) over 4 h.

Radiolabeling with ^{99m}Tc. For radiolabeling with Tc-99m 1 mg of the ligand **5C-5** was dissolved in 150 µl of water and than 30 µl of ethanol was added. Next 100 mg of tricine and 1.5 ml of technetium eluate were added together with 25 µl $SnCl_2$ in ethanol (1mg/ml). After 30 minutes incubation at room temperature quality control was performed by HPLC analysis. HPLC analysis was performed on Agilent System 1100 Series with UV and radiometric detection, with LiChrocart column 250-3 LiChrospher 100 RP-18 (5 µm). Flow rate 1ml/min. (Table 1) Quality control of ^{99m}Tc-labeled HYNIC-compounds with tricine as coligand on HPLC confirmed pure product, without unbound technetium (pertechnetate or hydrolyzed form which would appear at a short elution time) (Table 1).

Biodistribution studies in rats

Animals. For biological experiments radiolabelled compound was dissolved in saline to concentration of the ligand 100 µg/ml. An intravenous dose was 20 µg per animal. For biodistribution studies, male Wistar rats weighing 190-260 g were used. The animals were fasted overnight before the experiment (to empty the bowels) but had free access to water. All animal experiments were carried out according to the National Institute of Health Guidelines for the care and use of laboratory animals and the European Council Directive on 24 November 1986 for Care and Use of Laboratory Animals (86/609/EEC), and approved by the Local Ethics Committee of the Faculty of Pharmacy, Charles University, Hradec Kralove.

Table 1. Gradients for HPLC analysis

Gradient I:	Gradient II :
Mobile phase A: 0.9% NaCl, B. CH_3CN. 1-25 min 50%B 25-30min 50-100%B 30-35 min 100%B 35-40 min 100-0%B.	Mobile phase A: 0.9% NaCl, B. CH_3CN. 0-10 min 0% B 10-25 min 0-100% B 25-30 min 100% B30-35 min 100-0 % B.

Biodistribution in rats. The agent was administered to rats intravenously in a volume of 0.2 ml. During the course of experiments, the animals were placed singly in cages. At various time points after injection, the carotid artery was exposed under ether anesthesia and a blood sample was collected in glass tubes containing dry heparin. The rats were sacrificed and dissected. The organs of interest were weighed and counted for radioactivity in an automatic gamma counter 1480 Wizard 3. The results were expressed as mean standard deviations of minimally four animals.

RESULTS AND DISCUSSION

The important role of central cholinergic neurotransmission in learning and memory processes and the correlation of cholinergic deficits with cognitive impairment of patients with AD have led to the development of symptomatic cholinergic therapies. Acetylcholinesterase inhibitors (AChEIs) act by inhibiting the enzyme AChE, which hydrolyzes the cholinergic neurotransmitter, acetylcholine (ACh). Approved for the treatment of AD AChE inhibitors such as tacrine, galantamine, rivastigmine and donepezil provide comparatively little chance of prolonged improvement of cognitive functions. Therefore, there is still the requirement to search for new compounds with anticholinesterase activity, and many scientific teams pick up a long-standing challenge to discover new compounds, which would improve cholinergic neurotransmission. There is also an urgent need to find novel diagnostic agents which certainly have a profound impact on the scientific study of AD.

Compound **5C-5** was designed for radiolabeling. Its synthesis and biological evaluation towards inhibition of AChE and BChE was described previously [11]. Structure

of this compound is presented in Figure 1. In addition, spectrophotometric experiments were performed to determine its stability in water (Figure 2). Figure 3 presents radiochromatograms of complexes formed by technetium-99m with tricine and Hynic. In comparison with 6-hydrazino-*N*-[5-(2,3-dihydro-1*H*-cyclopenta[*b*]quinolin-9-ylamino)ethyl]nicotinamide hydrochloride (compound **5C-2**) radiochromatograms of compound examined within this study **5C-5** (more lipophilic structure than **5C-2**) show more forms which corresponds probably to different isomers of HYNIC-Tc-tricine complex (Figure 4) [5].

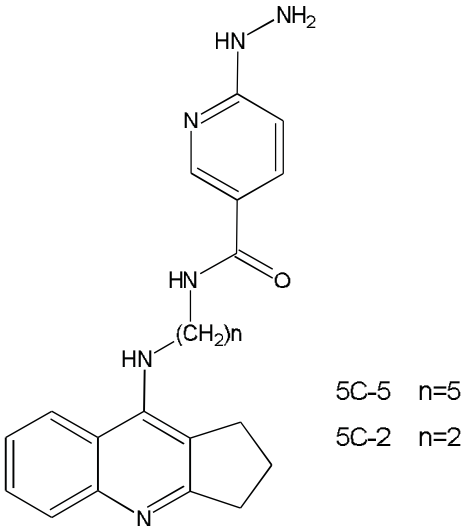

Fig. 1. Structures of compounds which were radiolabeled; within this study 5C-5 and published previously 5C-2 [11]

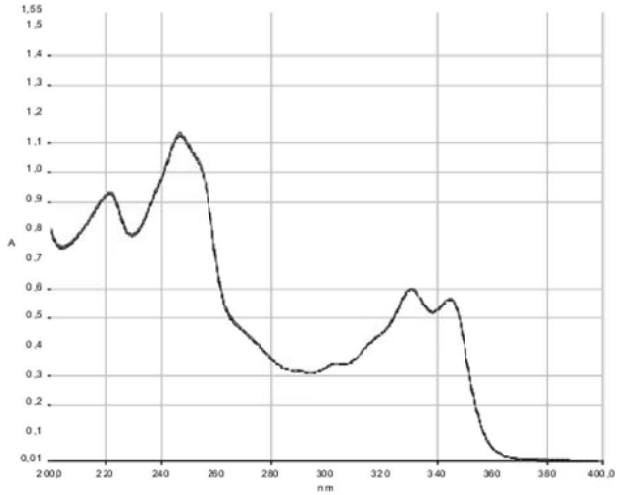

Fig. 2. UV spectra of compound **5C-5** in aqueous solution at different times of incubation (0 to 4 h, measured every 15 min)

Table 2 presents the tissue distribution of radioactivity after intravenous administration of ^{99m}Tc-5C-5 to rats. Collectively, the agent under study exhibited relatively rapid blood radioactivity clearance. Furthermore, a large percentage of ^{99m}Tc-radioactivity was localized in the

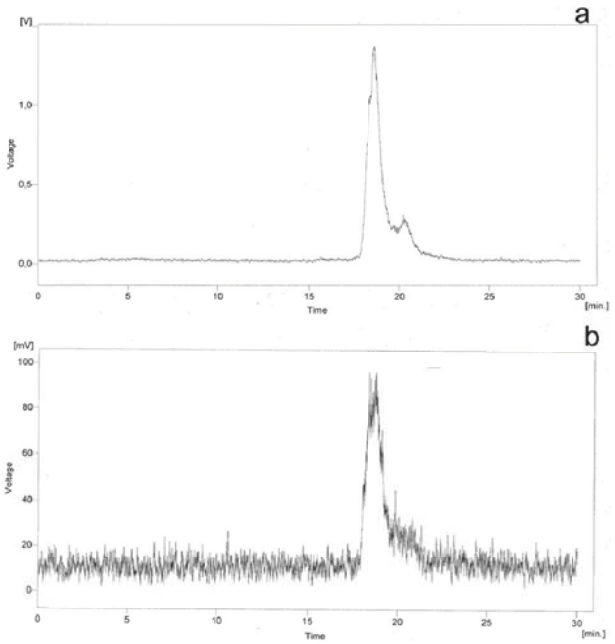

Fig. 3. Radiochromatograms of complexes formed by technetium-99m with tricine and Hynic; a) Compound 5C-5, b) Compound 5C-5 after 24 h

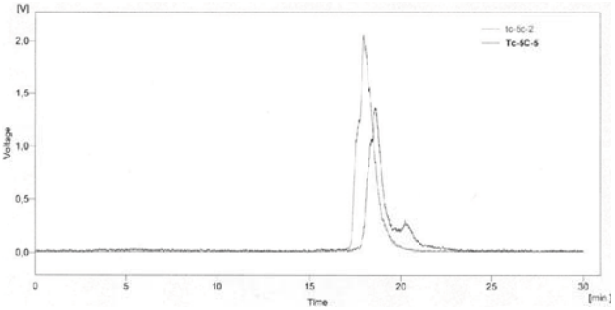

Fig. 4. Comparison of radiochromatograms of complexes formed by technetium-99m with tricine and Hynic for compound 5C-5 and compound 5C-2 [11]

liver, partly also in the kidney, lung and gastrointestinal tract. Radioactivity found in the kidney and gastrointestinal tract is most likely connected with elimination of the parent compounds and/or their metabolites from the body. Unfortunately, radioactivity concentrations in the brain were very low; 60 min. after the intravenous administration of ^{99m}Tc-5C-5 the percentage of the initial dose was only 0.014. It might be due to hydrophilicity of radiolabelled compounds (the effect of technetium and co-ligands attached to the 2,3-dihydro-1*H*-cyclopenta[*b*]quinoline analogues).

On the other hand, selectivity of the compound **5C-5** for AChE**,** 1200-fold higher than selectivity of tacrine, is very promising because moiety of 6-hydrazinenicotinic acid (HYNIC) is responsible for the binding of the radiotracer, which is ^{99m}Tc (reported previously) [1].

AChE inhibitors approved by FDA for the treatment of AD and their analogues were examined as radioligands for *in vivo* mapping of AChE. In case of [^{11}C]donepezil

mice biodistribution studies exhibited a high uptake of radioactivity in the brain (6.34 %ID) and a rapid blood clearance. However, regional distribution studies in rabbit did not show any correlation between the uptake of radioactivity and the amount of AChE. Therefore, it was concluded that despite good properties in the treatment of AD, donepezil does not seem to be a good ligand for the visualization of AChE by PET [16].

Table 2. Distribution of radioactivity in selected organs and systems of rats after intravenous administration of ^{99m}Tc-5C-5

^{99m}Tc-5C-5				
	%Dose/g			
	5 min	60 min	120 min	24 h
Organs				
Blood	0.680 ± 0.038	0.207 ± 0.022	0.156 ± 0.007	0.062 ± 0.009
Plasma	1.173 ± 0.073	0.338 ± 0.030	0.267 ± 0.012	0.105 ± 0.015
Pancreas	1.105 ± 0.244	0.157 ± 0.039	0.101 ± 0.010	0.083 ± 0.012
Liver	6.084 ± 0.413	3.234 ± 0.384	3.182 ± 0.218	2.224 ± 0.245
Adrenals	0.723 ± 0.113	0.428 ± 0.059	0.372 ± 0.020	0.376 ± 0.024
Kidney	6.863 ± 1.123	3.880 ± 0.790	2.693 ± 0.389	2.071 ± 0.501
Lung	0.978 ± 0.085	0.571 ± 0.136	0.435 ± 0.039	0.328 ± 0.031
Heart	0.472 ± 0.052	0.181 ± 0.025	0.144 ± 0.011	0.098 ± 0.012
Spleen	0.489 ± 0.060	0.362 ± 0.073	0.317 ± 0.009	0.363 ± 0.016
Stomach	1.982 ± 3.031	0.227 ± 0.104	1.673 ± 1.903	0.266 ± 0.297
Intestine	3.340 ± 0.916	10.393 ± 0.336	7.200 ± 0.583	0.201 ± 0.094
Colon	0.131 ± 0.028	0.076 ± 0.023	5.029 ± 2.790	2.999 ± 1.886
Testes	0.067 ± 0.018	0.045 ± 0.003	0.039 ± 0.002	0.037 ± 0.004
Skin	0.312 ± 0.015	0.178 ± 0.013	0.149 ± 0.015	0.120 ± 0.015
Muscle	0.146 ± 0.026	0.060 ± 0.010	0.048 ± 0.001	0.038 ± 0.008
Thyroid	0.507 ± 0.101	0.239 ± 0.008	0.270 ± 0.079	0.141 ± 0.011
Brain	0.031 ± 0.003	0.014 ± 0.001	0.011 ± 0.000	0.006 ± 0.001
Fat	0.391 ± 0.110	0.131 ± 0.013	0.078 ± 0.026	0.075 ± 0.005
Femur	0.271 ± 0.037	0.152 ± 0.024	0.129 ± 0.019	0.108 ± 0.001

1-(4-[^{18}F]fluorobenzyl)-4-[(5,6-dimethoxy-1-oxoindan-2yl)methyl]-piperidine (4-[^{18}F]FDP), an analogue of donepezil, was evaluated as an agent for *in vivo* studies of AChE. [4] It was reported that the brain uptake of the radioligand reached 1.6 %ID at 5 min, however, region-to-cerebellum (R/C) uptake ratios were uniform at all points similarly to [^{11}C]donepezil, suggesting that 4-[^{18}F]FDP may not be a suitable agent for *in vivo* studies of AChE, despite its potent *in vitro* biological activity. [4]

Also in case of [^{11}C]methyltetrahydroacridine ([^{11}C]MTHA) its high concentrations in the brain, nevertheless, the regional brain distribution of [^{11}C]MTHA does not parallel that of *in vivo* AChE concentrations. [13]

CONCLUSIONS

At the current stage of medical knowledge diagnosis of AD is made by clinical, neuropsychological, and neuroimaging evaluations. Routine neuroimaging evaluation is based on non-specific features such as atrophy, which is a late feature giving evidence of the progression of AD. Therefore, it is of vital importance to develop new approaches for early and specific recognition of AD. During the last 20 years, functional neuroimaging techniques such as PET and SPECT have proven to be valuable in the differential diagnosis of AD.

In our earlier paper we presented synthesis and biological evaluation of 2,3-dihydro-1*H*-cyclopenta[*b*]quinoline derivatives with hydrazine nicotinate (HYNIC) moiety. [14] Compound **5C-5** evaluated within this study possesses the fragment of cyclopenta[*b*]quinoline which has the possibility to inhibit both acetylcholinesterases and HYNIC moiety which could be utilized as a co-ligand for radiolabeling.

Biodistribution studies in rats revealed that compound ^{99m}Tc-5C-5 exhibited comparatively rapid blood radioactivity clearance. ^{99m}Tc-radioactivity was mainly localized in the liver, and to the lesser extent in the kidney, lung and gastrointestinal tract. Unfortunately, low radioactivity concentrations in the brain suggest that this agent does not cross the blood-brain barrier and, therefore, may not be a suitable agent for diagnosis of AD.

However, similarly to [^{11}C]choline which is an oncologic PET radiopharmaceutical utilized with good results in the diagnosis of lung cancer, colon cancer, or prostate cancer [11], compound **5C-5** might be further evaluated as an element for the detection of cancers in certain organs (*e.g.* liver, kidney or lungs) or to monitor the response to various therapies.

Acknowledgements: This work was supported by the grant (N N405 669940) from National Science Centre in Poland and by the Grant Agency of the Czech Republic (grant P304/10/1738).

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