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Radioiodinated esmolol as a highly selective radiotracer for myocardial perfusion imaging: *In silico* study and preclinical evaluation

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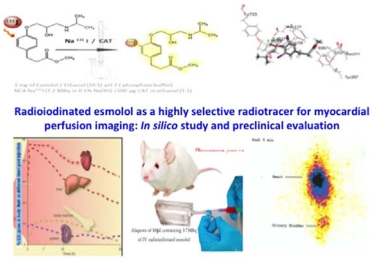
HIGHLIGHTS

- Radioiodination of esmolol for heart imaging evaluation.
- In vivo evaluation of radio iodinated esmolol in normal mice model.
- Gamma scintigraphy radio iodinated esmolol in normal mice model.
- Blocking studies to evaluate the *in vivo* targeting ability to β1-adrenergic receptors.
- Molecular modeling and docking studies to evaluated the in silico affinity of radio iodinted esmolol to β1-adrenergic receptors.

GRAPHICAL ABSTRACT



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ABSTRACT

Challenges facing cardiovascular imaging necessitate innovation of better radiopharmaceuticals to augment or replace the existing ones. This research assesses the ability and competency of radioiodinated esmolol as a potential cardio selective imaging agent. Radioiodinated esmolol was synthesized with 97.3 ± 0.3% radiochemical yield and with high stability up to 48 h at room temperature as well as in rat serum. Molecular modeling study was performed to confirm the binding of iodinated esmolol to β_1-adrenergic receptor. Its biodistribution studies in normal Swiss albino mice showed high heart uptake (38.5 ± 0.11%ID/g at 5 min p.i.), heart/liver ratio nearly 3.85:1 and heart/lungs ratio was about 7:1 at 5 min p.i. The evidenced selectivity of the radioiodinated esmolol to β_1-adrenoceptor was confirmed by prior injection of cold esmolol. Gamma camera biodistribution pattern showed that radioiodinated esmolol accumulated selectively in heart.

1. Introduction

The term "Heart diseases" includes a variety of heart pathological abnormalities. The most recognized one in the US is coronary artery disease (CAD), which influences blood flow into the heart muscle and heart diseases mortality is high in the US, interpreted as 1 among 4 deaths (CDC, 2014). In Egypt, ischemic heart disease is one of top 10 causes of death representing 21% of mortality (WHO, 2016). In 2012 cardiovascular diseases (CVDs) represented 31% of all global deaths (WHO, 2016). On the other hand, diagnostic vague cut-offs could be one of the most challenging causes behind mis or under treatment (Cleland, 2016; Mozaffarian et al., 2015). Single-center studies found

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that heart failure mis-detection in a corresponding community was about 50% of cases; as a result, appropriate treatment for heart failure is missed (Van Riet et al., 2016; Cleland, 2011).

High mortality and disabilities corresponding to cardiac diseases necessitate early diagnosis and management. A very important detection method of cardiac abnormalities is imaging (Van Riet et al., 2016; Mozaffarian et al., 2015; Cleland, 2011). Modalities for heart imaging have been developed to measure cardiac perfusion, ventricular wall function and coronary morphology for both management and research purposes that is done through imaging the myocardium and its adjacent cavity (Picano, 2011). An ultimate goal of cardiac imaging is providing an efficient investigation for the right patient at the right time. So it is crucial to be accurate and certain especially regards regarding differential diagnosis purposes. In that purposes, avoidance of overlapping interpretations is a must abandon repeated testing, delay of timely decision-making, and increased cost (Einstein et al., 2007). In contrast to other imaging modalities, nuclear medicine procedures are fit for mapping organ physiology and metabolic activity, thereby giving more accurate data about organ dysfunction (Cleland, 2002; Ohnesorge et al., 2000; Cerqueira, 2003).

The mapping of the radiopharmaceutical distribution *in vivo* gives information in a non-invasive technique, making it a golden choice in diagnostics era. The radiolabelled drug is intravenously injected, and its distribution and pharmacokinetics are measured (Cleland, 2002; Ohnesorge et al., 2000). Radiopharmaceuticals represent a class of drugs that are very safe preparations such as ^{18}F-FDG, ^{131}I and $^{99m}TcO_4^-$ with few adverse reactions and unexpected bio-distributions, making them powerful chances for research fields (Taylor et al., 2001; Swidan et al., 2014; Cerqueira, 2003). Within the past 40 years, using nuclear diagnostic cardiology has been encountered an exponential development to cope up with of diagnostic needs. Positron emission tomography (PET) beside single photon emission computed tomography (SPECT) enabled assessment of drug concentration in different tissues and organs and with magnetic resonance imaging (MRI) are considered now crucial ways to help in expanding cardiac conditions diagnose capacity (Budoff et al., 2005; Bateman et al., 2016; Baggish and Boucher, 2008). Technetium-99m or thallium-201 radiotracers are highly suitable for myocardial perfusion imaging (MPI) (Saraste et al., 2009; Tahara et al., 2014; Taylor et al., 2001).

Several challenges are facing cardiovascular imaging, such as small size and movement of the structures as well as few pathological substrates. Despite these challenges, markers imaging in the myocardium and vasculature, including altered energy metabolism, thrombosis, inflammation, neuronal function, apoptosis and angiogenesis is greatly promising nowadays (Baggish and Boucher, 2008; Taylor et al., 2001). That is why Innovation of better radiopharmaceuticals to augment or replace the existing ones, allows the acquisition of higher-quality, detailed diagnostic information and accuracy (Baggish and Boucher, 2008; Taylor et al., 2001). Radiopharmaceuticals for neuronal function imaging (metabolism, transmitter uptake, storage and release) may open the way to image dysfunction of cardiac sympathetic innervation to help in non-cardiac diseases diagnosis (diabetes and neurodegenerative disorders) (Baggish and Boucher, 2008). It is important to perform complete *in vitro*, *ex vivo* and *in vivo* studies for potential myocardial perfusion imaging (MPI) radiotracer to ensure its initial heart uptake, its fast liver clearance and obtaining heart images with good quality (Baggish and Boucher, 2008; Taylor et al., 2001).

^{99m}Tc-annexin-A5, ^{11}C-hydroxyephedrine, ^{111}In-GSAO, ^{11}C-CGP12177, radioiodinated-candesartan, ^{99m}Tc-losartan, radioiodinated-acebutolol, ^{99m}Tc-nebivolol, ^{99m}Tc-valsartan and ^{18}F-FDG had been examined in acute ischemia–reperfusion injury, tissue transplant rejection, myocarditis, and in a few advanced non-ischemic heart diseases (Taylor et al., 2001; Sogbein et al., 2014; Sanad et al., 2016a, 2016b, 2014; Ibrahim and Sanad, 2013; Swidan et al., 2014; Sakr et al., 2013).

Esmolol (Brevibloc®), Fig. 1., is a cardioselective agent. It is a class II antiarrhythmic. Esmolol represents one of good candidates for research as a selective cardiac nuclear imaging. It has a specific affinity for β_1-adrenergic receptors. Esmolol acts by blocking the action of the endogenous catecholamines, epinephrine and norepinephrine, at β_1-adrenoceptors. Its metabolites are clinically inactive and are eliminated renally (Wolman and Fiedler, 1991; Fita et al., 1999; Turlapaty et al., 1987; Sum et al., 1983). Accordingly, The aim of this study is to determine the probability of using radioiodinated esmolol as potential preclinical cardioselective imaging agent. This was attained through preparation of radioiodinated esmolol, studying its *in-vitro/in-vivo* stability, performing molecular modeling and evaluating its biodistribution pattern besides performing gamma scintigraphy on mice to confirm its ability to accumulate selectively in heart.

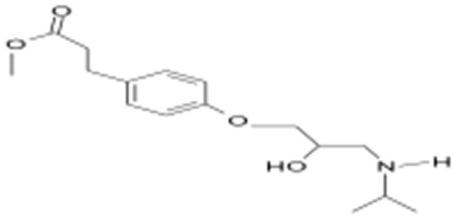

Fig. 1. Chemical structure of Esmolol.

2. Materials and methods

Esmolol, Chloramine-T, Sodium Metabisulfite, Ethanol and Chloroform were purchased from Sigma-Aldrich Company. No-carrier-added sodium iodide (NCA Na^{131}I, 3.7 GBq/mL in 0.1 N NaOH) was obtained as a gift from Radioisotope Production Facility (RPF), Egypt. γ-counter: Model Scaler Rate meter SR7, Nuclear enterprises LTD, USA. Electrophoresis apparatus: EC 3000P-series 90 programmable (E-C apparatus corporation) power supply and chamber unit. Shimadzu HPLC with 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, Shimadzu, C-R4A chromatopac, Pressure controller Dgu 2A and Fraction Collector-LKB, Bromma.

γ-Camera: A dual-head variable angle gamma camera from Siemens, Germany. The γ-Camera with a 5-mm pinhole collimator, window setting of suitable energy of I-131 (190–364 keV) γ-rays and 20% width, in the Nuclear Medicine Unit of the National Cancer Institute, Cairo University, Egypt,

2.1. Radioiodinated esmolol preparation and assay

2.1.1. Radiolabeling procedure

Radioiodinated esmolol, Fig. 2., was prepared by the direct electrophilic radioiodination using NCA ^{131}I ($t_{1/2}$ = 8 days) in the presence of chloramine-T (CAT) as oxidizing agent. Radioiodination conditions were optimized to obtain the highest radiochemical yield by using CAT (25–200 μg), concentration of esmolol (100–5000 μg), temperature (25 °C), pH of the reaction (2–12) and reaction time (5–60 min).

In a two-neck 25 mL round-bottomed flask fitted with a reflux condenser, radiolabeling was carried out. Firstly, 1 mg of esmolol dissolved in ethanol (10:1) was transferred to the reaction system and the pH was adjusted at 7 using phosphate buffer solution. Then NCA Na^{131}I (7.2 MBq in 0.1% NaOH) was added followed by accurately weighed 100 μg CAT dissolved in ethanol (1:1) was added to the reaction flask. The reaction mixture was stirred at ambient temperature for 15 min. A drop of saturated sodium metabisulphate (30 mg/mL saline) was added to decompose the excess of iodine in order to stop the reaction then the radioiodinated esmolol product was isolated (Greenwood and Earnshaw, 1988; Sanad et al., 2016a, 2016b; Ibrahim et al., 2015; Swidan et al., 2015; Motaleb et al., 2012; Rashed et al., 2014).