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Analysis of [^{11}C]methyl-candesartan kinetics in the rat kidney for the assessment of angiotensin II type 1 receptor density in vivo with PET☆

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ABSTRACT

Introduction: Angiotensin II type 1 (AT_1) receptors play a key role in the regulation of renal and cardiovascular functions and have been implicated in the pathogenesis of many diseases. The aim of this study was to assess binding of the novel radioligand [^{11}C]methyl-candesartan to AT_1 receptors in the rat kidney in vivo with PET.

Methods: Dynamic PET images were acquired for 60 min at baseline, with coinjection of candesartan (5 mg/kg), and after injection of PD123,319 (5 mg/kg). Volumes of distribution ($R_C \cdot V_T$) were estimated with a two-compartment model, by Logan analysis, and by taking the tissue-to-plasma activity ratio at 50–60 min post-injection.

Results: The two-compartment model did not describe the kinetics at baseline adequately and the baseline scans were too short to obtain accurate estimates of $R_C \cdot V_T$ with the Logan approach. Based on the tissue-to-plasma ratios, roughly one-third of V_T at baseline could be attributed to AT_1 receptor binding. There were no indications of AT_2 receptor binding in the rat kidney.

Conclusion: It may be possible to detect changes in AT_1 receptor density in the rat kidney in vivo with [^{11}C]methyl-candesartan and PET. Imaging AT_1 receptors with PET may provide valuable information on the role of these receptors in the pathogenesis of diseases such as hypertension, diabetic nephropathy, ventricular remodeling, and heart failure.

1. Introduction

The renin–angiotensin system (RAS) plays an important role in the regulation of renal and cardiovascular functions. Angiotensin II (Ang II), the biologically active component of this system, acts by stimulating membrane receptors in a number of tissues, including kidney, adrenal gland, blood vessel, heart, brain, lung, and liver [1]. The G-protein-coupled angiotensin II type 1 (AT_1) receptors mediate most of the physiological effects of Ang II, including vasoconstriction, elevation of blood pressure, increase in cardiac contractility, renal water and sodium absorption, and aldosterone secretion [1]. The RAS has been implicated in the pathogenesis of hypertension, diabetic nephropathy, ventricular remodeling, and heart failure [2–6].

Positron emission tomography (PET) may allow the investigation of the pathophysiology of diseases involving AT_1 receptors in vivo and may be helpful in the diagnosis of these diseases and in the assessment of their treatment. Several studies have been performed on experimental animals with the AT_1 receptor ligands [^{11}C]L-159,884 [7–11] and [^{11}C]KR31173 [12–14]. Binding specificity and selectivity of these radioligands for AT_1 receptors in the renal cortex have been demonstrated in mice [14], dogs [9,14], and baboons [14]. The effects of dietary sodium, estrogen, and chronic angiotensin-converting enzyme (ACE) inhibitor treatment on AT_1 receptor density in the dog kidney and the regulation of AT_1 receptor density in a swine model of renal artery stenosis have been investigated [8,10,11,13]. However, no PET studies of human AT_1 receptors have been reported to date.

We have recently described the synthesis of an alternative AT_1 receptor ligand, [^{11}C]methyl-candesartan, and of its desethyl derivative, [^{11}C]TH4 [15]. An important characteristic of [^{11}C]methyl-candesartan as a ligand for AT_1 receptor imaging is that it is a derivative of a compound (candesartan) already used widely in humans for treatment of hypertension and heart failure. The methyl-ester derivative was previously shown to exhibit a binding profile (IC_{50} for AT_1 receptors: methyl-candesartan 66 nM, candesartan 110 nM) and antagonistic activity similar to those of the parent candesartan [16]. Ex vivo experiments in rats have shown greater uptake of [^{11}C]methyl-candesartan and [^{11}C]TH4 in the renal cortex and outer medulla than in other tissues with the exception of the liver [15]. Furthermore, significant reductions in tracer retention in the renal cortex and outer medulla were observed after administration of AT_1 receptor antagonists, suggesting that both [^{11}C]methyl-

☆ The authors declare that they have no conflict of interest.

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candesartan and [^{11}C]TH4 bind specifically to renal AT_1 receptors [15]. The objective of this study was to assess binding of [^{11}C]methyl-candesartan to AT_1 receptors in the rat kidney in vivo with PET.

2. Methods

2.1. Radiochemistry

[^{11}C]Methyl-candesartan was synthesized as described previously [15] with high radiochemical purity. The specific activity at the time of injection ranged from 1.1 to 137.3 GBq/μmol.

2.2. Animals

The study was conducted in accordance with the recommendations of the Canadian Council on Animal Care and was approved by the Animal Care Committee of the University of Ottawa. Male Sprague–Dawley rats (Charles River, Montreal, Canada) were housed in pairs and were maintained on a 12-h light/dark cycle with free access to food and water.

2.3. Plasma-to-blood activity ratio

Arterial blood samples were collected from four rats to measure the ratio of activity in plasma to that in blood over time. Before cannulation of the carotid artery, the rats were given buprenorphine (0.04 mg/kg s.c., analgesic) and were anesthetized with 1%–2% isoflurane. [^{11}C]Methyl-candesartan (2 mCi) was injected into the tail vein and blood samples (200 μL) were collected at 0.5, 1, 5, 10, 20, 30, 45, and 60 min post-injection. The blood samples were weighed, counted in a Packard Cobra II gamma counter (Perkin Elmer, Boston, MA), and centrifuged (4000 rpm, 5 min) to obtain plasma. The plasma samples were in turn weighed and counted in the gamma counter. The ratio of activity (expressed in counts per min per g) in plasma to that in blood was calculated for each time point for each rat and then averaged across rats.

2.4. Labeled metabolites in plasma and kidney

A column-switching high-performance liquid chromatography (HPLC) method [17] was used to measure the percentages of unmetabolized [^{11}C]methyl-candesartan and labeled metabolites in plasma and kidney tissue (capture column: In-Line refillable guard column 2×20 mm with 2-mm 0.2-μm filter elements (Alltech, Deerfield, IL) hand-packed with Oasis HLB polymeric reverse phase sorbent (Waters Corp., Milford, MA), 1/99 acetonitrile/water, 2 mL/min; analytical column: Luna C18 10 μm 100 A 250×4.60 mm (Phenomenex, Torrance, CA), 35/65 acetonitrile/0.1 M ammonium formate, 1 mL/min, retention time of unmetabolized [^{11}C]methyl-candesartan = 15.8 min post-switch). Rats received 222–740 MBq of [^{11}C]methyl-candesartan and were sacrificed at 3, 15, and 20 min post-injection for collection of trunk blood and removal of kidneys. Blood was centrifuged at 4000 rpm for 5 min to obtain plasma. Dissected tissue samples were homogenized in 80:20 ethanol/water (v/v) using a polytron homogenizer and centrifuged (82,000×g) for 15 min. Supernatant was collected and evaporated, then reconstituted in 1:99 MeCN/water (v/v). Before injection into the HPLC system, plasma and kidney homogenate samples were microfiltered through 0.22 μm Cathivex-GS syringe filters and urea (1 g/mL and 0.4 g/mL, respectively) was added to disrupt plasma protein binding. Three or four measurements were obtained for each time point. Similar procedures were followed for six additional rats who received a coinjection of [^{11}C]methyl-candesartan and a blocking dose (5 mg/kg) of candesartan. These rats were sacrificed at 15 min post-injection.

2.5. Plasma protein binding

Plasma protein binding of [^{11}C]methyl-candesartan was measured in duplicate using a commercially available kit (Micon Bioseparations). Authentic [^{11}C]methyl-candesartan was added to plasma obtained from one rat sacrificed by decapitation without anesthesia. The plasma was centrifuged (2000×g, 30 min) at room temperature and the resulting filtrate (protein-free fraction) counted in the gamma counter.

2.6. PET data acquisition

Rats (231–405 g) were anesthetized with 1%–2% isoflurane and were placed supine in an Inveon DPET scanner (Siemens, Knoxville, TN). A 10-min ^{57}Co transmission scan was acquired to measure attenuation. [^{11}C]Methyl-candesartan (15–107 MBq) was injected into the tail vein and dynamic PET data were acquired for 60 min (12×10 s, 3×60 s, 11×300 s). Fourteen rats were scanned under baseline conditions. Of these, six were scanned a second time within 14 days to assess test–retest repeatability. In addition, candesartan (5 mg/kg dissolved in 10% sodium bicarbonate in saline) was coinjected with [^{11}C]methyl-candesartan in four rats and PD123,319 (5 mg/kg dissolved in saline) was injected intravenously 5 min before tracer injection in six rats. Heart rate, respiratory rate, and temperature were monitored throughout the scans. PET images were reconstructed using the vendor-provided 3-dimensional ordered-subset expectation maximization maximum a posteriori algorithm ($\beta = 1$) with corrections for detector efficiency and dead time, isotope decay, photon attenuation, and random and scattered coincidences.

2.7. Image analysis

The Inveon Research Workplace software v3.0 (Siemens, Knoxville, TN) was used to determine the time-course of activity concentration in arterial blood and in the left kidney. A sphere was drawn over the left atrium in an early frame and a region of interest was defined as those pixels within the sphere with intensity greater than 80% of the maximal intensity. Similarly, a three-dimensional ellipsoid was drawn over the lower two-thirds of the left kidney in a later frame and a region of interest was defined as those pixels within the ellipsoid with intensity greater than 50% of the maximal intensity. The time–activity curves for arterial blood and for the left kidney were obtained by averaging the activity in these regions of interest for each frame. The remainder of the analysis was performed using custom software in Matlab (The MathWorks, Natick, USA).

2.8. Compartmental modeling

HPLC analysis revealed that, under baseline conditions, at 20 min post-injection, ~66% of the radioactivity in kidney samples can be attributed to unmetabolized [^{11}C]methyl-candesartan and that the desethyl derivative [^{11}C]TH4 accounts for most of the remaining radioactivity (~28%). In contrast, nearly all the radioactivity in plasma at this time can be attributed to the unmetabolized tracer, with only a small percentage (~4%) due to [^{11}C]TH4 (see Results). It appears, therefore, that the transformation of [^{11}C]methyl-candesartan to [^{11}C]TH4 takes place within the kidney. The results of previous ex vivo experiments suggest that [^{11}C]methyl-candesartan and [^{11}C]TH4 bind reversibly to AT_1 receptors in the renal cortex and outer medulla [15]. In addition, dosimetry studies revealed the presence of radioactivity in urine after the injection of [^{11}C]methyl-candesartan in rats (unpublished). The diagram in Fig. 1A illustrates the postulated tracer kinetics. Because [^{11}C]TH4 accounts for a small portion of the radioactivity in plasma and to simplify the model and the analysis considerably, we assume that the concentration of [^{11}C]TH4 in plasma, $C_{P\cdot MET}(t)$, is negligible.