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Preliminary assessment of radioiodinated fenoterol and reproterol as potential scintigraphic agents for lung imaging

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Abstract Radioiodinated fenoterol and reproterol were prepared by electrophilic radioiodination reaction using chloramin-T as oxidizing agent with radiochemical yields of 97.7 ± 0.7 and 95.2 ± 0.3 %, respectively, and in vitro stability up to 72 h. Biodistribution study performed in male Albino Swiss mice showed maximum radioactivity accumulation in lungs tissue to the extent of 52 ± 1.03 and 50.6 ± 1.2 % ID/g at 15 and 30 min post injection (p.i.) for radioiodinated fenoterol and reproterol, respectively, with low accumulation in heart and blood. The clearance pathway of both iodo-compounds was through renal and hepatobiliary routes. The selectivity of iodo-compounds to lung was examined by in vivo receptor blocking study. Radioiodinated fenoterol and reproterol are not a blood products and so they are more safer than the currently available ^{99m}Tc-MAA, and their lungs uptake is higher than that of the recently discovered $^{125/123}$I-IPMPD, ^{99m}Tc(CO)$_5$I, ^{99m}Tc-DHPM and $^{125/123}$I-paroxetine. So, radioiodinated fenoterol and reproterol could be introduced as a new compromising radiopharmaceuticals for lung perfusion scintigraphy more safe than the currently available ^{99m}Tc-MAA and more potential than the recently discovered $^{125/123}$I-IPMPD, ^{99m}Tc(CO)$_5$I, ^{99m}Tc-DHPM and $^{125/123}$I-paroxetine.

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Introduction

Lung scanning is a test of great value for the detection of blood clot (pulmonary embolism), evaluation of lung function prior to lung surgery and diagnosis of lung tumor [1–3]. There are two types of lung scanning techniques: Ventilation and perfusion scans which are usually done separately or together to evaluate the circulation of air and blood within a patient's lungs [4–7].Ventilation scanning has the ability to evaluate the movement of the air into and out of the bronchi and bronchioles and can be done by inhalation of radioactive gases such as xenon or ^{99m}Tc-DTPA in aerosols [8–14].While perfusion scanning has the ability to produce a picture of blood flow to the lung helping in the diagnosis of blockages in the pulmonary arteries, so detecting pulmonary embolisms. Perfusion scanning can be performed by intravenous injection of the radiotracer which passes through the larger blood vessels and become temporarily trapped in small blood vessels giving rise to images which reflect blood perfusion in the lungs [3, 15]. Macro-aggregated albumin (MAA) labeled with ^{99m}Tc has been established as the most currently used radiopharmaceutical for lung perfusion scanning. But it shows two main disadvantages, the first one is related to its particles size (≈30 microns) which may lead to particles trapping in the pre-capillary arterioles of the lungs after intravenous administration [16, 17]. Besides, MAA is derived from human serum albumin (HSA) which is collected from the pooled blood of human donors. This intensively increases the possibility of contamination by infective agents such as variant Creutzfeldt Jakob disease, hepatitis B, hepatitis C and HIV [18–20]. While recombinant DNA technology is a promising method of albumin production avoiding problems

associated with human-derived HSA, but this would substantially increase the cost of routine imaging [21]. As a result, finding a non-biological material (radiopharmaceutical products) is of very importance to ensure the continued supply of these diagnostic reagents [22]. In recent years, there have been extensive studies on the biological behavior of ^{99m}Tc-carbonyls and other radiotracers as potential lung perfusion agents [23]. $^{125/123}$I-IPMPD, ^{99m}Tc(CO)$_5$I, ^{99m}Tc-DHPM and $^{125/123}$I-paroxetine are recently discovered radiotracers to be used as potential lung perfusion agents showing maximum lungs uptake of 21.4 ± 1.7, 12.8 ± 2.87, 10.12, 27.9 ± 1.0 ID/g at 15, 60, 2 and 15 min post injection, respectively [8, 16, 24, 25]. Although fluorine (^{18}F) derivatives of fenoterol as PET radiotracers could be used for the in vivo visualization and quantification of the β_2-adrenergic receptor status in lung [26, 27], But it shows limitations related to high cost and the availability of fluorine. So, research continues for developing and introducing highly promising new radiotracers of high lung affinity as molecular imaging agents for pulmonary circulation.

As lung tissues are rich in β_2-receptors [28, 29], so radiopharmaceuticals based upon β_2-adrenoceptor selective agents labeled with radioactive isotopes are expected to show high β_2-receptor binding affinity and high radioactivity accumulation in lungs as introduced in this research. Fenoterol (5-(1-Hydroxy-2-{[2-(4-hydroxyphenyl)-1-methylethyl] amino} ethyl) benzene-1, 3-diol) and reproterol (7-(3-{[2-(3, 5- dihydroxyphenyl)-2-hydroxyethyl] amino} propyl)-1, 3-dimethyl-3, 7-dihydro-1*H*-purine-2, 6-Dione) are β_2-adrenoreceptor agonists acting by the activation of β_2-adrenoreceptor resulting in relaxation of pulmonary smooth muscle. That will facilitate the dilation of bronchial passages and the opening up the airways to the lungs helping in the treatment of asthma and other pulmonary disorders [28, 30, 31].

In the presented study, fenoterol and reproterol were radiolabelled with radioactive iodine (Fig. 1). The factors affecting the radioiodination process to obtain the highest radiochemical yield were studied in details and the biological distribution pattern of radioiodinated fenoterol and reproterol were evaluated and examined as novel highly promising tools for lung perfusion imaging.

Experiment

Materials and equipments

All chemicals were of analytical grade and were used directly without further purification unless otherwise stated. Deionized water was used in all experiments for the preparation of solutions, dilution and washing purposes. Fenoterol, M.wt. = 303.35 g/mol and reproterol, M.wt. = 389.4 g/mol were obtained as gifts from Sigma Pharmaceutical Industries,

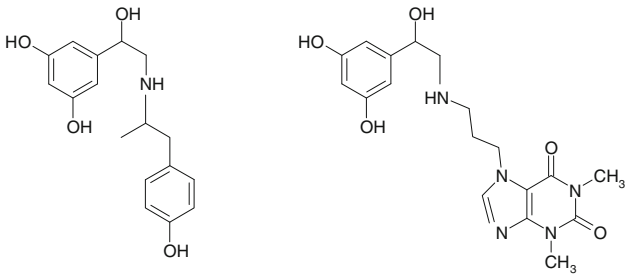


Fig. 1 The chemical structure of fenoterol and reproterol

Menoufya, Egypt and Cairo Pharmaceuticals Industries, Cairo, Egypt, respectively. Chloramine-T (CAT) [ArSO2NClNa)], M.wt. = 227.65 g/mol, sodium metabisulfite [$Na_2S_2O_5$], M.wt. = 190.107 g/mol, methanol [C_2H_6O], M.wt. = 46.07 g/mol and chloroform [$CHCl_3$], M.wt. = 119.38 g/mol were purchased from Sigma-Aldrich Company. No-carrier-added sodium iodide (NCA Na^{125}I, 3.7 GBq/ml in 0.1 N NaOH) was obtained as a gift from the radioisotope production factory (RPF), Egyptian Atomic Energy Authority (EAEA). Whatman paper number 1: international LTD was purchased from Merck Company, Germany. A NaI(Tl) γ-ray scintillation counter (Scaler Ratemeter SR7 model, the United Kingdom) was used for the measurement of γ-ray radioactivity. Electrophoresis apparatus (EC 3000P-series 90 programmable (E-C apparatus corporation) power supply and chamber unit) was used to determine the radiochemical yield. High performance liquid chromatography, (Shimadzu HPLC) which consists of U.V. spectrophotometer detector SpD-6A, Reversed phase Waters Symmetry C18 (RP-18) column (250 × 4.6 mm, 5 μm), Lischrosorb, Merck, pump LC-9A and fraction Collector-LKB, Bromma was used for determining the radiochemical yield.

Animal model

Animal studies were conducted in accordance with the guidelines set out by the EAEA and were approved by the animal ethics committee. Normal Swiss albino mice of body mass 20–40 g were purchased from Helwan University, Egypt. They were housed in groups of five and kept at a constant ambient temperature with a 12 h light/dark cycle and free access to food and water.

Method

Radioiodination

Different amounts of substrate in water (50–300 μg) were placed in amber color vial and then the addition of 100 μL of buffer solution to adjust pH of reaction medium (2–11)

followed by addition of aqueous solution of freshly prepared CAT (10–150 μg) and then 10 μL of radioactive ^{125}I(7.2 MBq) was added. The reaction was allowed to proceed for a chosen different time intervals at different temperature. The radioiodination reaction was quenched by the addition of a drop of high concentration sodium metabisulfite solution (10 mg/ml) [32].

Radiochemical yield analysis

After the preparation of ^{125}I-fenoterol and ^{125}I-reproterol formulations, the radiochemical yields were analyzed using different chromatographic techniques such as paper chromatography (PC), electrophoresis and HPLC.

Paper chromatographic (PC) analysis

At 2 cm above the lower edge of 13 cm length and 2 cm width paper sheet of Whatman paper no. 1, suitable portion of reaction mixture was positioned and left to dry then the paper was developed using fresh mixture of chloroform: methanol (9:1v/v) as mobile phase. After complete development, paper sheet was removed, dried, and cut into 1 cm segments which were counted by well type γ-counter.

^{125}I-fenoterol and ^{125}I-reproterolmoved with the solvent front ($R_f = 0.8$), while radioiodide (I^-) remained near the origin ($R_f = 0$–0.1). The percentage of radiochemical yield was calculated as the ratio of the radioactivity of iodo-compounds to the total activity multiplied by 100.

Electrophoresis analysis

The radiochemical yield of iodo-compounds was also determined using strips of Whatman paper no. 1 sheets (43 cm long, 2 cm width).The strips were moistened with normal saline and putted in the electrophoresis chamber followed by sampling of suitable portions of reaction mixture. The application point of reaction spotted was positioned at 12 cm far from the cathode edge of the paper sheet. The operation time was 1 h at voltage of 300 V using normal saline (0.9 % w/v NaCl solution) as electrolytes source solution, and then the developed strips were dried, cut into strips of 1 cm segment and counted by well type γ-counter.

Electrophoresis radiochromatogram at optimum labeling conditions are shown in Fig. 2 revealing that ^{125}I-fenoterol and ^{125}I-reproterol kept at distance 1 and 2 cm from spotting point, respectively while the free radioiodide moved to distance 13 cm far from the spotting point towards the anode. This is depending on molecular charge and ionic mobility [33].The percentage of radiochemical yield was calculated as the ratio of the radioactivity of iodo-compound to the total activity multiplied by 100.

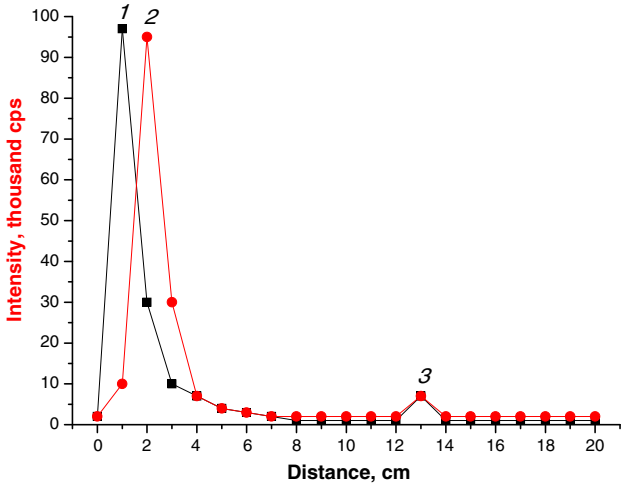


Fig. 2 Electrophoresis radiochromatogram of radioiodinated fenoterol and reproterol

HPLC analysis

The radiochemical yield of ^{125}I-fenoterol and ^{125}I-reproterol was confirmed by the injection of 50 μL of reaction mixture into RP-18 column of the HPLC system. After that the reaction mixture was loaded and eluted by methanol: H_2O (70: 30v/v) as a mobile phase and at flow rate of 1 ml/min with wave length of 254 nm in case of ^{125}I-fenoterol while acetonitrile: H_2O (85:15v/v) as a mobile phase and at flow rate of 1 ml/min with wave length of 282 nm in case of ^{125}I-reproterol. Then the eluted fractions of each reaction mixture were collected and counted using a well type γ-counter.

HPLC radiochromatogram of ^{125}I-fenoterol is represented in Fig. 3 showing that three peaks were obtained where the first peak at 3.1 min retention time corresponding to free iodide, while the second peak at 4.8 min corresponds to cold fenoterol and the third peak at 5.1 min which correspond to ^{125}I-fenoterol. While in

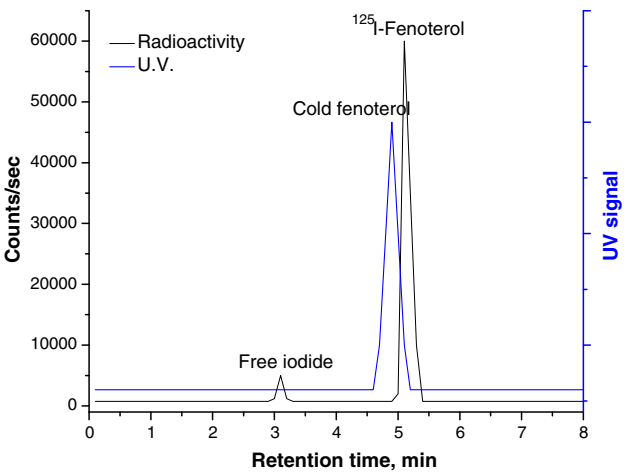


Fig. 3 HPLC radiochromatogram of radioiodinated fenoterol

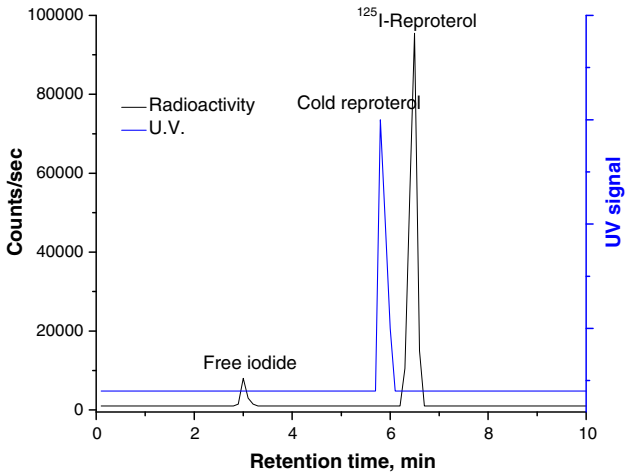


Fig. 4 HPLC radiochromatogram of radioiodinated reproterol

radiochromatogram of ^{125}I-reproterol (Fig. 4), free iodide is obtained at retention time of 3 min while cold reproterol is obtained at 5.8 min retention time and ^{125}I-reproterol is obtained at 6.5 min retention time.

In vitro stability study

The in vitro stability of ^{125}I-fenoterol and ^{125}I-reproterol was studied at ambient temperature for up to 72 h during which suitable fraction of samples were taken from reaction mixture at different time intervals in order to be assayed.

Biodistribution study

Preliminary biodistribution studies of ^{125}I-fenoterol and ^{125}I-reproterol were carried out in normal Swiss albino mice. After the I.V. administration of the iodo-compounds, the preferential uptake and clearance in most relevant organs was evaluated. Aliquots of 10 μL containing 3.7 MBq of the ^{125}I-fenoterol and ^{125}I-reproterol were separately injected intravenously into the tail vein of mice; the mice were anaesthetized then weighed at 5, 15, 30, 60 and 120 min post-injection (p.i.). Whole organs were immediately harvested, rinsed with saline and weighed in pre-weighed plastic vials and their radioactivity as well as background was measured using a well type γ-counter. The results were expressed as the percentage injected dose per gram (%ID/g ± SEM.) in a population of five mice for each time point. Blood, bone and muscles were assumed to be 7, 10 and 40 % of the total body weight, respectively [34].

Drug inhibition study

To confirm that the radioiodinated fenoterol and reproterol were taken up specifically with high affinity binding to β_2-receptor located in lungs, different amounts of cold fenoterol and reproterol (50 μg/kg of mouse) were I.V injected separately into the mice just exactly before the injection of the radioiodinated fenoterol and reproterol then mice was biologically evaluated for lung uptake at 15 and 30 min p.i., respectively.

Statistical analysis

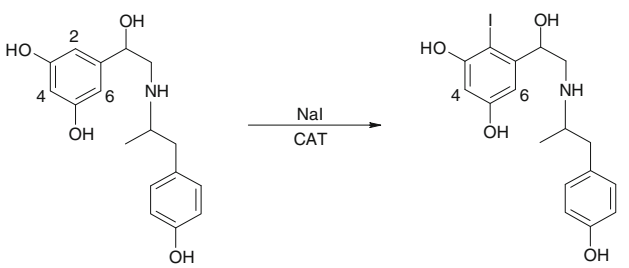
Data were evaluated with one way ANOVA test. Results for P are reported and all the results are given as mean ± SEM. The level of significance was set at $P < 0.05$.

Result and discussion

The radioiodination reaction was done by electrophilic substitution. The free molecular iodine (I_2) has the structure of I^+-I^- in aqueous solution [35]. The hydrated iodonium ion (H_2OI^+) and the hypoiodous acid (HOI) are believed to be highly reactive electrophilic species resulting in iodination reaction through electrophilic substitution of a hydrogen ion in a molecule of interest (fenoterol or reproterol) (Figs. 5, 6) [36–40].

Effect of chloramine-T (CAT) amount

Chloramine-T is considered as an oxidizing agent that has the ability to oxidize the iodine (I_2) generating the highly reactive electrophilic species (H_2OI^+ and HOI) which have important role in iodination reaction [39, 41]. So the amount of chloramine-T is very important and critical in the iodination reactions. The data presented in Fig. 7 clearly reveal that the radiochemical yield of ^{125}I-fenoterol increased by increasing the amount of chloramine-T from 10 to 20 μg resulting in the highest radiochemical yield of 97.7 ± 0.7 %, while in case of ^{125}I-reproterol, the radiochemical yield reached maximum yield of 95.2 ± 0.3 % by increasing amount of chloramine-T from 10 to 50 μg.



Fenoterol Proposed iodinated fenoterol

Fig. 5 The proposed structure of radioiodinated fenoterol

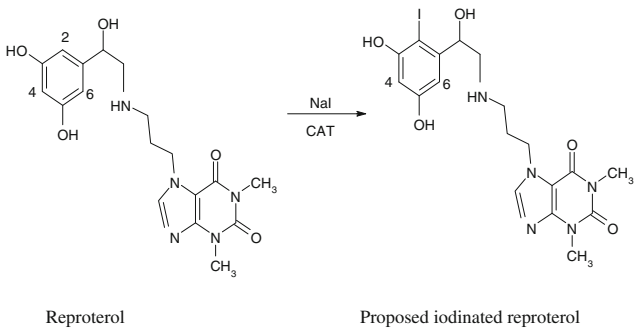


Fig. 6 The proposed structure of radioiodinated reproterol

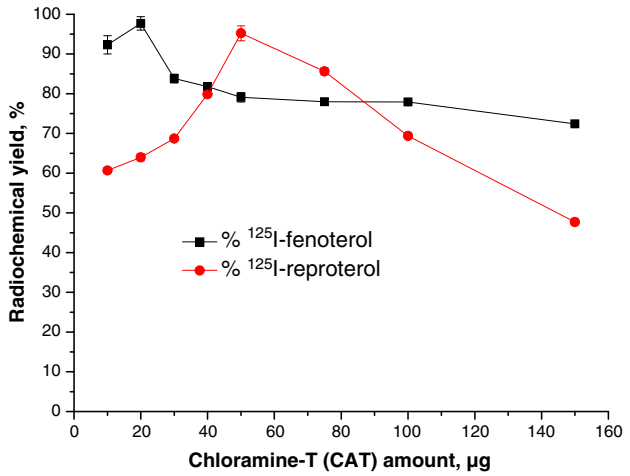


Fig. 7 Variation of the radiochemical yield of iodo-compounds as a function of CAT; reaction conditions: 10 µL (~3.7 MBq) $Na^{125}I$, 150 µg of fenoterol and reproterol, (x µg) of CAT, at pH 3 and 4 respectively, the reaction mixtures were kept at room temperature for 15 and 30 min, respectively

Increasing the amounts of chloramine-T above the optimum values (20 and 50 µg, respectively)up to 150 µg was resulting in a significant decrease of the radiochemical yield of iodo-compounds. This may be due to the formation of oxidative side products [42, 43].

Effect of pH

The pH of the reaction medium was also found to be a critical factor affecting the radiochemical yield of ^{125}I-fenoterol and ^{125}I-reproterol. The radioiodination process was carried out through the electrophilic substitution of H^+ by iodonium ion (I^+) [36, 38]. This is depending on the oxidizing power of chloramine-T which decreases by increasing the pH of the reaction medium [36]. The data presented in Fig. 8 clearly shows that at pH 2, the radiochemical yields of ^{125}I-fenoterol and ^{125}I-reproterol were very poor while highest yield of 97.7 ± 0.7 and 95.2 ± 0.3 % were obtained at pH 3 and 4 for fenoterol

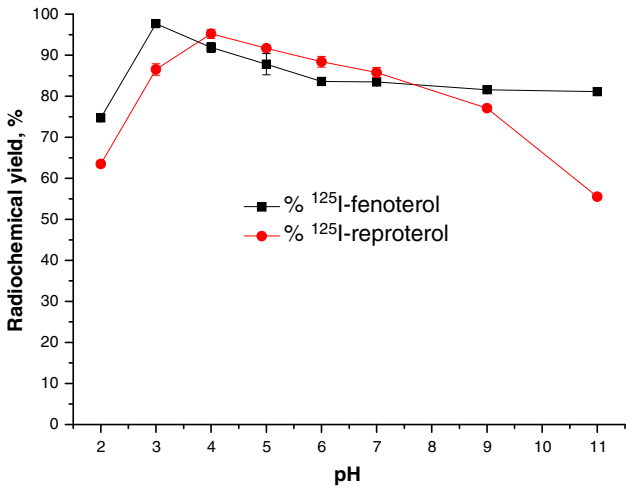


Fig. 8 Variation of the radiochemical yield of iodo-compounds as a function of pH; reaction conditions: 10 µL (~3.7 MBq) $Na^{125}I$, 150 µg of fenoterol and reproterol, 20 and 50 µg of CAT respectively, at different pH, the reaction mixtures were kept at room temperature for 15 min and 30 min respectively

and reproterol, respectively. By shifting pH medium towards neutral and alkaline medium, the radiochemical yield decreased significantly reaching 81.1 ± 0.9 and 55.5 ± 0.7 % at pH 10 for fenoterol and reproterol, respectively. This may be attributed to the formation of hypoiodite ion (IO^-) and iodate (IO^{-3}) ions which are not the suitable forms for radioiodination process [44, 45].

Effect of substrate (fenoterol and reproterol) amount

The amount of fenoterol and reproterol used during this study varied between 50 and 300 µg. The data presented in Fig. 9 clearly show that the radiochemical yield increased from 90.4 ± 0.8 to 97.7 ± 0.7 % and from 88.3 ± 0.6 to 95.2 ± 0.3 % as fenoterol and reproterol amount increased from 50 to 150 µg, respectively. Further increase in the amount of fenoterol and reproterol beyond 150 µg were resulting in slight decrease of radiochemical yield till reached 94.8 ± 1 and 93.8 ± 0.7 %, respectively at 300 µg. So the optimum concentration of fenoterol and reproterol which could give the highest radiochemical yield is 150 µg, which may be attributed to 150 µg of fenoterol and reproterol is enough to capture all the entire generated iodonium ions as a result of the oxidation of the radioactive iodine [45, 46].

Effect of reaction time

The radiochemical yields of ^{125}I-fenoterol and ^{125}I-reproterol were determined at different time intervals ranging from 5 to 60 min. It is clear from Fig. 10 that the radiochemical yield of ^{125}I-fenoterol was slightly increased by

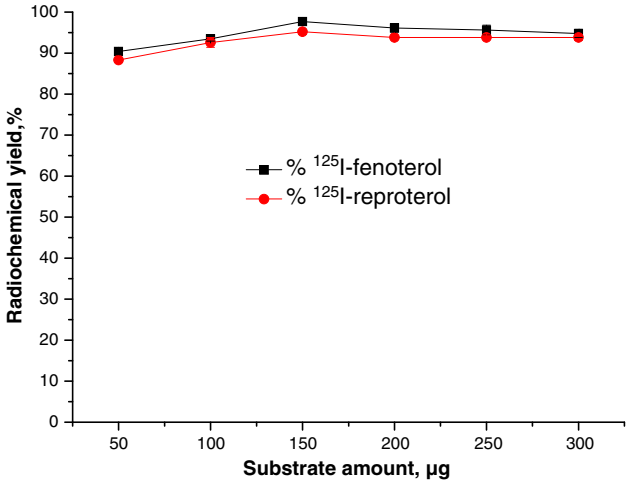


Fig. 9 Variation of the radiochemical yield of iodo-compounds as a function of different substrate amounts; reaction conditions: 10 µL (~3.7 MBq) $Na^{125}I$, (x µg) fenoterol and reproterol, 20 and 50 µg of CAT, at pH 3 and 4 respectively, the reaction mixtures were kept at room temperature for 15 and 30 min respectively

increasing the reaction time from 5 to 15 min at which the highest radiochemical yield of 97.7 ± 0.7 % was obtained while the highest radiochemical yield (95.2 ± 0.3 %) of ^{125}I-reproterol was obtained at 30 min. Increasing reaction time longer than 15 and 30 min for ^{125}I-fenoterol and ^{125}I-reproterol, respectively was resulting in slight decrease of radiochemical yields, which could be attributed to long exposure time of fenoterol and reproterol to the highly reactive CAT which can result in oxidative side reactions [8]. Whereas, at shorter reaction time (5 min), the time

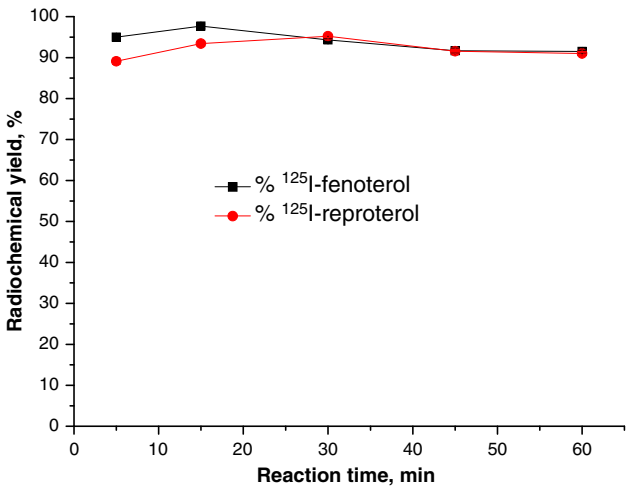


Fig. 10 Variation of the radiochemical yield of iodo-compounds as a function of reaction time; reaction conditions: 10 µL (~3.7 MBq) $Na^{125}I$, 150 µg of fenoterol and reproterol, 20 and 50 µg of CAT, at pH3 and 4 respectively, the reaction mixtures were kept at room temperature for different intervals of time

required for reaction between chloramine-T and iodide to produce the iodonium ion is minimal [46].

In vitro stability of ^{125}I-fenoterol and ^{125}I-reproterol

It was observed that both ^{125}I-fenoterol and ^{125}I-reproterol were stable up to 72 h without detection of any by-products in the reaction mixture affecting the radiochemical yields, thus no significant change in the radiochemical yields were observed.

Biodistribution

The preclinical biodistribution studies of radioiodinated fenoterol and reproterol in normal mice at 5, 15, 30, 60 and 120 min post injection (p.i.) were performed. The in vivo instability of radioiodinated compounds is commonly reflected by a high amount of radioactivity accumulation in the thyroid [47]. So the low thyroid levels found at all the experiment time points indicate that these radioiodinated compounds were relatively stable in vivo. Biodistribution study of radioiodinated fenoterol (Fig. 11;Table 1) showed high accumulation of radioactivity (52 ± 1.03 %) within the lungs at 15 min p.i., while in case of radioiodinated reproterol (Fig. 12; Table 2) showed 50.6 ± 1.2 % ID/g lungs uptake at 30 min p.i. This high accumulation of these iodo-compounds within lung is much greater in comparison with the recently discovered lung perfusion radiotracers as $^{125/123}$I-IPMPD, ^{99m}Tc(CO)$_5$I, ^{99m}Tc-DHPM and ^{125}I-paroxetine which show lung accumulation of 21.4 ± 1.7,

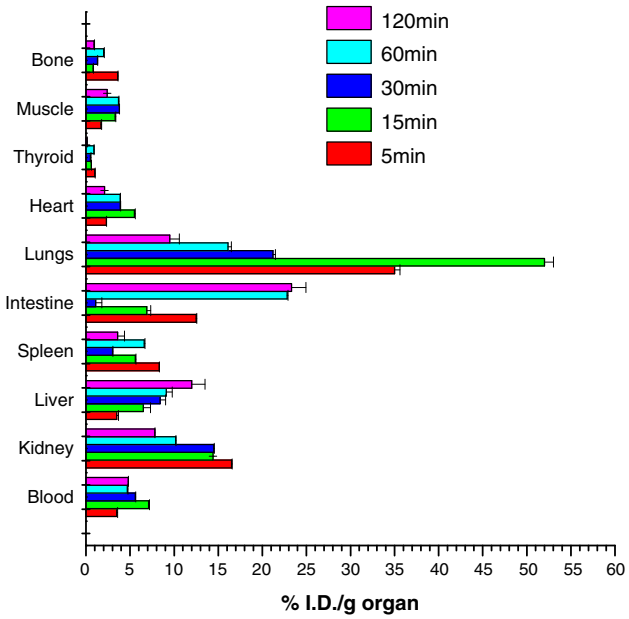


Fig. 11 Biodistribution of radioiodinated fenoterol in normal Swiss Albino mice at different time intervals post-injection (%ID/gram ± SEM, $n = 5$)

Table 1 In vivo biodistribution of radioiodinated fenoterol in normal Swiss Abino mice at different time intervals post-injection. (% ID/gram ± SEM, $n = 5$)

Organs and body fluids	% Injected dose/gram at different time intervals (min)				
	5 min	15 min	30 min	60 min	120 min
Blood	3.5 ± 0.1	7.1 ± 0.09	5.6 ± 0.04	4.7 ± 0.06	4.8 ± 0.02
Kidneys	16.5 ± 0.08	14.4 ± 0.01	14.5 ± 0.05	10.2 ± 0.02	7.8 ± 0.04
Liver	3.5 ± 0.19	6.5 ± 0.82	8.4 ± 0.63	9.1 ± 0.68	12.0 ± 1.50
Spleen	8.3 ± 0.02	5.6 ± 0.06	3.0 ± 0.06	6.6 ± 0.09	3.6 ± 0.76
Intestine	12.5 ± 0.07	6.9 ± 0.46	1.1 ± 0.71	22.8 ± 0.12	23.3 ± 1.64
Lungs	35 ± 0.61	52.0 ± 1.03	21.2 ± 0.28	16.1 ± 0.39	9.5 ± 1.08
Heart	2.3 ± 0.03	5.5 ± 0.09	3.9 ± 0.05	3.9 ± 0.2	2.1 ± 0.01
Thyroid	1.0 ± 0.05	0.6 ± 0.03	0.5 ± 0.07	0.9 ± 0.04	0.1 ± 0.05
Muscle	1.7 ± 0.09	3.3 ± 0.08	3.8 ± 0.02	3.7 ± 0.03	2.4 ± 0.02
Bone	3.6 ± 0.04	0.8 ± 0.06	1.3 ± 0.04	2.0 ± 0.08	0.9 ± 0.07

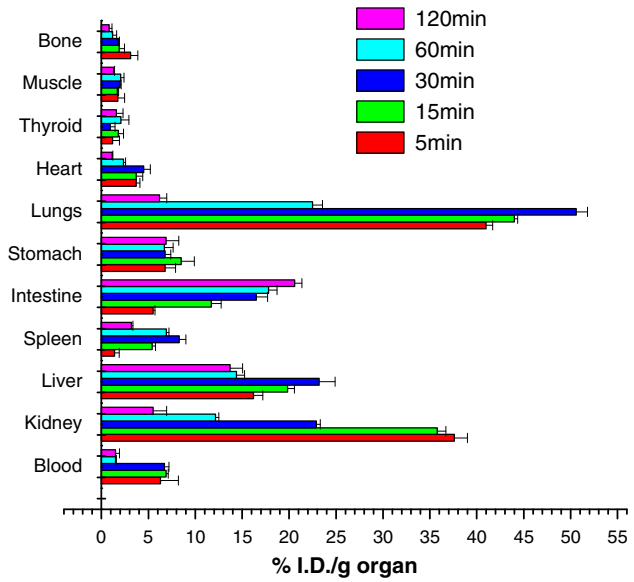


Fig. 12 Biodistribution of radioiodinated reproterol in normal Swiss Albino mice at different time intervals post-injection (%ID/gram ± SEM, $n = 5$)

12.8 ± 2.87, 10.12, 27.9 ± 1.0 ID/g at 15, 60, 2 and 15 min p.i., respectively [8, 16, 24, 25]. Furthermore, both iodo-compounds are not blood-derived products so there is no possibility of contamination by different infective agents as in case of ^{99m}Tc-MAA which suffers from high biological hazard risk. MAA is a HSA derived product collected from the pooled blood of human donors, so there is high possibility of contamination by infective agents such as variant Creutzfeldt Jakob disease, hepatitis B, hepatitis C and HIV [18–20]. Although radioiodinated fenoterol and reproterol are accumulating within the lung by different mechanism other than ^{99m}Tc-MAA, these iodo-compounds have the ability to overcome the drawbacks of ^{99m}Tc-MAA. The biological data show low radioactivity accumulation within heart and circulating blood all over the studies time, which will help in having high quality lung imaging. It was noticed that the kidney uptake was high at 5 min p.i., besides there were gradual increase of liver and intestine uptakes through experiment time points, which indicated that the clearance mechanisms of these iodo-compounds were through the renal and hepatobiliary

Table 2 In vivo biodistribution of radioiodinated reproterol in normal Swiss Abino mice at different time intervals post-injection. (% ID/gram ± SEM, $n = 5$)

Organs and body fluids	% Injected dose/gram at different time intervals (min)				
	5 min	15 min	5 min	60 min	5 min
Blood	6.3 ± 1.90	6.9 ± 0.21	6.7 ± 0.49	1.5 ± 0.09	1.5 ± 0.42
Kidneys	37.6 ± 1.41	35.8 ± 0.91	22.9 ± 0.42	12.1 ± 0.35	5.5 ± 1.41
Liver	16.2 ± 1.01	19.8 ± 0.72	23.2 ± 1.71	14.4 ± 0.84	13.7 ± 1.34
Spleen	1.4 ± 0.49	5.4 ± 0.35	8.3 ± 0.71	6.9 ± 0.28	3.2 ± 0.14
Intestine	5.5 ± 0.20	11.7 ± 1.06	16.5 ± 1.21	17.8 ± 0.91	20.6 ± 0.77
Stomach	6.8 ± 1.10	8.5 ± 1.40	6.8 ± 0.60	6.7 ± 0.93	6.9 ± 1.34
Lungs	41.0 ± 0.70	44.0 ± 0.35	50.6 ± 1.20	22.5 ± 1.06	6.2 ± 0.76
Heart	3.7 ± 0.40	3.7 ± 0.71	4.5 ± 0.70	2.3 ± 0.22	1.1 ± 0.08
Thyroid	1.2 ± 0.71	1.8 ± 0.56	0.9 ± 0.49	2.1 ± 0.84	1.6 ± 0.69
Muscle	1.7 ± 0.72	1.7 ± 0.11	2.0 ± 0.10	2.1 ± 0.35	1.3 ± 0.10

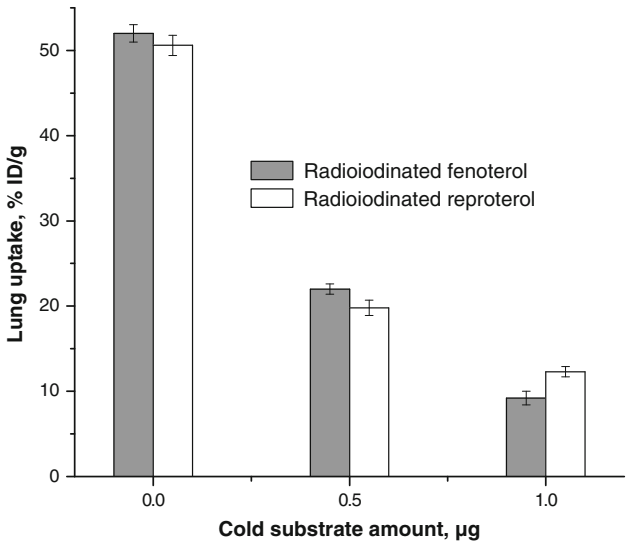


Fig. 13 Radioiodinated fenoterol and reproterol inhibition lung uptake in normal Swiss Albino mice at 15 and 30 min p.i., respectively (%ID/gram ± SEM, $n = 5$)

pathways which is matched with the excretion pathways of cold fenoterol and reproterol [48, 49].

Drug inhibition study

The blocking of β_2-receptor by the intravenous injection of cold fenoterol and reproterol result in extensive decrease in the accumulation of radioactivity of radiodinated fenoterol and reproterol within the lungs as it dropped from 52 ± 1.03 to 9.2 ± 0.8 % and from 50.6 ± 1.2 to 12.1 ± 0.6 % ID/g, respectively by the injection of 1 µg of the cold substrate (Fig. 13). These intensive decreases confirm the selectivity and high binding affinity of these iodo-compounds to β_2-receptor located in lung.

Conclusion

Radioiodinated fenoterol and reproterol were prepared by radioiodination reaction through elelctrophilic substitution mechanism under oxidative conditions using CAT with high radiochemical yield of 97.7 ± 0.7 and 95.2 ± 0.3 %, respectively. Due to both iodo-compounds are not blood products, we can conclude that radioiodinated fenoterol and reproterol could overcome all the drawbacks of ^{99m}Tc-MAA obtained from HSA. Both iodo-compounds had showed in vitro stability up to 72 h and in vivo stability higher than 2 h. The preclinical biodistribution studies showed that radioiodinated fenoterol and reproterol were potentially accumulated with high selectivity to β_2-receptors located in lungs (52 ± 1.03 % at 15 min p.i. and 50.6 ± 1.2 % ID/g at 30 min p.i, respectively) In comparison with the recently discovered perfusion scintigraphic agents ($^{125/123}$I-IPMPD, ^{99m}Tc(CO)$_5$I, ^{99m}Tc-DHPM and ^{125}I-paroxetine). These selectivity to lung tissue was confirmed by blocking study which result in sever reduction of lungs uptake. As a result, radioiodinated fenoterol and reproterol could be introduced as potential perfusion scintigraphic agents and have the advance over the commercially available ^{99m}Tc-MAA and recently discovered perfusion scintigraphic agents ($^{125/123}$I-IPMPD, ^{99m}Tc(CO)$_5$I, ^{99m}Tc-DHPM and ^{125}I-paroxetine).

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