DEVELOPMENT OF A NOVEL ^{99m}Tc-LABELLED BRAIN PERFUSION AGENT

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Abstract

A method has been described for radiolabeling of salbutamol with technetium-99m (^{99m}Tc). To a 1 ml solution, containing 5 mg salbutamol and 2 mg of ascorbic acid, was added a clear solution (10 µl) of $SnCl_2.2H_2O$ (100 µg) in distilled water. The pH of the solution was adjusted to 8.5, mixed gently with 10 mCi (370 MBq) of ^{99m}Tc elute and incubated at room temperature for 15 minutes. The resulting solution was passed through 0.22 µ filter and radiolabeled fraction was quantified using paper chromatography. The radiochemical analysis, employing the use of above-mentioned radioanalytical technique, revealed that greater than 97% of the radioactivity was bound to salbutamol and rest of the activity was in the form of free pertechnitate ($^{99m}TcO_4$).

Biological data, obtained after i.v. injection of ^{99m}Tc-salbutamol to female albino rabbits, revealed a fair uptake in the brain at 30 min and 60 min post injection time intervals. On the basis of animal biodistribution data it is suggested that ^{99m}Tc-salbutamol can be successfully applied as a brain perfusion agent.

Introduction

The clinical value of measuring regional cerebral blood flow (rCBF) has long been recognized [1]. Nuclear medicine is rapidly establishing itself as an important tool in the invivo study of the brain neurochemistry. Several neuroligands have been developed which offer valuable new insights in various neural disorders from brain injury to vascular diseases [2-4]. ^{99m}Tc and iodine-123 (^{123}I) are well suited radionuclides for single photon emission computed tomography (SPECT) studies [5]. Compared to currently used ^{123}I-labeled brain perfusion agents [6-14], the ready availability, low cost, instant kit formulation at the clinical site and ideal nuclear emission are the advantages of ^{99m}Tc-labeled rCBF agents [5].

Several neutral, lipophilic ^{99m}Tc chelates have been known to cross the blood-brain barrier (BBB) but only a few are efficiently extracted by the brain and retained there for long enough to be useful as brain perfusion agents with SPECT. Many derivatives of these chelates have been evaluated. Among these, hexamethylpropyleneamine oxime (^{99m}Tc-HMPAO), the first Food and Drug Administration (FDA) approved ^{99m}Tc-labeled rCBF agent, has considerable potential for routine clinical studies using SPECT [15]. HMPAO has two stereoisomers due to two asymmetric carbon atoms (at 3 and 9 positions) and these two isomers have dramatic differences in their

cerebral retention characteristics [16]. Synthesis of d,l-HMPAO requires prolonged purification steps and ^{99m}Tc-HMPAO is unstable in vitro [17]. ^{99m}Tc-l,l-ethylcysteinate dimer (^{99m}Tc-ECD) have been shown to be quite suitable for human SPECT brain imaging studies [18] but its synthesis involving the use of liquid ammonia is complex.

Salbutamol, 2-tert-butylamino-1-(4-hydroxy-3-3-hydroxymethylphenyl)-ethanol, is a direct acting sympathomimetic agent with predominantly beta-adrenergic activity and has a selective action on β_2 receptors. Salbutamol, a bronchodilator, may be inhaled, given by mouth or injected [19]. A molecule of salbutamol contains three oxygen and one nitrogen as donor atoms and thus was expected to form complex with reduced ^{99m}Tc.

Keeping in view the need for development of a safe, convenient, stable and particularly cheep radiopharmaceutical, salbutamol, closely resembling the structure of dopamine, has been labeled with ^{99m}Tc for rCBF SPECT studies. The aim of the present study is to develop a sensitive and stable nuclear neural diagnostic agent with optimum imaging ability which would help to monitor the cerebral flow disorders. This article describes the details of a recently developed ^{99m}Tc-salbutamol brain perfusion agent.

Experimental

1 Material and Methods

Salbutamol was a gift from Glaxo Laboratories. All the chemicals used in this study were of analytical grade and were procured from commercial sources. ^{99m}Tc generator was imported from Amersham International Ltd. UK The equipment used was composed of gamma camera GCA 40A interfaced to a nuclear medicine computer (Toshiba Japan) for imaging studies; freeze-dryer, consol 12, The VirTis Co. NY for preparation of lyophilized kits; dose calibrator, Nuclear Associates, UK for assessment of radioactivity and Albino rabbits (female) for biodistribution studies. Chromatographic separations were carried out on Whatman No. 1 paper. Round bottom glass developing chambers were employed to develop radiochromatograms. Binding efficacy of ^{99m}Tc-salbutamol complex was determined by ascending chromatography using appropriate solvents. Well-type gamma counter by Thorn EMI UK was used for radioactivity counting.

2 Radiolabeling

To a 1 ml solution, containing 5 mg salbutamol and 2 mg of ascorbic acid, was added a clear solution (10 µl) of $SnCl_2.2H_2O$ (100 µg) in distilled water. The pH of the solution was adjusted to 8.5, mixed gently with 10 mCi (370 MBq) of ^{99m}Tc elute and incubated at room temperature for 15 minutes. The resulting solution was passed through 0.22 µ filter and radiolabeled fraction was quantified using paper chromatography.

3 Quality Control

Following preparation of ^{99m}Tc-labeled salbutamol, the radiochemical purity of the final product (^{99m}Tc-labeled salbutamol) was determined using paper chromatography. The radiochromatograms were developed in a circular development chamber for a period of 10-15 minutes using chloroform and acetone as eluents. The paper was removed from the development tank and 2 cm segments were cut and radioactivity was measured using gamma counter. All kits used had radiochemical purity greater than 97%. The results of radiochromatography, obtained from kits up to 4 hr post reconstitution, were identical to those obtained at injection time and confirmed the stable and pure labeling of salbutamol with ^{99m}Tc.

4 Biodistribution studies

The distribution of ^{99m}Tc-salbutamol was evaluated in four 1-2 kg female rabbits that were allowed food and water. One mCi (37 MBq) of ^{99m}Tc-salbutamol complex was injected in the marginal ear vein of the rabbit. Immediately after i.v. injection, gamma camera was started for

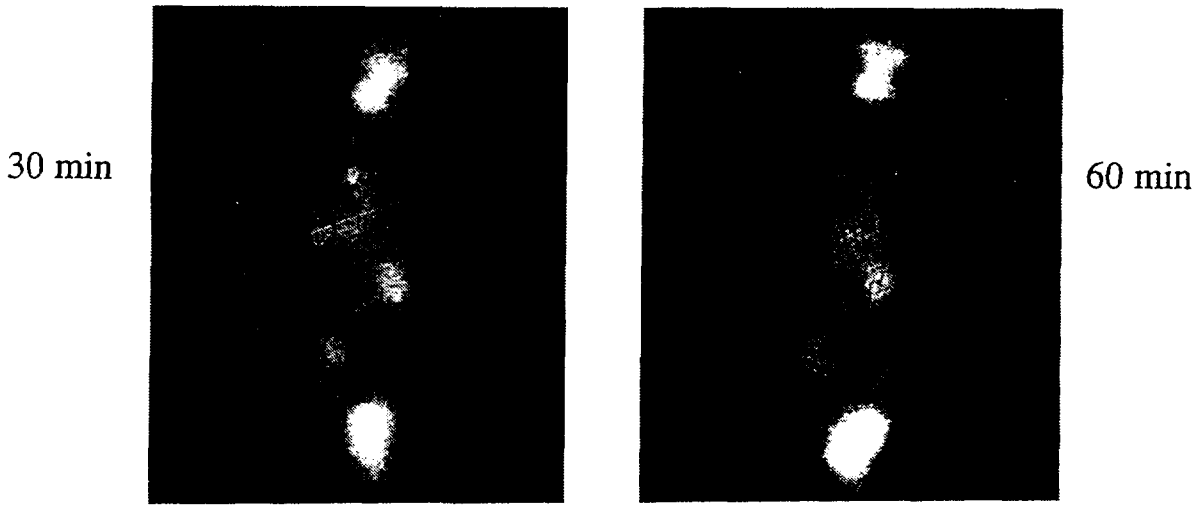

FIG. 1. Posterior views of Rabbit, after intravenous injection of ^{99m}Tc-salbutamol at 30 min and 60 min post injection time intervals, showing uptake in brain.

dynamic study and the static images were taken after 15 min, 30 min and 90 min using computerized gamma camera. Organ uptake at different time intervals was calculated using computer interfaced to gamma camera and detailed biodistribution studies are in progress. Preliminary results of biodistribution studies, indicating a fair uptake in the brain, are in depicted in Fig. 1.

Discussion

^{99m}Tc-labeled propyleneamineoxime (^{99m}Tc-PnAO), initially developed at the University of Missouri [20, 21], was found to be a stable neutral lipophilic chelate. ^{99m}Tc-PnAO freely diffuses across the BBB with a high first-pass extraction efficiency that is similar to ^{123}I-IMP. However, it was not retained in the brain long enough for brain SPECT studies.

It was recognized that simple N_4 ligand chemistry could be utilized to prepare a number of small neutral, lipophilic compounds that could potentially be more useful as rCBF agents. A series of this type of agents was synthesized and screened at Amersham International. UK [22]. One of these agents, HMPAO (4,8-diaza-3,6,6,9-tetramethyl-undecance-2-10, dione-bisoxime) has been found to have high cerebral extraction and long retention in the brain. These properties make it quite suitable for brain SPECT studies [15]. Initial clinical brain imaging studies with these neutral lipophilic chelates have been reported [23]. HMPAO has two stereoisomers due to two asymmetric carbon atoms (at the 3 and 9 positions), and these two isomers have dramatic differences in their cerebral retention characteristics [16]. The meso form is cleared rapidly from the brain; however, d, l-HMPAO demonstrated excellent cortical retention. ^{99m}Tc-d,l-HMPAO is somewhat unstable in aqueous solution [17]. Thus, it is recommended that the product be used within 30 min of preparation. Causes of this instability and ways to correct it are being explored.

Diamine dithiol (N_2S_2) ligands are known to form single, stable, neutral lipophilic complexes with ^{99m}Tc. Several derivatives have been synthesized and evaluated. Among them, ^{99m}Tc-N-piperidinylethyl-diaminodithiol (NEP-DADT) and methyl-NEP-DADT have given promising results in experimental studies [24]. In humans, the most promising results have been obtained with ^{99m}Tc-ethyl cysteinate dimer (ECD; DuPont, Billerica, Mass.). Only the l,l-ECD distereoisomer is retained in the brain whereas ^{99m}Tc d,d-ECD is not. ^{99m}Tc l,l-ECD crosses the BBB and rapid conversion of the lipophilic chelate to a hydrophilic form takes place inside the cells, it cannot diffuse back and is retained in the brain for a long period of time ($T_{1/2}$> 24h) [25].

QNB(R)-3quinuclidinyl-4-iodo-benxilate has been labeled with ^{123}I with high specific activity [26]. Radioiodinated QNB has been used to image the receptor distribution in normal subjects and in patients with Alzheimer's disease [27]. The work on ^{123}I-epidepride, presented by Komhuber et al. 1995 [28], is an important effort to improve SPECT imaging of neuroreceptors. Recently, Leslie [2] has compared ^{123}I-IBZM and ^{123}I-epidepride for SPECT imaging of dopamine D_2 receptors and showed that latter provided higher quality images of striatum with enhanced target-to-background ratios. Further evaluation of the clinical utility of these agents is awaited. Radioiodinated (^{123}I-IMP, ^{123}I-IBZM and ^{123}I-epidepride) as well as ^{99m}Tc-labeled (^{99m}Tc-HMPAO, ^{99m}Tc-ECD) agents, that are excellent for brain perfusion studies, are now available.

It is important to note that the use of ^{123}I-labeled radiopharmaceuticals is limited to the countries where the cylotrons are part and parcel of the nuclear medicine establishments because ^{123}I is cyclotron produced and due to short half life ($T_{1/2}$ = 13 hr) it is not feasible to import this radionuclide. Several countries such as Pakistan don't have even single cyclotron facility in the country. As cyclotron is an expensive technology, the use of ^{123}I-labeled radiotracers for brain perfusion studies is not expected in the near future in developing countries. Furthermore, the cold kits ready to be labeled with ^{99m}Tc are quite expensive and it is not feasible for nuclear medicine departments in Pakistan to use these brain perfusion agents on regular basis for brain SPECT studies. Salbutamol is a cheep drug and animal studies have revealed encouraging results. In case it works well in human studies, it would be possible to use ^{99m}Tc-salbutamol as a brain perfusion agent in developing countries.

REFERENCES

[1] Lassen,N.A. Hoedt-Rasmussen, K., Sorensen, S. et al. Regional cerebral blood flow in man determined by krypton. Neurology. 13 (1963) 719-727.

[2] Leslie, W.D., Abrams, D.N., Greenberg, C.R. and Hobson, D. Comparison of iodine-123-epidepride and iodine-123-IBZM for dopamine D2 receptor imaging. J. Nucl. Med. 37 (1996) 1589-1591.

[3] Ishizu, K., Yonekura, Y., Magata, Y., Okazawa, H., Fukuyama, H., Tanaka, F., Hatorri, N., Kitano, H., Fujita, T., Tanaka, N. and Konishi, J. Extraction and retention of Technetium-99mTc-ECD in human brain: Dynamic SPECT and Oxygen-15-water PET studies. J. Nucl. Med. 37 (1996) 1600-1604

[4] Jacobs, A., Put ,E., Ingels, M., Put ,T. and Bossuyt, A. One year follow up of Technetium-99m-HMPAO SPECT in mild head injury. J. Nucl. Med. 37 (1996) 1605-1609.

[5] Kulkarni, P.V. Recent developments in ^{99m}Tc and 123I-radiopharmaceuticals for SPECT imaging. Nucl. Med. Biol. 18 (1991) 647-654.

[6] Kuhl, D.E., Barrio, J.R., Huang, S-C., Selin, C., Ackermann, R.F., Lear, J.L., Wu, J.L., Lin, T.H. and Phelps, M.E. Quantifying local cerebral blood flow by N-isopropyl-p-[123I]iodoamphetamine (IMP) tomography. J. Nucl. Med. 23 (1982) 196-203.

[7] Baldwin, R. M. and Wu -L. In vivo chemistry of iofetamine HCI iodine-123 (IMP). J. Nucl Med. 29 (1988) 122-124.

[8] Royal, H.D., Hill, T.H. and Holmes, B.L. Clinical brain imaging with isopropyl-iodoamphetamine and SPECT. Semin. Nucl. Med. 15 (1985) 357-376.

[9] Kung, H. F., Tramposch, K.M. and Blau, M. A new brain perfusion imaging agent:[123I]HIPDM: N, N,N'-trimethyl-,'-[2-hydroxy-3-methyl-5-iodobenzyl]-1,3-propanediamine. J. Nucl Med. 24 (1983) 66-72.

[10] Holman, B. L., Lee, R. G. L., Hill, T.C., Lovett, R.D. and Lister-James, J. A comparison of two cerebral perfusion tracers N-isopropyl 123I p-iodoamphetamine and I-123 HIPDM, in the human. J. Nucl. Med. 25 (1984) 25-30.

[11] Lee R, G. L., Hill ,T. C., Holman, B. L., Royal, H. D., O' Leary, D. H., and Clouse, M. E. Predictive value of perfusion defect size using N-isopropyl-(I-123)-p-iodoamphetamine emission tomography in acute stroke. J. Neurosurg. 61 (1984) 449-452.

[12] Lucignani, G., Nehlig, A., Blasberg, R., Patlak, C.S., Anderson, L., Feischi, C., Fazio, F. and Sokoloff, L. Metabolic and kinetic considerations in the use of [123I]HIPDM for a quantitative measurement of regional cerebral blood flow. J. Cereb. Bld. Flow Metab. 5 (1985) 86-96.

[13] Cohen, M. B., Graham, S., Lake, R., Metter, E. J., fitten, J., Kulkarni, M. K., Sevrin, R., Yamada, L., Change, C.C., Woodruff, N. and Kling, A.S. Diagnosis of Alzeimer's disease and multiple infarct dementia by tomographic imaging of iodine-123 IMP. J. Nucl. Med. 27 (1986) 769-774.

[14] Blau, M. Radiotracers for functional brain imaging. Semin. Nucl. Med. 15 (1985) 329-334.

[15] Neirinckx, R.D., Canning, L.R., Piper, I.M., Nowotnik, D.P., Picket, R.O., Holmes, R.A., Volkert, W.A., Forster, A.M., Weisner, P.S., Marriott, J.A. and Chaplin, S.B. Technetium-99m d,l-HMPAO: a new radiopharmaceutical for SPECT imaging of regional cerebral blood flow. J. Nucl. Med. 28 (1987) 191-202.

[16] Sharp, P.F., Smith, F.W., Gemell, H.G., Lyall, D., Evans, N.T.S., Gvozdanovic, E.D., Davidson, J., Tyrell, D.A., Pickett, R.D. and Neirinckx, R.D. Technetium-99m HMPAO stereoisomers as potential agents for imaging regional cerebral blood flow: human volunteer study. J. Nucl. Med. 27 (1986) 171-177.

[17] Hung, J.C., Corlija, M., Volkert, W.A. and Holmes, R.A. Kinetic analysis of Technetium-99m d,l-HMPAO decomposition in aqueous media. J. Nucl. Med. 29 (1986) 1568-1576.

[18] Chessman, E. H., Blanchette, M. A, Ganey, M. V., Meheu, L. J., Miller, S. J. and Watson, A. D. Technetium-99m ECD: ester-derivatized diamine-dithiol Tc complexes for imaging brain perfusion. J. Nucl. Med. 29 (1988) 788 .

[19] Price, A.H. and Clissold, S.P. Salbutamol in 1980's: a reappraisal of it's clinical efficacy. Drugs 38 (1989) 77-122.

[20] Troutner, D.E., Simon, J., Ketring, A.R., Volkert, W.A., Holmes, R.A. Complexing of ^{99m}Tc with cyclam: concise communication. J. Nucl. Med. 21 (1980) 443-448.

[21] Volkert, W.A., Hoffman, T.J., Seger, R.M., Troutner, D.E. and Holmes, R.A. ^{99m}Tc-propylene amine oxime (^{99m}Tc-PnAO): a potential brain radiopharmaceutical. Eur. J. Nucl. Med. 9 (1984) 511-516.

[22] Chaplin, S.B., Oberle, P.A. McKenzie, E.H., Hoffman, T.J., Piper, I.M., Volkert, W.A., Holmes, R.A., Canning L.R., Picket, R.D., Nowotnik, D.P. and Neirinckx, R.D. Regional cerebral uptake and retention of ^{99m}Tc-tetramethyl- and pentamethylpropyleneamine oxime chelates. Nucl. Med. Biol. 13 (1986) 261-267.

[23] Podreka, I., Suess, E., Goldenberg, G. Initial experience with Technetium-99m HMPAO brain SPECT. J. Nucl. Med. 28 (1987) 1657-1666.

[24] Bok, B. D., scheffel, U., Goldfarb, H. W., Burns, H. D., Lever, S. Z., Wong, D.F. and Wagner, H. N. Jr. Comparison of ^{99m}Tc complexes (NEP-DADT, ME-NEP-DADT, and HMPAO) with 123I-IMP for brain SPECT imaging in dogs. Nucl. Med. Commun. 8 (1987) 631-641.

[25] Liveille, J., Demonceau, G., DeRoo, M., Rigo, P., Taillefer, R., Morgan, R. A., Kupranick, D. and Walovitch, R. C. Characterization of technetium-99m-L,L-ECD for brain perfusion imaging, Part 2: biodistribution and brain imaging in humans. J. Nucl. Med. 30 (1989) 1902-1910.

[26] Gibson, R. E., Schneidau, T. A., cohen, V. I., Sodd, V., Ruch, J., Melograna, J., Eckelman, W. C. and Reba, R. C. In vitro and in vivo characteristics of [iodine-125]3-(R)-quinuclidinykl(S)-4-iodobenzilate. J. Nucl. Med. 30 (1989) 1079-1087.

[27] Holman, B. L., Gobson, R. E., Hill, T. C. et al. Muscarinic acetylcholine receptors in Alzheimers' disease: in vivo imaging with iodine 123I-labeled 3-quinuclidinyl-4-iodobenzilate and emission tomography. J. Am. Med. Ass. 254 (1985) 3063-3066.

[28] Komhuber, J., Brucke, T., Angelburger, P., and Podreka, I. SPECT imaging of dopamine receptors with [123I]-epidepride: characterization of uptake in human brain. J. Neurol. Trans. 101 (1995) 95-103.