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Development of ^{99m}Tc-SDP-choline SPECT radiopharmaceutical for imaging of cerebrovascular diseases

Rashid Rasheed[1], Syed Ali Raza Naqvi[1]*, Rida Siraj[1], Nosheen Aslam[2], Muhammad Waqas Nasir[2], Ali Abbas[1], Muhammad Yaseen[3], Abdul Qaddus[4] and Samina Roohi[5*]

[1]Department of Chemistry, Government College University, Faisalabad, Pakistan
[2]Department of Biochemistry, Government College University, Faisalabad, Pakistan
[3]Department of Chemistry, University of Education (Lahore), Faisalabad Campus, Faisalabad, Pakistan
[4]Government College University, Faisalabad, Pakistan
[5]Pakistan Institute of Nuclear Science and Technology (PINSTECH), Nilore, Islamabad, Pakistan

Abstract: Cerebrovascular diseases are known as serious public health problem worldwide, which can be addressed more precisely through molecular imaging of non-functional brain cells. CDP-choline is an active cerebrovascular chemotherapeutic agent that can be used for diagnosis of cerebrovascular diseases post radiolabeling with γ-emitter radioisotopes. In this study we developed ^{99m}Tc labeled CDP-choline for imaging of cerebrovascular diseases particularly alzheimer, stroke, and parkinson's diseases. The radiosynthesis reaction resulted 97.47±2.34% radiochemical with promising stability, that is, >95% up to 6 h in blood serum. The biodistribution study in healthy mice revealed non-accumulated uptake of radiochemical in key body organs; in brain it was 8.59±1.11% ID/g at 1h post-injection which washed-out leaving behind 0.87±0.61% ID/g at 24 h post-injection. The over-all data revealed the ^{99m}Tc-CDP-choline could be a good candidate for further imaging investigations in diseased animal model.

Keywords: Cerebrovascular disease, alzheimer disease, ^{99m}Tc-CDP-choline, radiopharmaceuticals, SPECT

INTRODUCTION

Instrumental procedures for diagnosis of diseases are considered backbone of imaging technology. X-rays, ultrasonography, computed tomography (CT) scan and magnetic resonance imaging (MRI) was developed for diagnosis of deep seated diseases; however, they are limited to anatomical/morphological changes which appear commonly at advance stage of disease and unable to chemical and functional changes which commonly appear as disease starts (Khan *et al*., 2019; Naqvi *et al*., 2013; Naqvi and Drlica, 2017).

Cerebrovascular or degenerative brain disease such as Parkinson disease, Huntington's disease, epilepsy, Alzheimer's disease, stroke, dementia, brain injury, and psychiatric are mainly chemical and functional disorders which poorly translate into anatomical alteration and hence could not be detected with instrumental modalities with high accuracy (Iulia *et al*., 2017). Nuclear medicine imaging technique (NMIT) offers precise detection of chemical and functional changes due to disease at early stage - in this regards the development of radiopharmaceuticals for cerebrovascular imaging is increasing continuously. The radiopharmaceuticals are administrated intravenously, and imaging is carried out either using single photon emission computed tomography (SPECT) or positron emission tomography (PET). Mainly four mechanisms are considered to develop radiopharmaceuticals for SPECT and PET brain imaging study; blood-brain-barrier (BBB) permeability, cerebral perfusion (CP), metabolism receptor-binding (MRB), and antigen-antibody binding (AAB) (Secades and Lorenzo, 2006). Lipophilic radiopharmaceuticals such as ^{123}I-IMP, ^{99m}Tc-HMPAO, ^{99m}Tc-ECD are well established SPECT perfusion agents which showed excellent ability to diffuse into normal brain while ^{123}I-QNE, ^{123}I-IBZM and ^{123}I-iomazenil are important MRB SPECT radiopharmaceuticals ^{13}N-glutamate, ^{68}Ga-EDTA and ^{82}Rb-RbCl are BBB permeability based PET imaging agent; ^{15}O-water, ^{13}N-ammonia, and ^{15}O-butanol are common CP based PET imaging agent (Nagamitsu *et al*., 2016). ^{18}F-fluorodeoxyglucose (^{18}F-FDG) is the most effective and common PET cerebral metabolic agent in clinical setup which successfully differentiate between recurrent tumors and radiation necrosis and the detection of Alzheimer's disease (Sarikaya *et al*., 2018).

Cytidine-5'-diphosphocholine (CDP-choline; generic name citicoline; fig. 1) is an endogenous compound mainly involve in synthesizing cellular membranes phosphatidylcholine that increase the level of neurotransmitter in the central nervous system (CNS) (Roberti *et al*., 2015) . After intravenous administration, CDP-choline accumulates inside the brain cells especially of Alzheimer disease, stroke, and Parkinson's disease, as well as in glaucoma and amblyopia disease (Roberti *et al*., 2015). The purpose of this study was to develop technetium-99m (^{99m}Tc) labeled CDP-choline for brain SPECT imaging agent.

**Corresponding author:* e-mail: draliraza@gcuf.edu.pk

MATERIALS AND METHODS

Cytidine-5'-diphosphocholine, stannous chloride dihydrate ($SnCl_2$. $2H_2O$), acetone, ethanol was purchased from Sigma-Aldrich (Germany). Sodium hydroxide (NaOH) and hydrochloric acid (HCl) were purchased from Merck, Germany. Whatman paper # 3 and Instant Thin Layer Chromatography strip impregnated with silica gel (ITLC-SG) were purchased from Agilent Technology (USA). Sprague-Dawley (SD) rats (150-200 g) were obtained from National Institute of Health (NIH), Islamabad, Pakistan and ethical approval for animal study was obtained from institute. Carrier-free ^{99m}Tc as $Na^{99m}TcO_4$ was obtained in the form of Mo/^{99m}Tc generator from Pakistan Institute of Nuclear Science & Technology (PINSTECH), Islamabad, Pakistan.

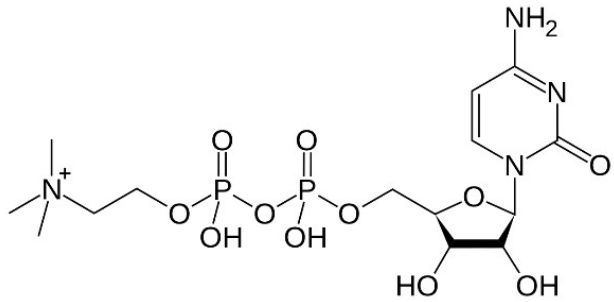

Fig. 1: Structure of CDP-choline

Radiosynthesis of ^{99m}Tc-CDP-choline

Initially, the radiosynthesis of ^{99m}Tc-CDP-choline was carried out by hit-and-trail method for adjusting the key parameters such as amount of CDP-choline, reducing agent, ^{99m}Tc-activity, pH, reaction time and temperature. Typically, the effect of reaction conditions was assessed by subsequent addition of 10-75µg CDP-choline, 2-10µg $SnCl_2$. $2H_2O$ as reducing agent, 10-20 mCi $^{99m}TcO_4^-$ in different batches and tested the reaction yield at 4-8 pH on 15-40 min incubation period at room temperature.

Quality control analysis

The percent yield of ^{99m}Tc-CDP-choline and radioactive impurities (free $^{99m}TcO_4^-$ and hydrolyzed fraction) were assessed using instant thin layer chromatography impregnated with silica gel (ITLC-SG) as described earlier with brief modification (Naqvi *et al*., 2019). An aliquot of 2 µL was spotted at base-line and run in two different mobile phase systems i.e. acetone and mixture of ethanol, water, and ammonium hydroxide with 2:5:1 ratio. Acetone as mobile phase was used to determined free $^{99m}TcO_4^-$; in this process the free $^{99m}TcO_4^-$ traveled to solvent front while ^{99m}Tc-CDP-choline and hydrolyzed fraction remained at base line. In second mobile phase system the ^{99m}Tc-CDP-choline and free $^{99m}TcO_4^-$ traveled to solvent front leaving hydrolyzed fraction at base line. From the determination of free $^{99m}TcO_4^-$ and hydrolyzed fraction on both strips the percent yield of ^{99m}Tc-CDP-choline was calculated. The strips that were developed in two different solvents analyzed under 2π-radioscanner for quantitative calculation of each radio-product.

Stability of ^{99m}Tc-CDP-choline Complex

Post radiolabeling stability of ^{99m}Tc-CDP-choline complex was assessed up to 24h using ITLC-SG. Typically, 2µL aliquot was spotted at the base line of chromatography strip at 0, 2, 4 and 24 h time points and developed with acetone and mixture of ethanol, water, and ammonium hydroxide solvent systems. Dried strips were then analyzed with 2π-radioscanner to assess intact radiochemical percentage.

^{99m}Tc-CDP-choline complex Stability in Blood Serum

The ^{99m}Tc-CDP-choline complex stability in blood serum was assessed in freshly harvested human blood serum. To a 0.8mL blood serum added 0.2mL radiolabeled compound, vortexed and incubated at 37ºC in CO_2-incubator. At different time intervals, typically 1, 2, 4, and 24 h time points, took 5µL incubated mixture and developed using ITLS-SG. Dried strips were then analyzed with 2π-radioscanner to assess intact radiochemical percentage.

Biodistribution study

Biodistribution study of ^{99m}Tc-CDP-choline complex was performed at 10 min post labeling reaction by following the reported protocol earlier (Iqbal *et al*., 2018). Briefly, an aliquot of 200µL was injected through tail vein and a group of three mice were sacrificed at 1, 4 and 24 h post chloroform anesthesia. Key body organs were removed, washed with saline, weighed, sealed in gamma tubes and then counted the gamma-ray counts with well type gamma counter. The results were then calculated in percent injected dose per gram organ (%ID/g organ).

STATISTICAL ANALYSIS

Statistical analysis of all readings was finished by ME 2010 and findings are offered as mean ± S.D.

RESULTS

Effect of reaction conditions on ^{99m}Tc-CDP-choline labeling

Fig. 2 showed the effect of different reaction parameters such as concentration of CDP-choline, reducing agent, pH and temperature on the yield of ^{99m}Tc-CDP-choline. In all radio synthesis reactions, change in one reaction parameter was assessed by keeping all other reaction parameters constant.

^{99m}Tc-CDP-choline labeling yield

The highest labeling yield, 97.47±2.34 was obtained by subsequent addition of 75mg CDP-choline, 4µg $SnCl_2{\cdot}2H_2O$ as reducing agent, 20 mCi of $^{99m}TcO_4^-$, room temperature, and at pH 7.

Shelf-life of ^{99m}Tc-CDP-choline complex and stability in blood serum

The radiosynthesis of ^{99m}Tc-CDP-choline at optimized reaction conditions was assessed for its shelf-life up to 24 h. Stability of ^{99m}Tc-CDP-choline in freshly harvested human blood serum was also tested at predefined time intervals. The results are shown in fig. 3.

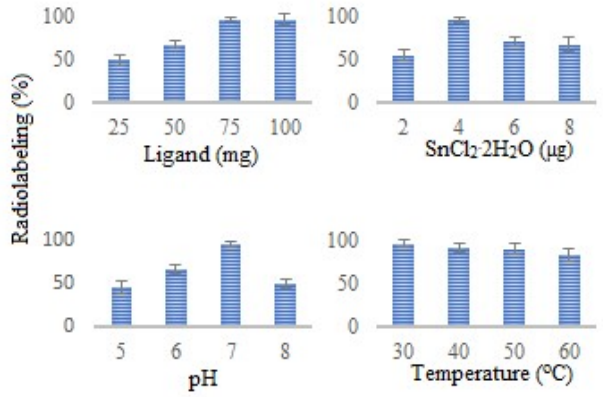

Fig. 2: Effect of reaction parameters on radio labeling yield.

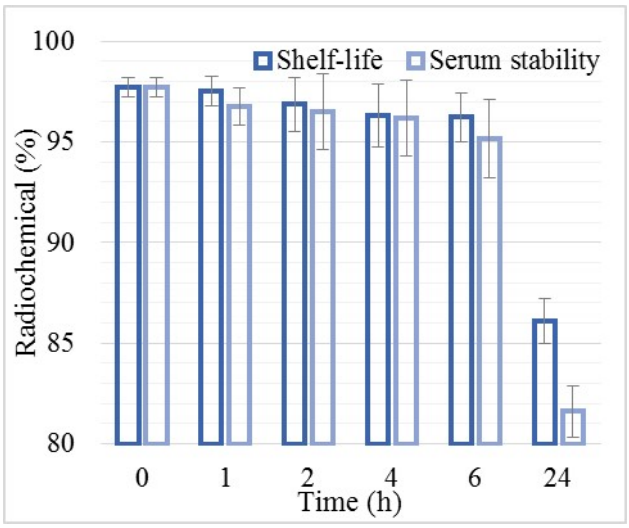

Fig. 3: Shelf-life of ^{99m}Tc-CDP-choline and stability in blood serum

Biodistribution Study

About 250 MBq activity of ^{99m}Tc-CDP-choline was injected through tail-vein of mice in a group of three. Results of radiopharmaceutical uptake in different body organs at 1, 4 and 24 h time points are shown in table 1.

DISCUSSION

The ^{99m}Tc-CDP-choline radiopharmaceutical was developed to diagnose cerebrovascular diseases especially Alzheimer, stroke, and Parkinson's diseases. The radiochemical purity for developing targeting radiopharmaceutical is a prime prerequisite to achieve high accuracy and sensitivity. To get maximum radiochemical yield, the effect of different parameters such as amount of CDP-choline & reducing agent, pH and reaction time were studied and optimized for maximum radiochemical yield. The radiolabeling reaction was found highly sensitive to pH, a slight change in pH significantly decreased the radiolabeling yield (fig. 2). The reducing agent is another more critical parameter that significantly affected the radiochemical yield, free 99mTc and colloid (Ahmed *et al*., 2018). Radiolabeling reactions in which more or less amount of $SnCl_2.2H_2O$ was chosen than optimized quantity, the percentage of hydrolyzed and free ^{99m}Tc radioactive impurities increased significantly. The main function of $SnCl_2.2H_2O$ is to reduce the oxidation state of ^{99m}Tc from +7 to +5 or +4 to make it suitable for radiolabeling (Naqvi *et al*., 2018). Highest radiochemical yield (97.47 ± 2.34%) was obtained at optimized conditions along with 1.21± 0.27% hydrolyzed ^{99m}Tc and 1.35± 0.19% free ^{99m}Tc. Prior to animal study, the stability study of radio labeled compound was also performed to ensure safe administration in living system. The shelf-life of radiochemical in saline medium was investigated up to 24 h at room temperature. The radiochemical was found highly stable up to 6 h; similarly, the complex stability in human blood serum at 37ºC revealed more that 95% intact radiochemical which ensure safe accumulation at target cells and tolerable radiotoxicity.

Table 1: Biodistribution study of ^{99m}Tc-CDP-choline in mice model

Organs	Biodistribution (%ID/g organ)		
	1 h	4 h	24 h
Brain	8.59±1.11	3.13±1.22	0.87±0.61
Heart	1.21±0.36	0.79±0.19	0.25±0.08
Liver	4.52±1.14	2.51±0.34	0.68±0.25
Spleen	0.63±0.18	0.29±0.16	0.21±0.11
Stomach	2.67±0.31	0.88±0.39	0.33±0.09
Kidney	10.41±2.34	4.51±1.24	1.52±0.61
Lungs	0.79±0.27	0.43±0.11	0.31±0.15
Intestine	2.31±0.54	1.61±0.91	0.72±0.19
Femur	0.28±0.08	0.22±0.07	0.17±0.04
Blood	2.91±0.31	1.09±0.29	0.35±0.04

Before performing pre-clinical studies, biodistribution study in normal and diseased animal model is carried out to generate uptake/washout profile by different organs (Naqvi *et al*., 2018). All organ showed normal uptake, however slightly higher uptake by liver and kidneys is due to the metabolic and excretory path of CDP-choline. Brain is the key organ on the bases on which the ^{99m}Tc-CDP-choline was developed showed 8.59±1.11% ID/g uptake at 1h post-injection which washed-out leaving behind 3.13±1.22 and 0.87±0.61% ID/g at 4 and 24h post-injection, respectively. The ^{99m}Tc-CDP-choline rapidly cross the BBB and accumulated in brain at 1h but due to

the absence of diseased cells, the ^{99m}Tc-CDP-choline no longer retained in brain and continuously drained off. The radiochemical yield, stability and biodistribution data of present study are in close agreement with the clinically approved brain imaging agents. Although, the quality control parameters and normal biodistribution study is looking encouraging but the application of ^{99m}Tc-CDP-choline merely depends on the pre-clinical studies (Lee, 2016).

CONCLUSION

Radiolabeling of CDP-choline showed good ^{99m}Tc-CDP-choline yield with promising shelf-life and stability in blood serum. Normal animal biodistribution study, rapid crossing of BBB to accumulate and in/washout-from brain cells indicates the efficiency of the radiochemical. However, no retention was due to the absence of cerebrovascular diseased cells. Based on the results obtained, ^{99m}Tc-CDP-choline can be assessed pre-clinically for imaging of cerebrovascular diseases particularly for Alzheimer, stroke and Parkinson's diseases by developing diseased animal models to investigate further in clinical set-up.

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