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PHYSICOCHEMICAL EVALUATION OF TECHNETIUM-99m COMPLEXES WITH BACLOFEN

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Abstract
The ^{99m}Tc-labeling of baclofen (Bac), a muscle relaxant, as well as physicochemical properties of the labeled compounds are investigated. Two different approaches for the labeling with ^{99m}Tc have been studied: direct reduction with tin(II)chloride and the 'organometallic approach' using $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor. The direct labeling approach was not successful and the yield was poor. The use of $[^{99m}Tc(CO)_3(H2O)_3]^+$ precursor pointed at the formation of ^{99m}Tc(I) coordinated complexes with high yield. In this approach, pH didn't influence the yields. Promising results of *in vitro* experiments suggest that ^{99m}Tc(I)-baclofen may be of potential use for diagnosis of some central nervous system disorders.

Introduction

Baclofen is a structural analogue of gamma-aminobutyric acid (GABA). It is a selective agonist of $GABA_B$ receptors in central nervous system and it's primarily used to treat spasticity. Also, it is in the early stages of use for the treatment of alcoholism. Baclofen is a muscle relaxant medicine commonly used to decrease spasticity related to multiple sclerosis, spinal cord injuries, or other neurological diseases. It could be used in oral use or intrathecal way. Its ways in organism are not well known nowadays [1].

^{99m}Tc has ideal physical properties for many applications in nuclear medicine therefore it is still the radionuclide of choice. A large number of techniques for radiolabeling with ^{99m}Tc have been developed. They are classified as: direct labeling, the preformed chelate approach and the bifunctional chelating or indirect labeling approach. The study of technetium radiopharmaceuticals has been stimulated by the availability of radioactive ^{99m}Tc(I) precursor $[Tc(CO)_3(H_2O)_3]^+$ [2]. As the three water ligands are labile, they can be readily exchanged with different mono-, bi- and tridentate donor ligands, like phosphines, thioethers and aromatic amines. Hydrophilic organometallic $[^{99m}Tc(H2O)_3(CO)_3]^+$ precursor allows the formation of Tc (I) radiopharmaceuticals based on the fac-$[^{99m}Tc(CO)_3]^+$ (I) core. This way is known as 'organometallic labeling approach'[3]. In this paper the possibility for the formation of ^{99m}Tc complexes with baclofen was studied.

Experimental

All chemicals used in our experiments were of analytical purity grade (Sigma Aldrich and Merck).The direct labeling method was investigated performing different experiments by varying the reducing agent amount, the labeling mixture pH (3.0-8.0) and the reaction temperature. An aqueous solution of baclofen (10^{-3}M) was prepared.

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To a 10 ml vial, 0.3 ml of baclofen solution in H_2O was added and followed by $SnCl_2x2H_2O$ solution (10^{-4}M) in 0.1 M HCl, thus molar ratios Sn(II):baclofen were 1:1, 1:10, 1:25 and 1:50. The stock solution of $SnCl_2x2H_2O$ was prepared by dissolving of the measure amount of the pure salt in concentrated HCl and then diluting it with doubly distilled water under the define volume. pH of these mixtures was adjusted at 3.0, 5.0 and 8.0, to the total volume of 3.0 ml. 18.5 MBq $^{99m}TcO_4^-$ eluate in 0.9% NaCl, from ^{99m}Tc-generator (Vinča) was added. The reaction mixtures were allowed to stand at room temperature (RT) or heated at boiling temperatures for 30min.

$[^{99m}Tc(CO)_3(H2O)_3]^+$ion was prepared by addition of 1ml of ^{99m}Tc-pertechnetate (740-1110 MBq$^{99m}TcO_4^-$) to a penicillin vial with lyophilized form of 7.15 mg sodium carbonate, 4.5 mg sodium boranocarbonate, 2.85 mg sodium tetraborate and 8.5 mg sodium tartarate (IsoLink^{TM}, Mallinckrodt Medical B.V., The Netherlands). After heating for 30 minutes in a boiling water bath and cooling, pH of solution was adjusted to desired value with 1M HCl. ^{99m}Tc-carbonyl-baclofen was prepared by addition of 0.1 ml of investigated muscle relaxant solutions to 0.9 ml of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor. The samples were labeled with ^{99m}Tc (I), without and with heating (30 min in boiling water bath). The complex stability after 24 hour time was investigated, too.

HPLC analysis was performed by isocratic HPLC. All measurements were made on liquid Chromatograph, Hewllet Packard 1050 (C18column (250x4,6mm)) with UV and Raytest gamma flow detector. The different methanol/water mixtures prepared from HPLC grade water, were used as mobile phases.

Results and Discusion

The direct labeling method was not successful and the labeling yield was poor. Varying of the reducing agent amount, the labeling mixture pH (3.0-8.0) and the reaction temperature did not improve the yield substantially. However the use of $[^{99m}Tc(CO)_3(H2O)_3]^+$ precursor, at pH 3.0, 5.0 and 8.0 with and without heating resulted in the formation of ^{99m}Tc(I) coordinated complexes of baclofen in high yields that was shown by radiochromatograms. The best results are obtained at pH 3.0 (Fig.1) and 8.0 at RT. The retention times together with the labeling yield are summarized in Table 1.

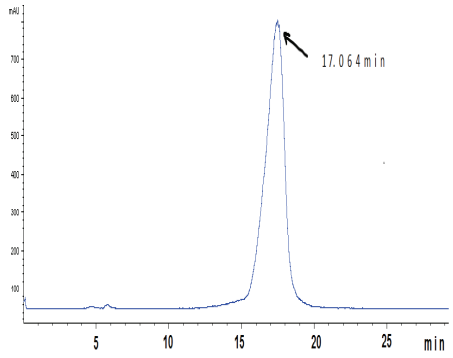

Figure1. HPLC radiochromatogram of ^{99m}Tc(I)-Bac labeled at RT, pH=3.0

Table 1. The influence of pH and heating on retention times (R_t) and labeling yield (Y) of baclofen coordinated to $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor.

Samples	pH	3.0		5.0		8.0	
	^{99m}Tc-species	R_t (min)	Y (%)	R_t (min)	Y (%)	R_t (min)	Y (%)
$[^{99m}Tc(CO)_3(H_2O)_3]^+$ +Bac, at RT	$^{99m}Tc(CO)_3$ ^{99m}Tc(I)-Bac	5.238 17.064	0.3 99.7	5.215 16.238	11.2 88.8	5.082 16.354	1.2 98.8
$[^{99m}Tc(CO)_3(H_2O)_3]^+$ +Bac, with heating at 95^0C	$^{99m}Tc(CO)_3$ ^{99m}Tc(I)-Bac	5.296 16.770	4.9 95.1	5.262 14.386	14.9 85.1	4.940 16.659	6.8 93.2
$[^{99m}Tc(CO)_3(H2O)_3]^+$ +Bac, at RT after 24h	$^{99m}Tc(CO)_3$ ^{99m}Tc(I)-Bac	5.257 17.076	1.6 98.4	5.317 15.277	14.8 85.2	5.267 14.417	7.1 92.8
$[^{99m}Tc(CO)_3(H2O)_3]^+$ +Bac, with heating at 95^0Cafter 24h	$^{99m}Tc(CO)_3$ ^{99m}Tc(I)-Bac	5.325 15.327	15.2 84.8	5.346 15.513	15.9 84.1	5.248 14.151	8.2 91.8

HPLC results revealed that radiolabeling yields were not significantly affected by pH values within the range pH 3.0–8.0 at RT. We assume that the different retention times of formed complexes are a consequence of the influence of pH and temperature on the structure and composition of these complexes. Bac contains both a carboxyl and an amino group and by a change in pH these groups are in their protonated or deprotonated forms. Depending on the forms, Bac can be mono- or bidentate ligand and easily forms different complexes with the *fac*-$[^{99m}Tc(CO)_3]^+$ (I) core. The following experiments on animals should show the impact of different structures of the complexes on their biological behavior. All complexes showed excellent stability in 24 hour time (>80%).

Conclusion

The results presented in this paper reveal that complexation of the baclofen are highly dependent on the labeling approach and the oxidation state of ^{99m}Tc. Direct ^{99m}Tc-labeling of baclofen, known to be weak-chelating agent, gave high concentrations of $^{99m}TcO_2^-$ and free $^{99m}TcO_4^-$. The best labeling yield was obtained with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor when labeling was done at RT.

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