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Radioiodination and biological evaluation of irbesartan as a tracer for cardiac imaging

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Abstract: Irbesartan was labeled using ^{125}I or ^{131}I with N-bromosuccinimide (NBS) as an oxidizing agent. A lot of operators such as, quantity of substrate, the quantity of oxidizing agent, reaction temperature, reaction time, and pH of reaction medium were studied to optimize high radiochemical yield of [^{125}I]IodoIrbesartan ([^{125}I]Irb.). The preclinical evaluation of IodoIrbesartan in experimental mice indicated high accumulation in target organ of heart with a high heart/blood ratio of 12.85 at 30 min post-injection. This study indicates the suitability of [^{125}I] IodoIrbesartan ([^{125}I]Irb.) for cardiac imaging.

Keywords: cardiac imaging; gamma camera; iodine-125; [^{125}I] IodoIrbesartan.

1 Introduction

Cardiac imaging in nuclear medicine can be used with high technology to estimate many defects of heart. Irbesartan (Figure 1), 2-butyl-3-([2′-(1H-tetrazol-5-yl) biphenyl-4-yl] methyl)-1,3 diazaspiro [4.4] non-1-en-4-one is determined as an (angiotensin II receptor blocker) that indicated to treat hypertension [1, 2]. Irbesartan blocks angiotensin II binding Angiotensin II receptor type 1 receptor in many tissues. In addition, it can bind the AT_1 receptor with 8500 times than it binds to the AT_2 receptor. Irbesartan can cause vascular smooth muscle relaxation and inhibits the aldosterone secretion that causing lowering blood pressure [3–6]. Due to the low energy of Iodine-125 (35 keV) therefore, Iodine-125 was utilized to achieve whole aspects of work excluding the imaging process in which Iodine-131 was used with high energy of 364 keV as gamma ray [7, 8]. This work was prepared to find out the possibility of using this radiotracer ([^{125}I]IodoIrbesartan, Figure 2) in radiography of the heart. The main operators that consider the responsible on the percent of the labeled compound were deliberated in giving the high radiochemical yield of the radioactive compound ([^{125}I]IodoIrbesartan). The biological distribution of radiotracer, [^{125}I]IodoIrbesartan and the extent of is uptake in the heart muscle compared to its concentration of blood was studied through empirical animals like mice [9–11]. Additionally, in recent study [12] a novel ^{18}F-labeled derivative of irbesartan was made for *in vivo* positron emission tomography (PET). To measure a angiotensin II type 1 receptor expression.

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2 Experimental

2.1 General

Irbesartan, N-bromosuccinimide (NBS), ethanol and methanol were bought from Sigma-Aldrich. TLC aluminum sheets (20 × 25 cm) SG-60 F_{254} were supplied by Merck. The chemicals were used as [8]. No-carrier-added (NCA) [^{125}I] as NaI (185 MBq/50 μL) diluted in 0.04 M NaOH, pH 9–11 was purchased from the institute of isotopes, Budapest, Hungary, <99%. Additionally, sodium [^{131}I]iodide (3.8 GBq/mL) diluted in 0.05 M NaOH, pH 8–11 for radiolabeling was given as a gift from radioisotopes production facility (RPF), AEA, Inshas, Egypt, <99%. High performance liquid chromatography (HPLC) was used to complete purification as [8] at 370 nm wavelength, the mobile phase, methanol-tetrahydrofuran-acetate buffer 47:10:43 v/v/v, pH 6.5 and a column temperature of 25 °C. [13] was delivered at 0.5 mL/min and the column Li Chrosorb (RP-C_{18}-150 × 4.6 mm, 5 μm) and the fractions were treated as [8]. The gamma camera (Philips axis gamma 2) to form two-dimensional images was used as [8, 12] at optimum conditions.

2.2 Radiolabeling procedure

The radiotracer, [^{125}I]IodoIrbesartan was synthesized by direct electrophilic radioiodination with Na ^{125}I in the presence of NBS as oxidizing agents. The quantity of sample was about ~ 600 μL in a two necked spherical bottomed flask outfitted with a reflux condenser and an elastic septum submerged in a thermostatically controlled water bath was added [^{125}I] NaI (7.2×10^{-3} GBq in 0.1% NaOH) up to dryness. Precisely amount of 100 μg NBS dissolved in ethanol (1 mg: 1 mL) adding to flask, followed by Irbesartan (100 μg) dissolved in ethanol (1 mg: 1 mL) with stirring by a magnetic stirrer at ambient temperature at optimum reaction time (15 min). At that point 150 μl of sodium metabisulphite (60 mg/ml H_2O) was added to stop the reaction.

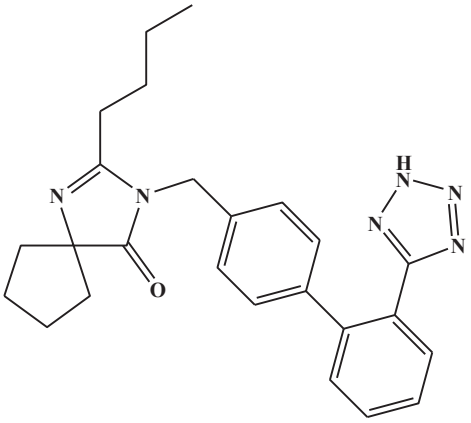

Figure 1: Radioiodination of irbesartan.

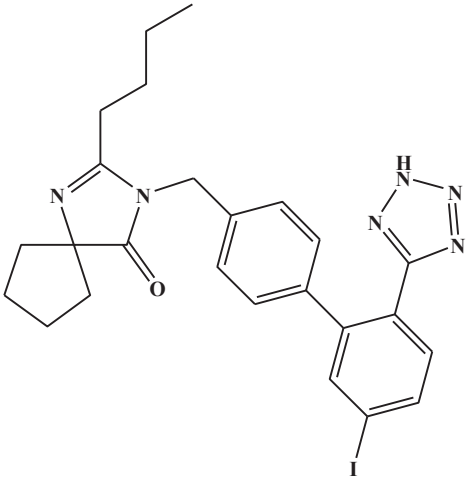

Figure 2: Propose structure of [^{125}I]IodoIrbesartan.

In addition, [^{131}I]IodoIrbesartan was prepared by the same method mentioned above of [^{125}I]IodoIrbesartan with the same yield. The radiochemical conversion to [^{125}I]IodoIrbesartan or [^{131}I]IodoIrbesartan was determined using TLC of aluminum-backed silica gel GF_{254} plates typically like [9]. A radiochemical conversion was further confirmed by HPLC analysis gave purity for [^{125}I]IodoIrbesartan reached to <99% after completely purified [14–17]. The specific activity under optimum conditions was 18 × 105 GBq/mmol for [^{125}I] NaI or [^{131}I] NaI.

2.3 Physicochemical evaluation

2.3.1 Stability in two different media: The stability of [^{125}I]IodoIrbesartan was made in two different media, rat serum and saline medium as follows: In human serum, purified [^{125}I]IodoIrbesartan or [^{131}I] IodoIrbesartan was detected by mixing 1.8 mL of serum with 0.2 mL of the radiotracer by volumes (v/v) and kept at 37 °C. Additionaly, [^{125}I] IodoIrbesartan was examined in saline medium. At time intervals, the stability was determined using TLC technique [15, 16, 18].

2.3.2 Biodistribution and animal studies: An adult mice were brought from animal house of Egyptian Company for production Vaccine, Sera, Biological Products and Drugs (EGY. VACSERA) in El-Dokei, the mice were weighed between 20 and 30 g. All animals were kept at a constant temperature (22 ± 0.5 °C) and they were treated like [8, 9]. To give 5 gps (5 mice for each group to give 25 mice in total, $n = 5$) intravenously injected with 0.2 mL ([^{125}I] NaI, 7.5×10^{-3} GBq) of [^{125}I] IodoIrbesartan adjusted to physiological pH. The study protocol, the ratios of organs (blood, bone, and muscles) and the corrections statics were tapically the same of [19, 20].

3 Results and discussion

3.1 Evaluation of radiochemical purity by TLC, and HPLC

The percent of labeled compound was confirmed on TLC silica gel by the percent proportion of free Iodide at retention factor (Rf) = 0.0–0.1 and for [^{125}I]IodoIrbesartan at Rf = 0.8–1.0. The yield percentage was deliberated as percent proportion activity of [^{125}I]IodoIrbesartan with regard to all out action on the thin layer chromatography strip. This method gives virtually equal values labeled compound percentage of 98.5%. The results were further confirmed by HPLC, in which the Rt values of free iodide, [^{125}I]IodoIrbesartan and UV were 3.5, 6.6, and 5.8 min respectively, as shown in Figure 3. The labeled compound percentage by HPLC of [^{125}I]IodoIrbesartan was 99%.

3.2 Reaction optimization

All factors were optimized at 37 °C to give high radiochemical purity. Figure 4a explains the impact of change in the quantity of Irbesartan. Indicating that the optimum radiochemical purity of [^{125}I]IodoIrbesartan was (98.5%) at 100 µg

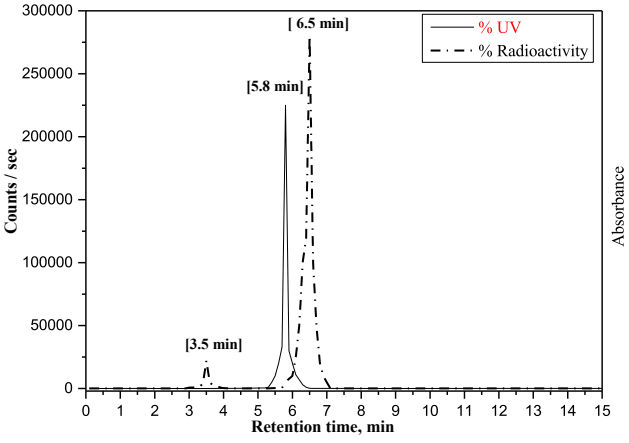

Figure 3: HPLC radiochromatogram of [^{125}I]IodoIrbesartan and its UV at optimum conditions.

of Irbesartan and ^{125}I-Na (7.5 MBq). Figure 4b indicates that the percent of labeled compound of [^{125}I]IodoIrbesartan was elevated up to amount of 100 µg of NBS, giving of 98.5%. Further increasing the quantity of NBS may lead to the consistence of undesirable by-product such as chlorination and polymerization [10–13]. Therefore, 100 µg of NBS gave optimum conversion to [^{125}I]IodoIrbesartan. The effect of reaction time was also studied, giving a maximum yield at 15 min in case of NBS (Figure 4c). In addition, pH of the medium is a critical factor in an optimum conversion to give high radiochemical yield of [^{125}I]IodoIrbesartan (Figure 4d) that need to be controlled, at pH 5 (acidic medium) gave high radiochemical yield of 98.5% which may reflect in part the stability of [^{125}I]IodoIrbesartan in acidic medium. In contrast in basic medium at pH 12 the percent of labeled compound of [^{125}I]IodoIrbesartan was decreased to give 56%.

Finally, *in vitro*: the stability of [^{125}I]IodoIrbesartan or [^{131}I]IodoIrbesartan was studied in two different media, rat serum, and saline solution. In rat serum, It was noticed that the stability of [^{125}I]IodoIrbesartan or [^{131}I]IodoIrbesartan was up to 12 h only to give 95.7 ± 0.56% and 96.32 ± 0.91%, then decreasing to give 80 ± 0.28% and 82 ± 0.18%, at 24 h respectively. but in saline up to 24 h to give 97.0 ± 0.34% and 98.2 ± 0.28% for [^{125}I]IodoIrbesartan or [^{131}I]IodoIrbesartan respectively too.

3.3 Gamma scintigraphy

It was shown (Figure 5) that the radiotracer, [^{131}I]IodoIrbesartan was located inside the heart muscle as target organ at 30 min. p.i., and this is harmonious to the biological studies.

3.4 Imaging and biodistribution

Table 1 shows the biodistribution of radio-IodoIrbesartan in different body organs and fluids. All radioactivity levels are expressed as average percent-injected tissue (%ID/gram ± S.D) [25–28]. Low thyroid uptake at all times indicated that the of [^{125}I]IodoIrbesartan was relatively stable *in vivo* [21–24]. Radioiodinated Irbesartan was parceled out speedily in most organs at 5 min p.i. [29–31]. The uptake within the kidneys increased from 3.22 ± 0.17% at 5 min p.i. to 22.12 ± 0.88% at 60 min p.i. which decline to 5.33 ± 0.44% at 2 h p.i. This indicated that the [^{125}I]radio-IodoIrbesartan was extracted through circulation of urinary pathways. The essential factors for dynamic heart imaging are the elevating of heart uptake and small heart/liver ratio with minimum lungs uptake. The radiotracer, [^{131}I]IodoIrbesartan exhibited an extensive uptake of 38.55 ± 0.67% ID/g in the cardiac muscle at 30 min and remained with time till 2 h p.i to give 5.44 ± 0.29% ID/g.,

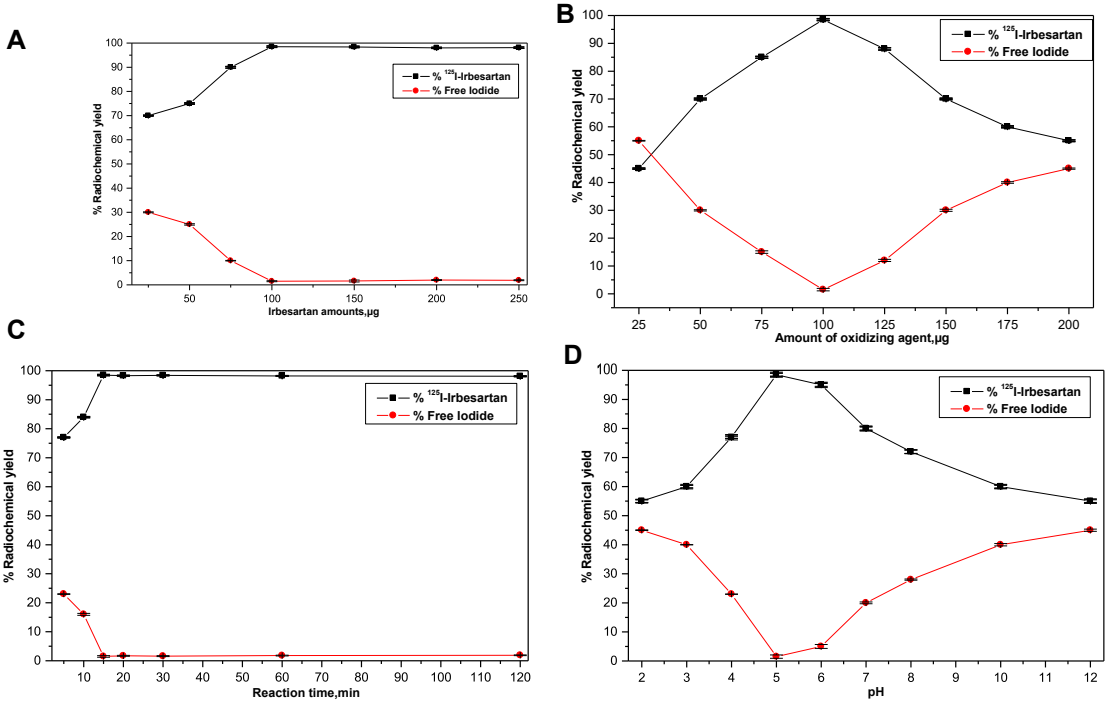

Figure 4: Variation of the radiochemical yield of radioiodinated Irbesartan as a function of olmesartan amount (A), NBS amount (B), reaction time (C) and pH (D).

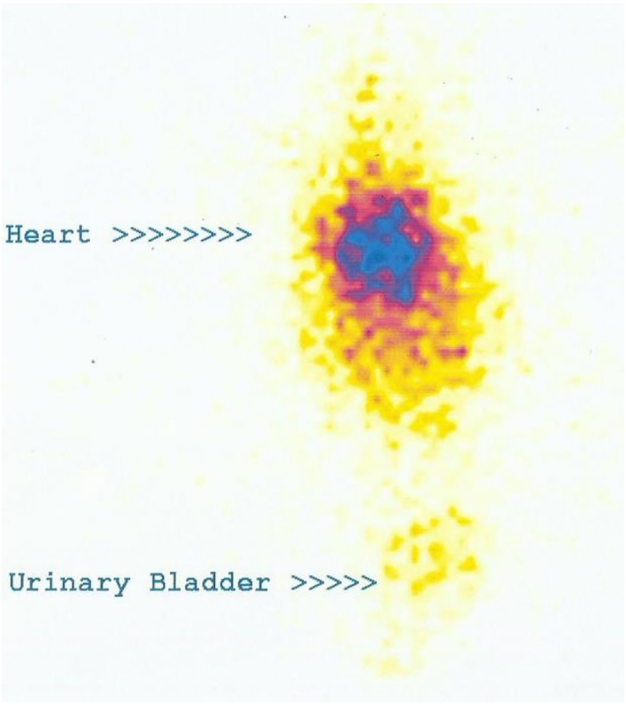

Figure 5: Gamma camera scintigraphy of radioiodinated Irbesartan at 30 min post injection.

where the tissue selective stability of labeled compound elucidated high receptor binding stability to A.R.B.

This result is confirmed by gamma camera analysis (Figure 5). It is necessary here to mention the ratio of the heart to blood of [^{125}I]IodoIrbesartan that was 1.21, 4.42, 12.85, 15.30, and 5.44 at five, ten, fifteen, thirty, sixty and hundred and twenty minutes, respectively. In a comparison, the heart to blood ratio of the radiotracer ([^{125}I]IodoIrbesartan) was higher than that of the following radiotracers of the same family like [^{125}I]olmesartan [9], ^{99m}Tc-losrtan [20], [^{125}I]olmesartan medoxomil [10] and [^{125}I]candesertan [11], it was found that they have less ratios than [^{125}I]IodoIrbesartan. In addition to that, all above radiotracers mentioned above have heart uptake less than that of [^{125}I]IodoIrbesartan which have maximum heart uptake of (35.54 ± 0.12% at 15 min), (8.2 ± 0.8% at 15 min), (33.80 ± 1.30% at 30 min), and (19.50 ± 0.51% at 5 min) respectively. Additionally, the heart to liver ratio of [^{125}I]IodoIrbesartan was 5.84, 8.84, 9.2, 5.57, and 2.9 at 5, 15, 30, 60, and 120 min, respectively. Hence the superiority of this radiotracer ([^{125}I]IodoIrbesartan) in radiographic imaging of the heart muscle with its use as a likely imaging agent with high accuracy is evident.

4 Conclusions

The synthesis of [^{125}I]IodoIrbesartan was made to give high radiochemical yield of 98.5% at optimum conditions. From biodistribution studies, it was concluded that the radioiodinated Irbesartan has a high heart uptake of 38.55 ± 0.67% Injected dose/gram at 30 min p.i. that confirmed by Gamma scintigraphy. Its uptake was much more than many radiotracers studied like [^{125}I]olmesartan, [^{99m}Tc] losrtan, and [^{125}I]olmesartan medoxomil. This puts in [^{125}I]IodoIrbesartan as a good radiotracer for heart imaging that could be attributed to its selectivity to A.R.B.

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Table 1: Biodistribution of [^{125}I]IodoIrbesartan in normal mice at different times.

Organs & body fluids	% I.D./g & body fluid at different times post injection				
	5 min	15 min	30 min	60 min	120 min
Blood	12.55 ± 0.19	6.15 ± 0.22	3.0 ± 0.19	1.20 ± 0.01	1.0 ± 0.03
Bone	1.11 ± 0.12	1.22 ± 0.01	1.1 ± 0.02	0.93 ± 0.001	0.88 ± 0.001
Muscle	1.12 ± 0.07	1.23 ± 0.11	1.21 ± 0.15	1.0 ± 0.02	0.95 ± 0.17
Brain	0.70 ± 0.02	0.66 ± 0.001	0.60 ± 0.02	0.52 ± 0.001	0.51 ± 0.001
Lungs	1.01 ± 0.01	1.18 ± 0.02	1.21 ± 0.01	1.0 ± 0.11	0.91 ± 0.01
Heart	15.18 ± 0.22	27.19 ± 0.88	38.55 ± 0.67	18.33 ± 0.77	5.44 ± 0.29
Liver	2.6 ± 0.17	3.22 ± 0.12	4.19 ± 0.15	3.29 ± 0.13	1.88 ± 0.25
Kidneys	3.22 ± 0.17	6.22 ± 0.19	12.67 ± 0.25	22.12 ± 0.88	5.33 ± 0.44
Spleen	1.23 ± 0.29	1.27 ± 0.01	1.11 ± 0.02	0.92 ± 0.03	0.89 ± 0.01
Intestine	1.19 ± 0.27	3.95 ± 0.01	5.34 ± 0.02	7.22 ± 0.28	4.29 ± 0.18
Stomach	1.01 ± 0.02	1.11 ± 0.05	0.95 ± 0.16	0.90 ± 0.04	0.82 ± 0.001
Thyroid	1.1 ± 0.01	1.20 ± 0.02	1.11 ± 0.14	0.89 ± 0.002	0.81 ± 0.001
Blood/Heart	1.21	4.42	12.85	15.30	5.44
Heart/Liver	5.84	8.44	9.2	5.57	2.9

Values represent mean ± SEM, n = 5.

Conflict of interest statement: The authors declare no conflicts of interest regarding this article.

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