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Original Article

PREPARATION AND MOLECULAR MODELING OF RADIOIODOPROPRANOLOL AS A NOVEL POTENTIAL RADIOPHARMACEUTICAL FOR LUNG PERFUSION SCAN

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ABSTRACT

Objective: Development of an easy method for radio iodination of propranolol with high percent labeling yield for the purpose of lung perfusion imaging.

Methods: Radioidination of propranolol was achieved using ^{125}I via electrophilic substitution under the oxidative conditions of Cholramine-T (CAT). All factors affecting the labeling procedure and labeling yield were studied. Paper electrophoresis and HPLC were performed to determine the radiochemical yield and purity of the ^{125}I-propranolol. Biodistribution studies were performed to determine the lung deposition of iodo propranolol by injecting the labeled propranolol into the tail vein of Swiss Albino mice. Molecular modeling and docking studies were performed to ensure the binding of the newly obtained ^{125}I-propranolol to beta-2 (β_2) adrenergic receptor.

Results: Radioiodination of propranolol has been successfully achieved with a high labeling yield (93.7±0.81%). ^{125}I-propranolol was stable for 24 h when kept away from light, at ambient temperature. Biodistribution studies showed lung uptake of 21.60±0.03% injected dose/g (%ID/g) at 30 min post-injection. Molecular modeling confirmed that radio iodination did not affect the binding of propranolol to β_2-receptor.

Conclusion: Iodopropranolol can be considered as good potential lung perfusion agent as suggested by the results of biodistribution and molecular modeling studies.

Keywords: Radiopharmaceutiacls, Propranolol, ^{125}I,Chloramine-T, Lung perfusion scan, Molecular modeling.

INTRODUCTION

Nuclear medicine imaging, non-invasively provides functional information at the molecular and cellular level that contributes to the determination of health status by measuring the uptake and turnover of target specific radiotracers in tissues [1].

A nuclear medicine examination reveals information on the physiological status of a specific organ or tissue rather than information on the anatomical outlines. A radiopharmaceutical is either a radioactive isotope alone, e. g. ^{131}I [2] or a radioactive isotope attached to a carrier molecule e. g. ^{99m}Tc-HMPAO [3].

Lung perfusion scan is a nuclear medicine imaging technique that produces a picture of blood flow to the lungs and so used to detect pulmonary embolisms, determine how much blood is flowing to the lungs and assess how well the lungs are functioning after surgery [4]. It also can be used for diagnosis of lung cancer as, patients with non-small cell lung cancer often have inhomogeneous lung perfusion [4]. Pulmonary perfusion is an important physiological parameter in the diagnostic work-up of various pulmonary diseases [5]. ^{99m}Tc-labeled macroaggregated albumin ($[99]^{m}Tc$-MAA) is a well established radiopharmaceutical for lung perfusion imaging [6]. A major drawback of ^{99m}Tc-MAA is that it is blood derived product which predisposes the injected patient to the risk of blood transmitted diseases, e. g. Variant Creutzfeldt Jakob Disease [6]. Due to this major drawback many non blood derived radiopharmaceuticals were developed. ^{99m}Tc $(CO)_5I$ and ^{99m}Tc-DHPM [^{99m}Tc-5-etoxycarbonyl-4-phenyl-6-methyl-3,4-dihydro-(1H)-pyrimidine-2-one] are two recently discovered radiopharmaceuticals and considered as potential lung perfusion agents. Both agents showed maximum lung uptake of 12.8±2.87 and 10.12±0.01 (% ID/g) at 1 h and 2 min post-injection, respectively [6, 7].

It's noted from the uptake of ^{99m}Tc $(CO)_5I$ that the lung uptake was only 8.82±0.75 (% ID/g) 15 min post administration and reached its maximum uptake 1 h post administration, this means that its lung deposition is too slow to reach its maximum which can prolong the time required for the image acquisition procedure [5]. On the other hand, ^{99m}Tc-DHPM reached its maximum uptake 2 min post-injection and the lung uptake reached approximately the half (5.01±0.03 % ID/g) 1 h after its administration [6]. Fast deposition and clearance of $[99]^{m}Tc$-DHPM give only a brief time for the image acquisition procedure.

Another recently discovered lung imaging agent is ^{123}I-ritodrine which has maximum uptake of 20.4±0.22 (% ID/g) after 30 min of administration with good pulmonary retention time [8]. Unfortunately, ^{123}I-ritodrine showed also very high cardiac uptake at all time points. High cardiac uptake may interfere with the quality of the produced image. Hence, the development of a new radiopharmaceuticals for lung perfusion imaging with proper lung affinity and retention is needed.

Propranolol (Inderal®) shown in fig. 1 is the prototype agent of a class of compounds known as aryloxypropanolamines [9]**.** Since propranolol is a non selective beta blocker it has affinity for both cardiac β_1 and β_2 adrenergic receptors. The ratio of cardiac to lung activity for propranolol was evaluated to be 2:1 [10]. The aim of this study is to determine the possibility of using radio iodinated propranolol as potential lung perfusion imaging agent.

MATERIALS AND METHODS

Materials

All chemicals and solutions used were of reactive grade. Bidistilled water was used in all experiments for the preparation of solutions, dilution, and washing purposes. Propranolol (RS)-1-(1-methylamino)-3-(1-naphthoxy)propan-2-ol. M. wt. = 259.34 g/mol was obtained as a gift from Pharco Pharmaceutical Industries, Alexandria, Egypt. No carrier-added sodium iodide (NCA $Na^{125}I$, 3.7 GBq/ml in 0.1 N NaOH) for radio iodination was purchased from the Institute of Isotopes, Budapest, Hungary. Chloramine-T (CAT) [$ArSO_2NClNa$], M. wt. = 227.65 g/mol, sodium metabisulfite [$Na_2S_2O_5$], M. wt. = 190.11 g/mol both were obtained from Aldrich chemical company. Citric acid, M. wt. = 192.124 g/mol and Methanol 99.5%, M. wt. = 32.04 g/mol were purchased from The British Drug

House (BDH) Chemicals LTD, England. Ethanol 95% M. wt. = 46.07 g/mol was obtained from Ubichem limited company, England.

Equipments

Vortex shaker, model-231, Fisher Scientific, U. S. A. Was used to shake all prepared mixtures and solutions. Whatman paper number 1: International LTD was purchased from Merck Company, Germany. A NaI (Tl) γ-ray scintillation counter (Scaler Ratemeter SR7 model, the UK) 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 a UV spectrophotometer detector SpD-6A, Reversed phase Waters Symmetry C18 (RP-18) column (250×4.6 mm, 5µm), Lischrosorb, Merck, pump LC-9A and fraction Collector-LKB, Bromma was used for determining the radiochemical yield.

Method

Radioiodination of propranolol

^{125}I-propranolol was synthesized by direct electrophilic substitution reaction with ^{125}I ($t_{1/2}$ =60 days) under oxidative conditions of Chloramine-T (CAT). The reaction was carried out in amber colored vials and was shaken by an electrical vortex shaker. The volume of the reaction mixture was fixed at 500 µl. For the labeling reaction, stock solutions of propranolol (1:1 w/v ethanol 95%) and of CAT (1:1 w/v H_2O) were prepared.

Factors affecting the efficiency of radio iodination reaction (amount of propranolol, an amount of CAT, the pH of the reaction mixture, reaction time and reaction temperature) were studied. The radiochemical yield of the product was determined by paper electrophoresis and HPLC. Experiment studying each factor was repeated three times and differences in the data were evaluated by the Student t-test. Results for *P* using the 2-tailed test are reported and all the results are given as mean±SEM. The level of significance was set at $P<0.05$.

Optimum conditions for radio iodination of propranolol were achieved by adding 50 µl of the propranolol stock solution, followed by the addition of 150 µl of citric acid buffer pH 3. Exactly 10 µl of Na^{125}I solution (7.2 MBq) was added afterward. Finally, 20 µl of CAT stock solution was added to start the reaction. The reaction was allowed to proceed for 30 min in a water bath (100 °C).

The iodination reaction was quenched using saturated sodium thiosulfate solution (10:1 w/v H_2O) to decompose the excess iodine (I_2) to iodide (I^-) [11].

Analysis of radiochemical yield and purity of ^{125}I-propranolol

Radiochemical yield and purity of the ^{125}I-propranolol were determined by paper electrophoresis method and HPLC

Paper electrophoresis

Using Whatman paper (no. 1) sheet (2.5x47 cm) and normal saline (0.9% NaCl solution) as a source of electrolytes. Two microliter of each reaction mixture were spotted 12 cm away from the cathode and allowed to dry at room temperature. Electrophoresis was conducted for 80 min under voltage of 300 Volt. After complete development, the paper was allowed to dry spontaneously and cut into slices (2.5 x 1 cm). Each slice was counted in a well type γ-counter.

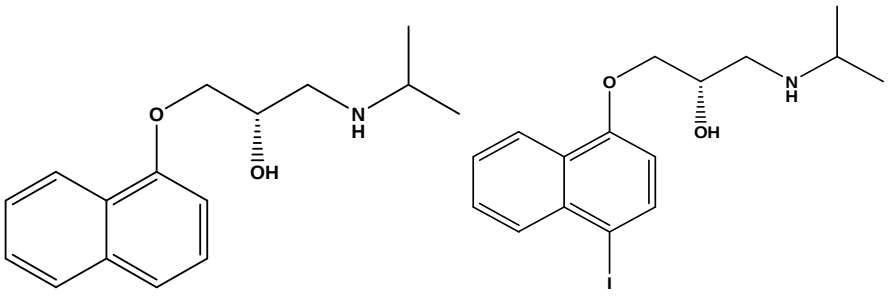
The percentage of radiochemical yield was calculated as the ratio of the radio activity of ^{125}I-propranolol to the total activity multiplied by 100 [4].

High performance liquid chromatography

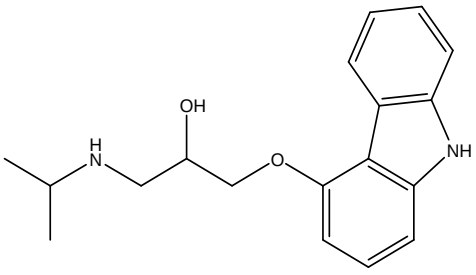
The radiochemical purity was further confirmed by HPLC. The HPLC analysis of ^{125}I-propranolol complex was done by injection of 2 µl of propranolol reaction mixture into the column and UV spectrophotometer detector wavelength was adjusted at 242 nm [12]. The used mobile phase was a mixture of methanol: ammonia (99.3: 0.7 v/v). The ability of the mobile phase to separate propranolol and the inactive KI solution was performed by injecting 2 µl of standard propranolol solution (1:1 w/v in ethanol) and standard KI solution, separately. Standard solutions of inactive iodo propranolol, CAT and sodium thio sulphate were also injected to avoid misinterpretation of the UV produced peaks. After injection of the reaction mixture, fractions of 1 ml were collected separately using a fraction collector up to 30 fractions and were counted in a well type NaI detector connected to a single channel analyzer.

Determination of *in vitro* stability of ^{125}I-propranolol

The reaction mixture was kept in amber colored vials at ambient temperature for 48 h. Two microliter samples were taken from each vial for analysis at each time point (6, 12, 24 and 48 h). The radiochemical yield and purity of the samples were measured by paper electrophoresis and HPLC.



Propranolol [12] **^{125}I-propranolol**



Carazolol

Fig. 1: Chemical structures of propranolol, ^{125}I-propranolol and carazolol

Biodistribution studies of the ^{125}I-propranolol

The experimental procedures of the biological studies were done in accordance with the guidelines set out by the Egyptian Atomic Energy Authority and were approved by the animal ethics committee, Labeled Compounds Department. The reaction mixture for biodistribution study was prepared in high radioactivity and purified using HPLC before injection. Exactly 0.1 ml of about 18 MBq of the^{125}I-propranolol solution was injected into the tail vein of normal Swiss Albino mice using an insulin syringe. After the administration of radio iodinated propranolol, animals were anesthetized by chloroform inhalation, then dissected at different time intervals (5, 15, 30 and 60 min).

Groups of five mice (20-25 g) were used for each time point. Samples of muscle, blood (obtained by cardiac puncture), liver, spleen, lung, kidneys, stomach, intestine, bone and heart were dissected washed with normal saline and weighed using digital balance [13]. The activity of each organ was measured in a shielded well type γ scintillation counter and expressed as percentage of injected dose per gram(% ID/g±SD). The % ID in the blood was estimated assuming a blood volume equal to 6.5% of the total body weight [14].

Molecular modeling studies

The X-ray crystallographic structure of β_2 adrenergic receptor (PDB code: 2RH1 downloaded) in complex with the Carazaolol, [1-(9H-carbazol-4-yloxy)-3-(propan-2-ylamino)propan-2-ol], was used in docking simulation [15]. Carazolol, propranolol and ^{125}I-propranolol structures were drawn using Chem Draw Ultra-8.0 as showed in fig. 1. The energy of the structures was minimized using Chem3D Ultra-8.0. Structures then were subjected to conformational search and energy minimization with Molecular Operating Environment (MOE®) 2008.10. Until RMSD gradient f 0.01 Kcal/mol and RMS distance of 0.1 Å with MMFF94X force-field and the partial charges were automatically calculated.

RESULTS AND DISSCUSSION

Analysis of radiochemical yield and purity of ^{125}I-propranolol

Paper electrophoresis

The radiochemical yield and purity of the ^{125}I-propranolol (fig. 1) was determined using paper electrophoresis. Free radioiodide and ^{125}I-propranolol moved to different distances away from the spotting point towards the anode (13 cm and 10 cm, respectively) as shown in fig. 2. To confirm the position of free iodide on the electrophoresis paper in the separation process, 2 µl of the inactive KI solution was added to the spotting point of the reaction mixture and allowed to dry before applying the electrical current.

Both hydrogen peroxide (H_2O_2) and starch solutions were added to the paper after the development process was completed to determine the place of free iodine by the appearance of a visible blue color. On a different electrophoresis paper, 2 µl of Na^{125}I solution was spotted and allowed to develop under the same conditions used previously for the assay of the position of the free radio iodide peak.

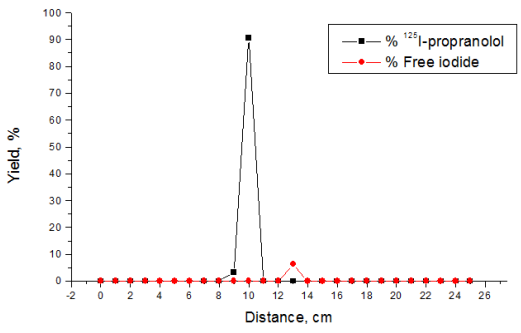


Fig. 2: Electrophoresis radiochromatogram of ^{125}I-propranolol

High performance liquid chromatography

HPLC analysis was performed to separate the radio iodinated propranolol form the free iodide. HPLC radiochromatogram of ^{125}I-propranolol is shown in fig. 3. The results showed a peak after 1.64 min that correspond to free iodide and another peak at 2.68 min that correspond to ^{125}I-propranolol.

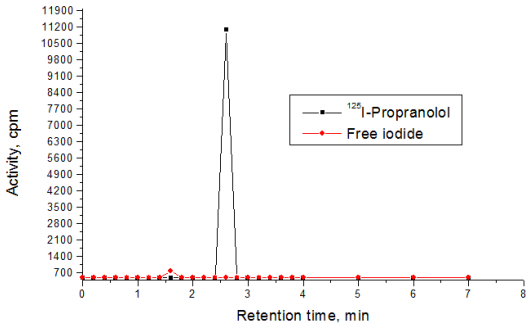


Fig. 3: HPLC radiochromatogram of ^{125}I-propranolol

Effect of propranolol amount

The dependence of the radiochemical yield on the amount of propranolol is depicted in fig. 4. The reaction was performed at different amounts of propranolol (10-200 µg). The radiochemical yield of the reaction was low at 10 µg of propranolol giving a radiochemical yield of 43.07±0.43%. The reaction yield then increased by increasing the substrate amount where a maximum yield of 93.7±0.81% was achieved using 100 µg of propranolol. The yield was then slightly decreased as the propranolol amount increased showing radiochemicalyield of 66.20±0.07% at 120 µg of propranolol. At low substrate amount, the low labeling yield was attributed to the insufficient molecules of the substrate to capture all of the generated iodonium ions [4].

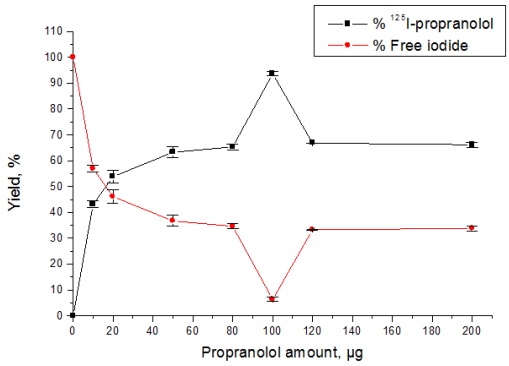


Fig. 4: Variation of the radiochemical yield of ^{125}I-propranolol as a function of substrate amount; reaction conditions: 10 µl (~7.2 MBq) Na^{125}I, (x µg) propranolol, 20 µg of CAT, at pH 3, the reaction mixtures were kept at 100 °C for 30 min

Effect of oxidizing agent amount

The effect of Chloramine-T amount on the labeling efficiency of ^{125}I-propranolol is demonstrated in fig. 5. CAT is a mild oxidizing agent that transforms iodide (I^-) to iodonium (I^+) allowing a spontaneous electrophilic substitution on the aromatic ring with a good leaving group such as H^+ [8].

When a low CAT amount (10 µg) was used, labeling yield was very poor (57.67±2.86%). This is because the used amount of CAT was not enough to produce sufficient iodonium species to attack all substrate molecules in the solution. A maximum yield of ^{125}I-

propranolol (93.70±0.81%) was achieved using 20 µg of CAT. Further increase in CAT amount resulted in decreasing the radiochemical yield to reach 38.30±5.20% at 200 µg of CAT. The decrease in the radio labeling yield can be attributed to the formation of chlorinated by-products, polymerization or decomposition of the substrate when the amount of CAT exceeded the optimum amount [16].

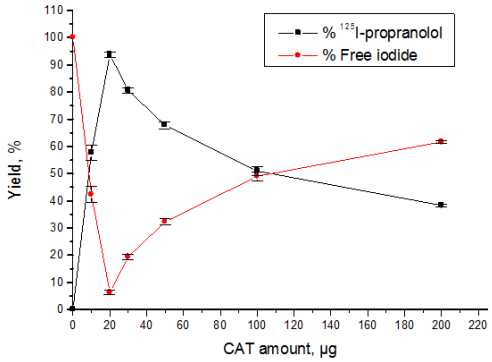


Fig. 5: Variation of the radiochemical yield of ^{125}I-propranolol as a function of CAT amount; reaction conditions: 10 µl (~7.5 MBq) Na^{125}I, 100 µg of propranolol, (x µg) of CAT, at pH 3, the reaction mixtures were kept at 100 °C for 30 min

Effect of reaction time

Reaction time is very important in order to give sufficient time for the reaction between CAT and iodine to produce iodonium ions and not affect the product stability at the same time [4]. After a reaction time of 5 min, radiochemical yield of 45.97±1.00% was obtained as demonstrated in fig. 6. The percent labeling yield of the reaction increased as the reaction time increased to reach a maximum yield of 93.7±0.81% after 30 min reaction time. A further increase in the reaction time resulted in decreasing the percentage labeling yield to reach 59.39±1.59% after 75 min. It was clear that 5 min was not sufficient for the reaction to be completed. This also explains the increase in the percent labeling yield as time increased. Decrease in the radio labeling yield was observed when the reaction time was further increased above optimum time. This can be attributed to the formation of oxidation side products as a result of the long exposure to CAT [16, 17].

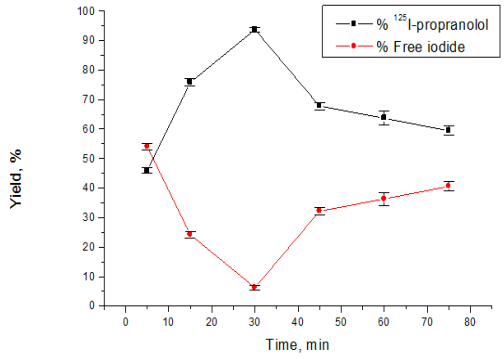


Fig. 6: Variation of the radiochemical yield of ^{125}I-propranolol as a function of reaction time; reaction conditions: 10 µl (~7.2 MBq) Na^{125}I, 100 µg of propranolol, 20 µg of CAT, at pH 3 the reaction mixtures were kept at 100 °C for different intervals of time

Effect of pH

Effect of pH of the reaction mixture was studied using buffers with different pH values. CAT generates different oxidizing species of radioiodine in acidic and alkaline medium. This gives a great importance of pH of the reaction on the labeling yield. In acidic medium, it undergoes hydrolysis to give hypochlorous acid (HOCl). Hypochlorous acid undergoes further hydrolysis to give H_2OCl^+. The HOCl or H_2OCl^+generated oxidize the iodine under acidic conditions to the oxidative iodonium (I^+) state and thus, rapidly reacts with any electron rich site [4, 18]. Results are depicted in fig. 7. At pH 1 a yield of 46.23±1.30% of ^{125}I-propranolol was obtained. Optimum yield of 93.70±0.81% was obtained at pH 3. This can be attributed to maximum stability of propranolol in pH 3 [19]. At pH 9, radio labeling yield decreased to reach 36.58±0.95%. This can be attributed to the instability of propranolol at neutral or alkaline pH. Another fact to be considered is that, CAT in alkaline medium decomposes to give HOCl and Hypochlorite (ClO^-) resulting in the generation of hypoiodite ion (IO^-) and iodate (IO_3^-) which are not the suitable forms for electrophilic radio iodination [20].

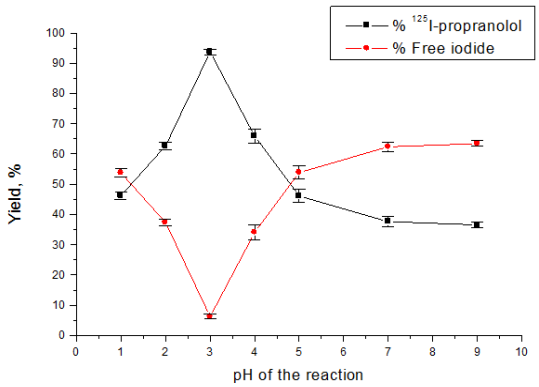


Fig. 7: Variation of the radiochemical yield of ^{125}I-propranolol as a function of pH; reaction conditions: 10 µl (~7.2 MBq) Na^{125}I, 100 µg of propranolol, 20 µg of CAT, at different pH, the reaction mixtures were kept at 100 °C for 30 min

Effect of reaction temperature

The influence of the temperature of the reaction mixture on the radiochemical yield of ^{125}I-propranolol is shown in fig. 8. The reaction was carried out at ambient temperature, 40, 60, 80 and 100 ºC. High temperature may be essential to initiate the labeling reaction as it helps in breaking C-H bond [21]. Only optimum temperature must be used in order to avoid decomposition of the substrate and/or the oxidizing agent. At room temperature a yield of 50.18±3.26% of ^{125}I-propranolol was obtained. The radiochemical yield of ^{125}I-propranolol showed gradual increase by elevating the temperature showing a maximum yield of 93.70±0.81% at 100 °C.

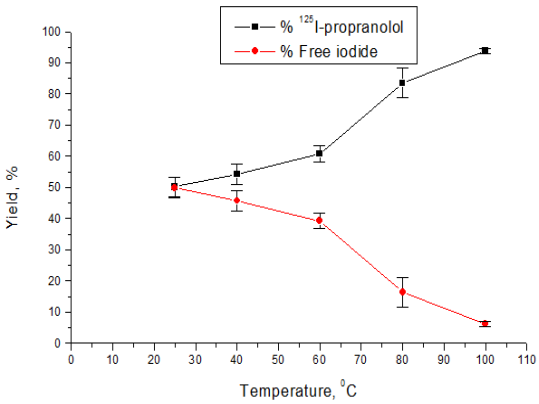


Fig. 8: Variation of the radiochemical yield of ^{125}I-propranolol as a function of reaction temperature; reaction conditions: 10 µl (~7.2MBq) Na^{125}I, 100 µg of propranolol, 20 µg of CAT, at pH 3, the reaction mixtures were kept at different temperatures for 30 min

In vitro stability of ^{125}I-propranolol

In vitro stability of ^{125}I-propranolol was studied in order to determine the suitable time for an injection to avoid the formation of the undesired products that result from the radiolysis of the labeled compound. These undesired radioactive products might be accumulated in non-target organs and interfere with the quality of the produced image [4]. Results of *in vitro* stability showed that ^{125}I-propranolol was stable up to 24 h if kept away from light at ambient temperature. The stability of the ^{125}I-propranolol is shown in fig. 9 and table 1.

Table 1: *In vitro* stability of ^{125}I-propranolol at ambient temperature

Time, h	%^{125}I-propranolol	% free iodide
0	93.70±0.81	6.30±0.81
6	93.10±0.29	6.9±0.29
12	91.83±0.16	8.17±0.16
24	91.60±0.81	8.40±0.81
48	54.91±0.78	45.09±0.78

Biodistribution of iodo propranolol

The lung uptake of radoiodinated propranolol was moderate (10.26±0.10 % ID/g) at 5 min post-injection as shown in table 2. A maximum lung uptake of 21.6±0.03 % ID/g was obtained at 30 min post-injection.

Also, the lung uptake of iodo propranolol at 5 min post-injection was higher than that of S-(-) [[11]C] CGP-12177 [(4-(3-t-butylamino-2-hydroxypropoxy)-benzimadazol-2-one)] which is a useful PET imaging agent for β-adreno receptor in the heart and lung showing lung uptake of 10.3±0.50 %ID/g at 20 min post-injection [22].

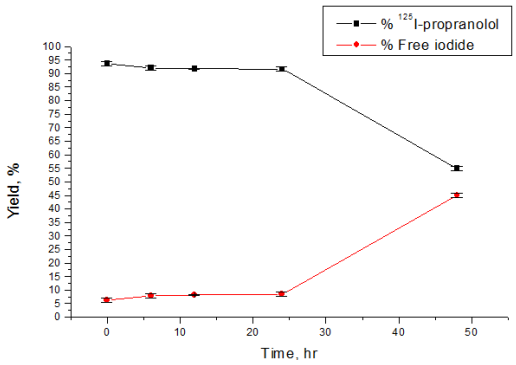


Fig. 9: *In vitro* stability of ^{125}I-propranolol at ambient temperature

This made iodo propranolol to be a potential imaging agent for β-adrenergic receptors in the lung. The biodistribution of ^{125}I-propranolol showed significantly higher heart uptake (18.33±0.01 %ID/g) at 5 min post-injection. Good retention in the heart was also observed showing uptake of 2.40±0.01% ID/g at 60 min post-injection. These results made iodo propranolol to be a potential cardiac β-adrenergic receptor imaging agent better than the currently used cardiac β-receptor imaging agent [[11]C] CGP-12177 which showed cardiac uptake of 4.57±1.43 and 2.40±0.10 %ID/g at 10 and 20 min post-injection, respectively [22, 23].

Table 2: Biodistribution of radio iodinated propranolol in normal Swiss Albino mice at different time intervals post-injection. (% ID/g±S. D, n = 5)

Tissue or body fluid	Biodistribution of radioiodinated Propranolol after			
	5 min	15 min	30 min	60 min
Blood	17.95±0.29	8.35±0.27	3.46±0.03	3.10±0.02
Kidneys	8.58±0.24	11.54±0.27	15.52±0.04	13.5±0.09
Liver	7.37±0.062	5.83±0.15	5.60±0.02	3.50±0.20
Spleen	0.37±0.01	3.56±0.15	3.20±0.07	6.30±0.09
Stomach	1.68±0.02	22.70±0.01	25.01±0.04	29.10±0.03
Intestine	2.31±0.10	6.20±0.30	7.19±0.01	10.09±0.2
Lungs	10.26±0.10	14.06±0.30	21.6±0.03	15.44±0.50
Heart	18.33±0.01	7.70±0.09	3.52±0.08	2.40±0.01
Thyroid	0.41±0.03	1.64±0.07	1.70±0.10	2.20±0.05
Muscle	0.14±0.06	2.68±0.05	2.00±0.02	2.00±0.25
Bone	2.81±0.04	2.63±0.00	3.20±0.01	3.10±0.07
Brain	2.13±0.05	2.71±0.01	1.06±0.02	0.90±0.01

Thyroid uptake of radio iodinated propranolol showed a gradual increase in the thyroid indicating peripheral de iodination of radio iodinated propranlol. It is well known that iodinated compounds undergo this peripheral deiodination by a group of enzymes called dehalogenases [24]. The activity of blood samples was fairly high (17.95±0.29% ID/g) 5 min post-injection and reached 3.10±0.02 % ID/g at 1 h post-injection. High radioactivity in the blood is attributed to plasma protein binding of propranolol [25]. The high renal uptake of radioactivity indicates that radio iodinated propranolol was excreted via renal pathway.

Molecular modeling studies

All structures were drawn using Chem Draw ultra. According to the chemistry of 1-substituted naphthalene, electrophilic substitution with radioiodine was expected to be homo nuclear (substitution on the same ring carrying the substituent) at C4 and less favorably at C2 [26]. Energy and torsion were calculated using MOE 2008.10 (MMF94X force field) for both 2-and 4-iodopropranolol. The results of energy calculation and minimization confirmed that 4-iodopropranolol is energetically more favored than 2-iodopropranolol.

Carazolol binding mode was studied to predict the possible binding mode of popranolol and ^{125}I-propranolol. Details of carazolol, propranolol and ^{125}I-propranolol hydrogen bonding and binding mode are shown in fig. 10 and table 3. From the docking data of the three compounds, the following can be deduced:

It was found from the binding mode of carazolol that Asn 312 and Asp 113 are important for high affinity binding of aryloxypropanolamines [15, 27]. Propranolol, binding to 2RH1 receptor is very similar to carazolol binding. Both compounds formed 4 hydrogen bonds with the receptor site. The docking score of propranolol-receptor complex (-7.65 Kcal/mol) was found to be lower than carazolol-receptor complex (-5.89 Kcal/mol) indicating that binding of propranolol was more favorable than carazolol. ^{125}I-propranolol formed 5 hydrogen bonds with the receptor site rather than 4. The docking score of ^{125}I-propranolol (-9.53 Kcal/mol) was also lower than both carazolol and propranolol. As a conclusion, the introduction of the heavy iodine atom didn't abolish binding of propranolol to its target receptor (2RH1). Rather, it caused inversion of the naphthalene ring in the binding pocket, higher number of hydrogen bonds and the more stable complex with the receptor.

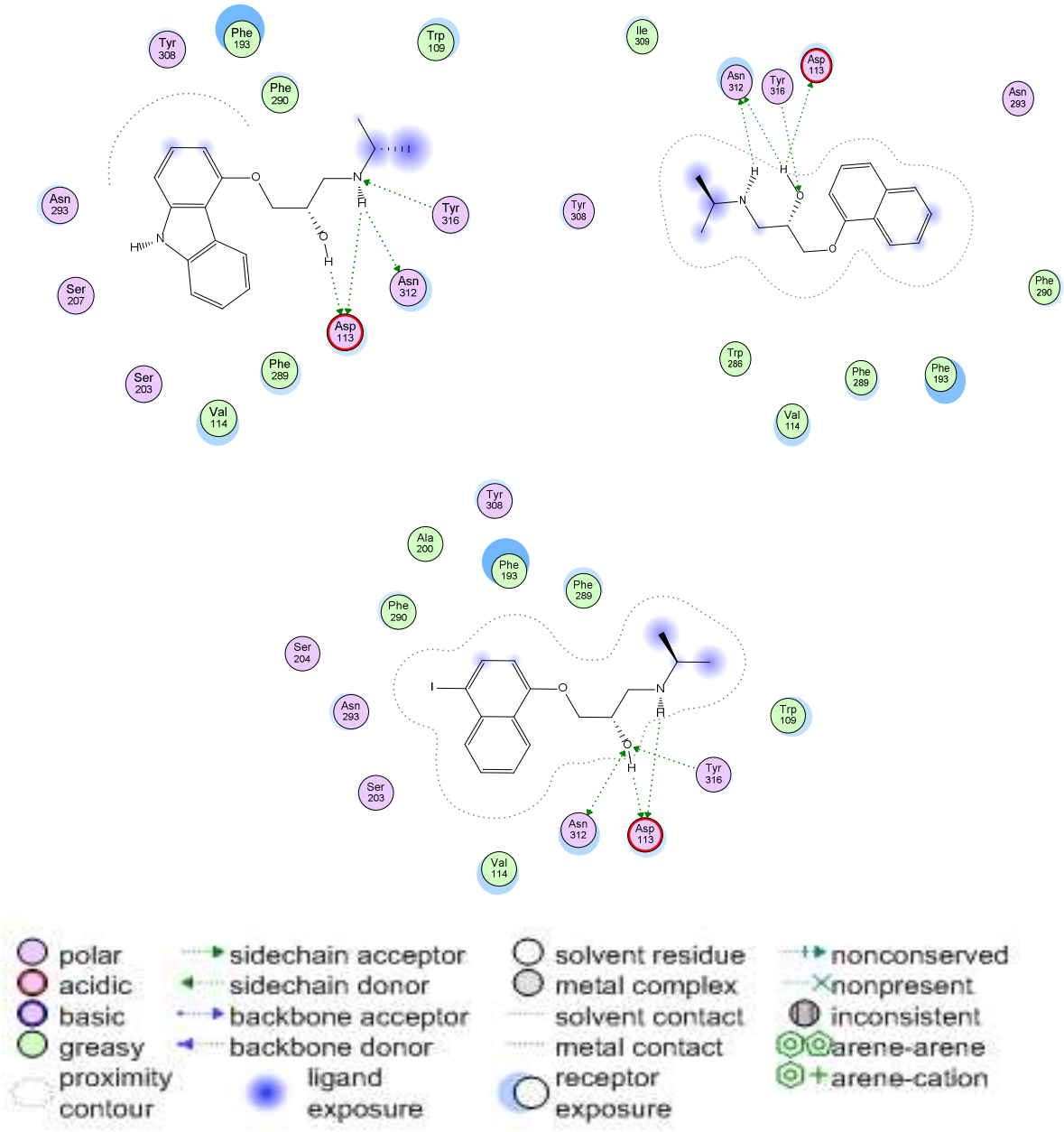


Fig. 10: Docking of carazolol, propranolol and ^{125}I-propranolol in the binding pocket of β2-adrenergic receptor (PDB 2RH1).

Table 3: Details of binding of Carazolol, propranolol and ^{125}I-propranolol in the binding pocket of β2 adrenergic receptor (PDB 2RH1)

Compound	Docking score (Kcal/mol)	No. of H-bonds	Amino acid residues forming hydrogen bonds (A °)
Carazolol	-5.89	4	Asp113 (2.37) Asp 113 (2.18) Asn 312 (3.13) Tyr 316 (3.13)
Propranolol	-7.65	4	Asp113 (2.48) Asn 312 (2.4) Asn 312 (2.82) Tyr 316 (2.79)
^{125}I-propranolol	-9.53	5	Asp 113 (2.7) Asn 113 (2.82) Asn 312 (2.4) Asn 312 (3.04) Tyr 316 (2.95)

CONFLICT OF INTERESTS

Declared None

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