Research Article

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Iodine-125-terbutaline (^{125}ITB): a new β_2-adrenoceptor probe for lung imaging

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Development of a selective β_2-adrenoceptor tracer for single photon emission tomography is important for imaging of the lungs. Iodine-125-terbutaline (^{125}ITB) was prepared by the reaction of terbutaline with iodine-125 in the presence of chloramine-T as the oxidizing agent. The reaction was completed by incubation of the reaction mixture at 70°C for 15 minutes at pH 7. The biodistribution study in mice indicated the ability of the tracer to bind β-adrenoceptors in lungs, liver, heart, and spleen. The localization of the tracer in the lungs was high (85%/g at 60 minutes post-injection) and the highest lung/blood/g ratio was 19.8% at 60 minutes post-injection. The selectivity of the tracer for the β_2-adrenoceptor was examined by blocking with both β_1 and β_2-adrenoceptor selective antagonists. The results showed reduction of both heart and lung uptake of the ^{125}ITB tracer, indicating a moderate (not absolute) selectivity for the β_2-adrenoceptor.

Keywords: terbutaline; Iodine-125; biodistribution; lungs imaging; radiolabelling; I-125

Introduction

Radionuclide imaging is one method by which physiological aspects of lung function are evaluated. The most widely used techniques are the following: lung perfusion imaging using technetium-99 m macroaggregates of albumin labeled perfusion agents and lung ventilation imaging using either radioactive gasses such as ^{133}Xe, ^{127}Xe, ^{81m}Kr, ^{13}N, or technetium-99 m labeled aerosols. The clinical indications are mainly the diagnosis of pulmonary embolism and the semi-quantitative assessment of the left–right distribution of pulmonary perfusion before performing major lung surgery such as lung transplantation or pulmonary volume reduction surgery in emphysema patients.[1]

A number of different imaging modalities can be employed to analyze ventilation and perfusion, including nuclear scintigraphy,[2] single photon emission computed tomography (SPECT),[3–5] magnetic resonance imaging (MRI),[6] and computed tomography (CT).[7,8] Positron emission tomography (PET) is another nuclear medicine imaging modality. It uses specially chosen positron emitters. The most commonly used radionuclide in PET is ^{18}F.[9,10]

The β-adrenergic receptors are present on the surface of mammalian cells. These receptors are stimulated physiologically by the neurotransmitter, norepinephrine, and the adrenal medullary hormone, epinephrine. There are three subtypes of adrenergic receptors, namely, β_1, β_2, and β_3; the pharmacological and physiological responses of an individual cell result from the particular mixture of the three β-adrenergic receptor subtypes present on that cell. Species-specific structure (amino acid sequence) also causes modification of the function of a given β-adrenergic receptors subtype.[11]

The structures of the stimulants closely mimic the structure of the neurotransmitters and are thus able to interact with the receptor site. Adrenergic stimulants may have three modes of action: direct interaction with specific receptors (examples are epinephrine and phenylephrine); indirect action by stimulating release of neurotransmitters; or a mixed action involving both of the aforementioned examples (examples are phenylpropanolamine and ephedrine).[12]

Lung function in the sense of gas exchange is determined by the regional matching of ventilation and perfusion. In addition, it is also controlled by the endothelial receptor systems, enzyme function, and metabolic processes. Neural control of airway smooth muscle resulting in contraction and relaxation is an important determinant of airway caliber in health and disease.[13] Several neurotransmitters in airway nerves and receptors on airway smooth muscle cells have been identified. One of them is β-adrenoceptors (β_2,bronchodilatation), which modulate their action via stimulation by β_2 agonists, including terbutaline, resulting in activation of adenylate cyclase via the stimulatory Gs protein followed by the formation of the second messenger, cyclic adenosine monophosphate (AMP).[14] Cyclic AMP accumulation leads via a cascade of processes to relaxation of smooth muscle cells in the wall of the airway. Such relaxation seems to be impaired in humans with pulmonary dysfunction, for example, in patients suffering from asthma. In addition, β-adrenoceptors are involved in numerous functions such as mucous secretion, ion transport across airway epithelium, and permeability of pulmonary blood vessels.

Several β-adrenoceptors ligands have been labeled with iodine-123 for SPECT imaging and with fluorine-18 and carbon-11 for PET imaging.[15–19] Radioactive tracer techniques are applied not only for diagnostic purposes but also for research and better understanding of normal physiology and pathology.

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The high incoming cost and isotopes unavailability for routinely producing receptor-specific imaging agents with these isotopes provide the impetus for similar agents labeled with the more widely available iodine.

Accordingly, there is an urgent need for improved PET and SPECT radioligands that home to specific tissue and allow detection and diagnosis. Because the chemistry and the pharmacologic effects of beta-adrenergic agonists have been well-documented, we have chosen one of these drugs (terbutaline - Figure 1) that selectively bind to and activate beta-adrenergic receptors as the compound for radio-iodination with the radioisotope ^{125}I, which is commercially available as a label for molecular probes and utilized by researchers in small animal studies.[20]

In this work, the factors affecting the radio-iodination of terbutaline were investigated to obtain a high radiochemical yield of radio-iodoterbutaline, and the biodistribution of the labeled product was studied.

Experimental

Materials

All chemicals and laboratory reagents used during this work were of the highest purity grade. In all cases, the water used was double distilled. Terbutaline and chloramine-T (CAT) (*N*-chloro-*p*-toluene sulfonamide sodium salt, CAT) were purchased from Sigma-Aldrich, Germany. Iodine-125 as no carrier added solution ($Na^{125}I$ (185 MBq/5 mL) in diluted NaOH, pH 7–11) was purchased from Institute of Isotopes, Budapest, Hungary. Animals, Male Swiss Albino mice weighing 25–30 gm were purchased from the Research Center, Cairo, Egypt.

Method

Preparation of labeled terbutaline

The iodination process was achieved by using CAT as oxidizing agent. All experiments were carried out in a convenient round bottom flask that could be tightly closed by rubber stopper. In the flask, 10 μl $Na^{125}I$ (7–14 MBq) were added and dried by a vacuum line, then a specific concentration of oxidizing agent in 100-μl double distilled water and a specific concentration of the terbutaline in 100-μl suitable medium (0.05 M phosphate buffer pH7) were added. The flask was immersed in a thermostatically controlled water bath. The reaction mixture was heated to specific temperature within suitable time. The pH value of the reaction mixture (pH 2–11) was varied using different buffer systems. After a specified interval of time, the reaction was quenched by using 100-μl [0.2 N] sodium metabisulphate ($Na_2S_2O_3$) solutions to ensure that the unreacted iodine is reduced before chromatographic analysis. The total reaction volume (210 μl) is constant in all experiments. The different parameters that affect the radiochemical yield of ^{125}ITB were investigated. In the process of labeling, trials and errors were performed for each factor under investigation till obtaining the optimum value. The experiment was repeated with all factors kept at optimum values except the factor under study until the optimal conditions were achieved.

Determination of radiochemical yield and purity

The radiochemical yield of the labeled terbutaline was determined using aluminum-backed silica gel-60 sheet (20 × 20 cm). It was cut into 1 × 13-cm strips. The strips were previously impregnated with $Na_2S_2O_3$ to inhibit the oxidation of radio-iodide to a volatile form. A volume of 5 μl of the reaction mixture was spotted on the start line, then the strip was chromatographed using n-butanol: acetic acid: water (4:1:1, v/v/v) as a developing system. The strips were removed, dried, cut into 1-cm segments, and assayed for radioactivity using gamma counter connected with a well-type NaI (Tl) crystal. The free iodide remains at the origin with $R_f = 0.1–0.2$, whereas the labeled terbutaline migrates with the solvent front with $R_f = 0.8–1.0$.

$$\text{Radiochemical yield}, \% = \frac{\text{Activity of labeled product} \times 100}{\text{Total activity}}$$

HPLC analysis

The radiochemical purity of ^{125}ITB was determined by direct injection of 5–10 μl, of the reaction mixture at the optimum conditions for obtaining the highest radiochemical yield, into the column (RP18 – 250 × 4 mm, 5 μm, Lischrosorb) built in HPLC Shimadzu model consisting of pumps LC-9A with a Rheohydron injector and U.V. spectrophotometer detector (SPD-6A) adjusted to the wave length 254 nm. The column was eluted with the isocratic solvent using methanol: H_2O (70: 30 v/v) as a mobile phase and the flow rate was adjusted to 1 ml/minute. The labeled compound was collected by using a fraction collector and its activity was counted by using a well-type NaI (Tl) crystal connected with single channel analyzer.

Radiochemical yield was determined by thin layer chromatography, and radiochemical purity was determined by HPLC.

Preparation and structure confirmation of non-radioactive iodoterbutaline

The non-radioactive iodoterbutaline is prepared by the same iodination method on a larger scale (to provide enough iodoterbutaline) to allow isolation and characterization. Unlabeled iodoterbutaline is characterized by proton NMR.

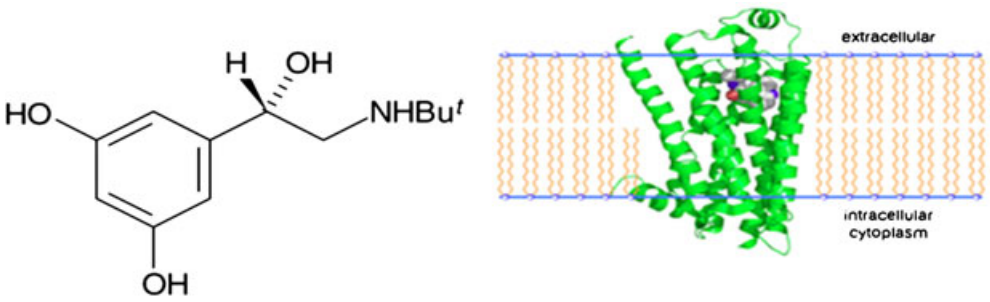

Figure 1. The chemical structure of terbutaline and adrenergic beta-2-receptor, surface crystallographic structure of the β_2-adrenergic receptor[21,22].

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^{1}HNMR spectrum of iodoterbutaline in (DMSO-d6) revealed signals at δ (ppm) = 5.99(m, 2H, benzene), 5.0(2H, OH. aromatic), 4.74(t,1H, CH. methine), 2.90–3.15(d,2H, CH_2.methylene), 2.0(1H, NH. amine), 2.0(1H,OH. alcohol), 1.10(3S, 9H, 3(CH_3) methyl)) as shown in Figure 2.

The resolution of preparative and analytical techniques was insufficient, so the uncertainty in the identity of the iodoterbutaline may affect the purification of the labeled compound from any iodo-isomers.

Biodistribution in mice

Three mice per group were used for each biodistribution study. A solution of 0.1 ml containing 2.5–3 MBq of radioactive tracer was injected into the tail vein. The mice were sacrificed at the time indicated by cardiac excision under ether anesthesia. Organs of interest were removed, weighed, and the radioactivity was counted. The results were expressed as percent injected dose per organ (%ID/organ) and percent injected dose per gram tissue (%ID/g) and were calculated by a comparison of the tissue counts to the standard solution of the labeled terbutaline measured at the same time.

The selectivity of ^{125}ITB for the β_1 and β_2 receptor subtypes was investigated by performing in vivo competitive binding experiments. The mice were injected with 2 mg/kg, I.V. atenolol (a $\beta_1$selective blocking agent) or 2 mg/kg and I.V. butoxamine (a β_2 selective blocking agent) at 30 minutes prior to the injection of ^{125}ITB. The animals were sacrificed and the distribution of radioactivity calculated as described earlier.

Results and discussion

Effect of substrate amount

This experiment was carried out by adding the iodine activity (10 MBq) to a mixture of terbutaline (20, 50, 100, 200, and 300 μg) in 100-μl phosphate buffer pH7 and 150-μg CAT. The reaction mixture was incubated in a water bath at 70°C for 15 minutes. The data of this experiment is presented in Figure 3, which clearly pointed to the possibility of using terbutaline in the range of 100–300 μg without significant change in the radiochemical yield of ^{125}ITB. This may be attributed to the fact that the yield reaches the saturation value because the entire generated iodonium ions in the reaction are captured at these concentrations of terbutaline. Below 100 μg of terbutaline, the radiochemical yield decreased to below 80%.

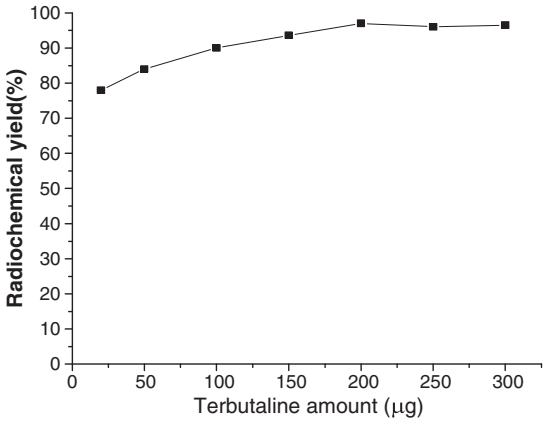

Figure 3. The radiochemical yield of ^{125}ITB as a function of substrate amount.

Effect of CAT amount

Because of its efficiency, CAT is the most widely used oxidizing agent in the labeling of organic compounds with iodine.[23,24] CAT is used in the range of 25–250 μg in this labeling reaction. The data presented in Figure 4 indicated the inability of 25 μg of CAT to oxidize all the iodine found in the reaction mixture, and as a result, the free iodine was detected in high percent (21.5%). These results may be explained by the fact that at low concentration of CAT, not all the iodide is converted to iodonium ion which is involved in the substitution reaction and thus the

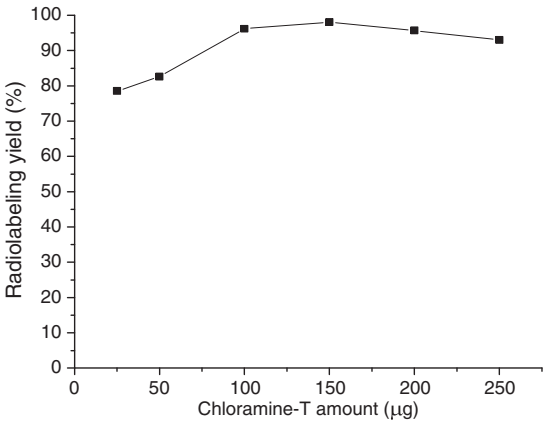

Figure 4. The radiochemical yield of ^{125}ITB as a function of chloramine-T amount.

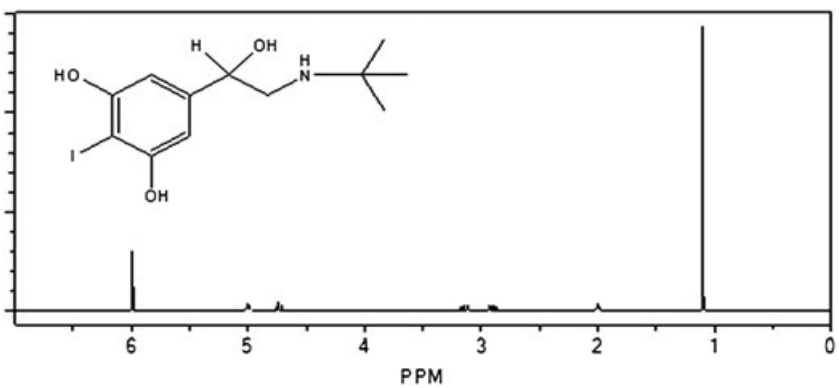

Figure 2. ^{1}HNMR spectrum of iodo-terbutaline.

yield decreased. Increasing the amount of CAT gradually caused an increase in the radiochemical yield up to 97% at 150 μg of oxidizing agent. As mentioned, 150 μg of oxidizing agent no significant increase in the radiochemical yield of ^{125}ITB was obtained. Excess of oxidizing agent is not recommended in this labeling reaction to avoid a number of undesirable side reactions including chlorination.[25–27]

Effect of reaction time

The experiment was carried out by adding (10 MBq) Na^{125}I to a mixture of 200-μg terbutaline and 150-μg CAT in phosphate buffer at pH 7 in a closed vial. The reaction mixture was kept at 70°C for different intervals of time up to 60 minutes. The gathered data is presented in Figure 5, which clearly shows that the optimum reaction time required to attain the maximum radiochemical yield is 15 minutes. Increasing the reaction time to 60 minutes does not affect the radiochemical of yield ^{125}ITB.

Effect of reaction temperature

The reaction temperature in most electrophilic substitution reactions plays an important role, as it is very important in the buildup of a new covalent bond between the iodonium ion and the carbon atom in the molecule. The radio-iodination reaction of terbutaline with iodine-125 was carried out using 200-μg terbutaline and 150-μg CAT at different temperatures, Figure 6. The radiochemical yield of ^{125}ITB was found to be 45.3% at 25°C and increased to 77.1% and 97% on heating the reaction mixture to 50 and 70°C, respectively at 15 minutes reaction time. This is because of the fact that the leaving hydronium ion requires some energy to break the C–H bond and to initiate the introduction of the radioactive iodonium ion into the TB ring. By raising the reaction temperature to 100°C, the radiochemical yield of ^{125}ITB decreased indicating the decomposition of the labeled compound.

Effect of pH

This experiment was carried out using different buffer systems to obtain the required pH values; citrate buffer for pH 2 and 4, phosphate buffer for pH 7 and 9, and bicarbonate buffer for pH 11. The optimum amount of terbutaline was added to the buffer system and then 150-μl CAT (150 μg) followed by Na^{125}I. The reaction mixture was heated to 70°C for 15 minutes. The results indicate the effectiveness of pH of the reaction mixture on the labeling yield. The radiochemical yield of ^{125}ITB was relatively poor at pH 2 and 4, as a result of the predominance of ICl species, which have low oxidation potential than HOCl species.[28] At pH 7, the radiochemical yield of ^{125}ITB reaches a maximum value of 97%. When the pH increased towards the alkaline side (9 and 11), the radiochemical yield decreased. This may be attributed to the decrease in HOI*, which is responsible for the electrophilic substitution reaction[29]. The data is summarized in Figure 7. The high radiochemical yield of ^{125}ITB at pH 7 may be because of the efficiency of CAT at this pH value.[30]

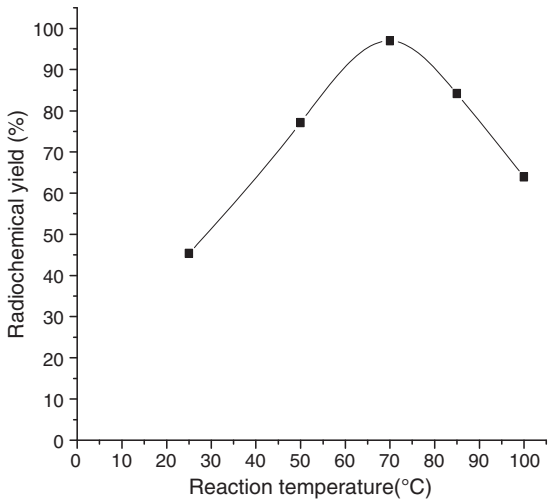

Figure 6. The radiochemical yield of ^{125}ITB as a function of reaction temperature.

HPLC analysis

A radiochromatogram for the iodination of terbutaline obtained after HPLC separation on RP-18 column at the optimum conditions (200-μg terbutaline in 100-μl phosphate buffer pH 7, 150 μg of CAT, and the reaction mixture was heated at 70°C for 15 minutes) is shown in Figure 8. Two peaks were obtained. The first peak was at 3 minutes, whereas the second peak was at 6 minutes retention time. The first peak corresponds to free iodide, whereas the second peak corresponds to the ^{125}ITB. ^{125}ITB is not completely separated from terbutaline or any iodo-isomers in Figure 8 that clarifies the short retentions. The eluted fractions containing the labeled compound are pooled

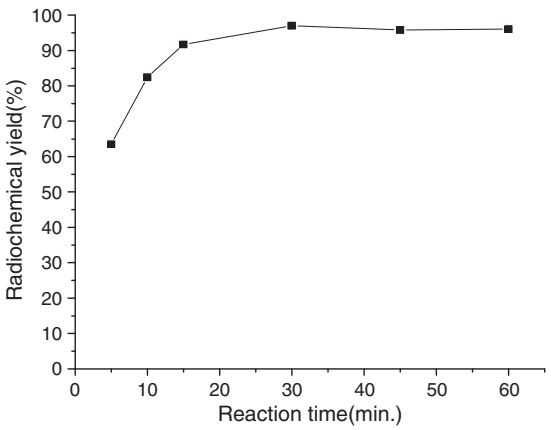

Figure 5. The radiochemical yield of ^{125}ITB as a function of reaction time.

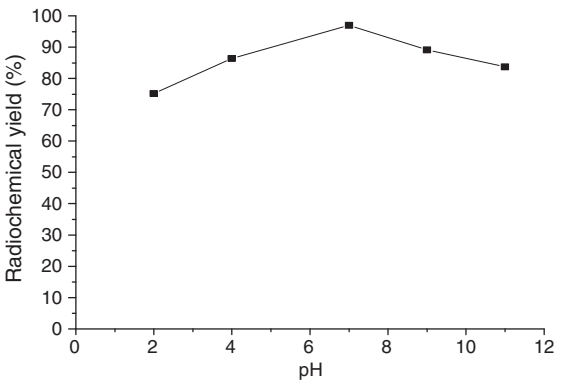

Figure 7. Effect of pH of the reaction medium on the percent radiochemical yield of ^{125}ITB.

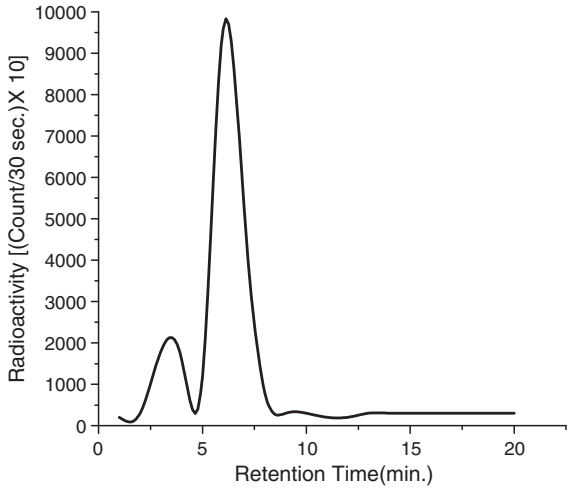

Figure 8. HPLC radiochromatogram of ^{125}I-terbutaline.

together, and the radiochemical purity of the isolated ^{125}ITB (99.1%) was determined using thin layer chromatography. These collected fractions were evaporated to dryness. The residue was dissolved in physiological saline and then sterilized by filtration through 0.22-μm Millipore filter to give specific activity of 15 MBq/mg of ^{125}ITB. The^{125}ITB is then suitable for use in biodistribution studies.

Biodistribution studies

The uptake of radioactivity in selected tissues was determined following intravenous administration of ^{125}ITB to albino mice. Mice were injected intravenously with 2.5–3 MBq of ^{125}ITB and sacrificed at designated time intervals (30 minutes, 1 hour, 2 hours, and 4 hours post-injection). The complete biodistribution results are presented in Table 1. The high initial ^{125}ITB uptake in kidney that reaches a maximum at 60 minutes before decreasing at 120 and 240 minutes is an indicative of the renal clearance. Although initial blood levels of radioactivity were moderately high (8.4% ID/g at 30 minutes), this tissue displayed ~40% radioactivity clearance between 30 and 60 minutes. Organs of interest, liver, spleen, heart, and lungs (organs that contain β-adrenoceptors) showed high accumulation of radioactivity 31.5%, 6.9%, 3.3%, and 60.5%/g at early time post-injection (30 minutes), respectively. Uptake in organs with different dominant receptor subtypes is relevant to receptor density and ratio of receptor subtypes. The lungs that are the main target of ^{125}ITB showed high binding percentage and reached its maximum at 60 minutes post-injection (85%ID/g) because β-adrenoceptors density in human peripheral lung is normally 100–130 fmol/mg protein and the ratio of the β_1: β_2 is about 30:70.[31] The built-up of radioactivity in the spleen, as time passed post-injection, was because of the dominance of β_2 subtype adrenoceptor,[32] whereas the less heart uptake was attributed to the dominance of β_1 subtype in the heart.[33] Blocking studies with atenolol and butoxamine were performed to determine a better selectivity of terbutaline to β_2-adrenoceptor.

Moderate radioactivity uptake was seen in thyroid (maximal uptake of 1.3% ID/g at 4 hours) indicating that the radioligand is relatively stable towards in vivo de-iodination.

Blocking studies

The ^{125}ITB binds to β_1 and β_2-adrenoceptors, then the uptake of the tracer may be non-selective which leads the need for in vivo blocking experiments to determine selectivity. The use of β_1 selective blocking agent (atenolol) diminished myocardial and pulmonary uptake of the radioligand as clear from Figure 9. In contrast, the use of β_2 selective blocking agent (Butoxamine) reduced the uptake of ^{125}ITB in the lungs (an organ that contains more β_2 receptors) with great rate and slight affect on binding in the heart (an organ that contains more β_1 receptors) as clear

Table 1. Biodistribution of ^{125}I-terbutaline in mice (x ± SD, $n = 3$), expressed as % injected dose/total tissue and injected dose/g tissue

	Time post-injection (minutes)							
	30		60		120		240	
Tissue	%ID/organ	% ID/g	%ID/organ	% ID/g	%ID/organ	% ID/g	%ID/organ	% ID/g
Blood	19.3 ± 2.1	8.4 ± 0.3	9.9 ± 0.4	4.3 ± 0.3	6.3 ± 0.4	2.7 ± 0.2	3.7 ± 0.2	1.6 ± 0.2
Bone	3.4 ± 0.3	1.1 ± 0.2	3.1 ± 0.1	1.0 ± 0.2	2.3 ± 0.2	0.72 ± 0.1	1.6 ± 0.1	0.5 ± 0.1
Muscle	3.6 ± 0.2	0.2 ± --	3.9 ± 0.2	0.3 ± ---	3.1 ± 0.3	0.19 ± --	2.3 ± 0.4	0.14 ± 0.1
Liver*	12.9 ± 1.2	31.5 ± 2.4	12.3 ± 1.1	30.0 ± 2.2	12.0 ± 1.5	29.3 ± 2.4	7.8 ± 0.8	19.0 ± 0.9
Spleen*	0.2 ± --	6.9 ± 0.3	0.39 ± 0.1	13.0 ± 1.3	0.58 ± 0.2	19.3 ± 1.6	0.88 ± 0.1	29.3 ± 2.4
Heart*	0.2 ± --	3.3 ± 0.2	0.3 ± 0.1	5.0 ± 0.4	0.2 ± --	3.3 ± 0.4	0.1 ± --	1.6 ± 0.3
Lungs*	12.1 ± 1.3	60.5 ± 2.5	11.6 ± 0.8	85.0 ± 1.9	9.1 ± 0.6	45.5 ± 2.7	3.6 ± 0.3	18.0 ± 1.2
Kidneys	10.1 ± 0.7	25.3 ± 2.1	26.9 ± 1.4	67.3 ± 2.4	4.7 ± 0.4	11.8 ± 1.2	4.3 ± 0.3	10.8 ± 1.5
Thyroid	0.1 ± --	0.7 ± 0.1	0.6 ± 0.1	4.0 ± 0.3	0.1 ± --	0.7 ± 0.3	0.2 ± --	1.3 ± 0.1
Brain	0.2 ± --	0.5 ± 0.1	0.2 ± --	0.5 ± 0. 1	0.6 ± 0.1	1.5 ± 0.2	1.3 ± 0.1	3.2 ± 0.2
Urine	15.8 ± 2.1	------	21.6 ± 1.7	----	26.3 ± 1.3	-----	38.0 ± 1.4	
L/Bl		7.2		19.8		16.9		11.25
B/Bl		0.06		0.1		0.55		2.0

*Organs of interest L: Lungs; Bl: Blood; B: Brain.

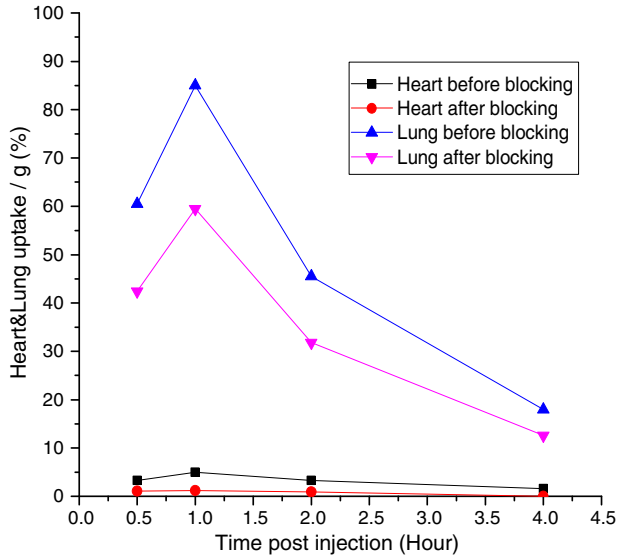

Figure 9. Lung & Heart uptake of ^{125}ITB tracer before and after the IV administration of atenolol (selective β_1 blocking agent) 30 minutes before the administration of ^{125}ITB.

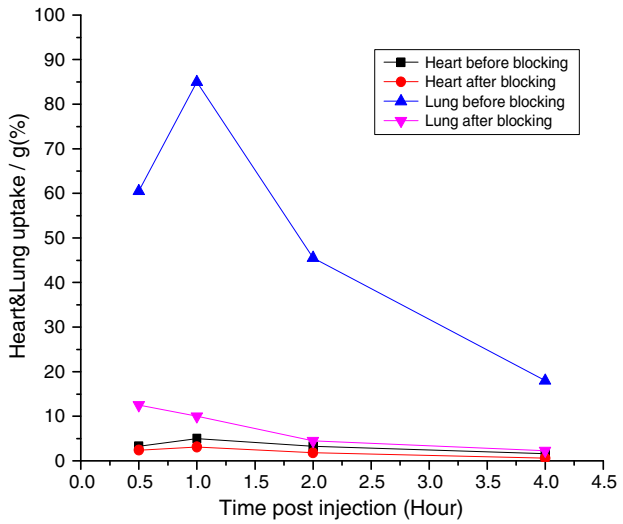

Figure 10. Lung & Heart uptake of ^{125}ITB tracer before and after the IV administration of Butoxamine (selective β_2 blocking agent)30 minutes before the administration of ^{125}ITB.

from Figure 10. The uptake of the heart in comparison with the uptake of the lungs was very low that may indicate the moderate selectivity of the tracer to β_2-adrenoceptor.

Conclusion

The receptor targeting agent molecules are even small in size and are generally organic ligands far from all restrictions keeping in view the need of a safe, convenient, stable, and particularly cheap radiopharmaceutical for lung scanning agent. Iodine-terbutaline is a partial selective radioligand suitable to study lung function and could be prepared easily under normal conditions. Terbutraline has been successfully labeled with radioactive iodine-125. The optimum conditions of the labeling of terbutaline to give a radiochemical yield of 97% were 200-μg terbutaline in 100-μl phosphate buffer pH 7, 150 μg of CAT, and the reaction mixture was heated at 70°C for 15 minutes. Biodistribution studies show uptake in organs known to contain adrenoceptors with high localization in the lungs (85%/g at 60 minutes post-injection). In vivo blocking experiments have indicated the moderate selectivity of the ^{125}ITB to β_2-adrenoceptor. For practical application,^{123}I-labeled terbutaline is used for lung imaging. A more efficient preparative purification system would be needed to ensure that the iodoterbutaline was separated from any iodo-isomers or any unlabeled terbutaline.

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Conflict of Interest

The authors did not report any conflict of interest.

References

[1] G. Zhang, T. J. Dilling, C. W. Stevens, K. M. Forster, Functional Lung Imaging in Thoracic Cancer Radiotherapy, April **2008**, Vol. *15*, No. 2, Cancer Control, p112–119.
[2] A. Gottschalk, H. D. Sostman, R. E. Coleman, et al. Ventilation-perfusion scintigraphy in the PIOPED study. Part II. Evaluation of the scintigraphic criteria and interpretations. *J. Nucl. Med.* **1993**, *34*, 1119–1126.
[3] B. Harris, D. Bailey, S. Miles, et al. Objective analysis of tomographic ventilation-perfusion scintigraphy in pulmonary embolism. *Am. J. Respir. Crit. Care Med.* **2007**, *175*, 1173–1180. Epub 2007 Mar 15.
[4] J. Petersson, A. Sánchez-Crespo, M. Rohdin, et al. Physiological evaluation of a new quantitative SPECT method measuring regional ventilation and perfusion. *J. Appl. Physiol.* **2004**, *96*, 1127–1136. Epub 2003 Nov 14.
[5] K. Suga. Technical and analytical advances in pulmonary ventilation SPECT with xenon-133 gas and Tc-99 m-Technegas. *Ann. Nucl. Med.* **2002**, *16*, 303–310.
[6] N. Ogasawara, K. Suga, Y. Kawakami, et al. Assessment of regional lung function impairment in airway obstruction and pulmonary embolic dogs with combined noncontrast electrocardiogram-gated perfusion and gadolinium diethylenetriaminepentaacetic acid aerosol magnetic resonance images. *J. Magn. Reson. Imaging* **2004**, *20*, 46–55.
[7] T. Guerrero, K. Sanders, E. Castillo, et al. Dynamic ventilation imaging from four-dimensional computed tomography. *Phys. Med. Biol.* **2006**, *51*, 777–791. Epub 2006 Jan 25.
[8] T. R. Johnson, B. Krauss, M. Sedlmair, et al. Material differentiation by dual energy CT: initial experience. *Eur. Radiol.* **2007**, *17*, 1510–1517. Epub 2006 Dec 7.
[9] M. F. Vidal Melo, D. Layfield, R. S. Harris, et al. Quantification of regional ventilation perfusion ratios with PET. *J. Nucl. Med.* **2003**, *44*, 1982–1991.
[10] G. Musch, J. D. Layfield, R. S. Harris, et al. Topographical distribution of pulmonary perfusion and ventilation, assessed by PET in supine and prone humans. *J. Appl. Physiol.* **2002**, *93*, 1841–1851.
[11] H. J. Mersmann. Overview of the effects of beta-adrenergic receptor agonists on animal growth. *J. Anim. Sci.* **1998**, *76*, 160–172.
[12] S. B. Liggett, J. R. Raymond. Pharmacology and molecular biology of adrenergic receptors. *Baillieres Clin. Endocrinol. Metab.* **1993**, *7*, 279–305.
[13] P. J. Brnes, Neural Control of Airway Smooth Muscle" In *The Lung* (Eds.: R. G. Crystal, J. B. West), Raven Publishers: Philadelphia, **1997**, pp. 1269.
[14] A. D. Strosberg. Biotechnology of b-adrenergic receptors. *Mol. Neurobiol.* **1990**, *4*, 211–250.
[15] F. Aigbirhio, V. W. Pike, E. Francotte, K. A. Jaeggi, S. L. Waters, *J. Label. Compd. Radiopharm.* **1992**, *31*, 159.
[16] P. H. Elsinga, A. van Waarde, K. A. Jaeggi, G. Schreiber, M. Heldoom, W. Vaalburg, *J. Med. Chem.* **1997**, *40*, 3829.

[17] P. H. Elsinga, A. van Waarde, P. Doze, P. K. Blanksma, R. M. Pieterman, A. T. M. Willemsan, W. Vaalburg, *J. Nucl. Med.* **1999**, *40*, 87.
[18] T. J. Visser, A. van Waarde, T. W. van der Mark, J. Kraan, P. H. Elsinga, J. Pruim, K. Ensing, T. Jansen, A. T. Willemsen, E. J. Franssen, G. M. Visser, A. M. Paans, W. Vaalburg, *J. Nucl. Med.* **1997**, *38*, 196.
[19] M. S. Berridge, A. D. Nelson, L. B. Zheng, G. P. Leisure, F. Miraldi, *J. Nucl. Med.* **1994**, *35*, 1665.
[20] M. N. Cinti, S. Majewski, M. B. Williams, C. Bachmann, F. Cominelli, B. K. Kundu, A. Stolin, V. Popov, B. L. Welch, G. DeVincentis, M. Betti, R. Pani, Biophys. PhD Sch., Univ. Rome La Sapienza, Italy, Iodine-125 imaging in mice using NaI(Tl)/Flat panel PMT integral assembly, Nuclear Science Symposium Conference Record, **2004** IEEE, 3916–3919 Vol. *6* .
[21] V. Cherezov, D. M. Rosenbaum, M. A. Hanson, S. G. Rasmussen, F. S. Thian, T. S. Kobilka, H. J. Choi, P. Kuhn, W. I. Weis, B. K. Kobilka, R. C. Stevens. High-resolution crystal structure of an engineered human β_2-adrenergic G protein-coupled receptor. *Science* **2007**, *318*(5854), 1258–65.
[22] D. M. Rosenbaum, V. Cherezov, M. A. Hanson, S. G. Rasmussen, F. S. Thian, T. S. Kobilka, H. J. Choi, X. J. Yao, W. I. Weis, R. C. Stevens, B. K. Kobilka. GPCR engineering yields high-resolution structural insights into β_2-adrenergic receptor function. *Science* **2007**, *318* (5854), 1266–73.
[23] W. M. Hunter, F. C. Greenwood, *Nature* **1962**, 194,495.
[24] F. C. Greenwood, W. M. Hunter, J. S. Glover, *Biochem. J.* **1963**, *89*, 114.
[25] R. M. Baldwin, T. H. Lin, *J. Radioanal. Chem.* **1981**, *65*, 163.
[26] C. N. M. Bakker, F. M. Kaspersen, *J. Labelled Compd. Radiopharm.* **1978**, *15*, 681.
[27] C. N. M. Bakker, F. M. Kaspersen, *Int. J. Appl. Radiat. Isot.*, **1981**, *32*, 176.
[28] E. J. Knust, K. Dutschka, H. J. Machulla, *J. Radioanal. Nucl. Chem. Lett.* **1990**, *144*, 107.
[29] B. F. Cynthia, K. D. Roger, A. J. Kaumann, L. S. Theodore, B. Lutz, *J. Mol. Pharmacol.* **1979**, 12,328.
[30] J. C. Saccavini, C. Bruneau, *IAEA. CN.* **1984**, *4519*, 153.
[31] O. E. Brodde, *Pharmacol. Rev.* **1991**, 43,203.
[32] A. F. Lo`pez, V. Revilla, M. A. Candelas, M. I. Aller, C. Soria, A. Pazos. Identification of β-adrenoceptors in lymph nodes and spleen an autoradiographic study. *Euro. J. pharmacology* **1994**, *262*(3), 283–286.
[33] O. E. Brodde, M. C. Michel. Adrenergic and muscarinic receptors in human heart. *Pharmacol. Rev.* **1999**, *51*(4), 651–690.