Research Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 18 December 2007, Revised 28 February 2008, Accepted 4 March 2008 Published online in Wiley Interscience

(www.interscience.wiley.com) DOI: 10.1002/jlcr.1517

Synthesis of ^{11}C-labelled metomidate analogues as adrenocortical imaging agents

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Clinical findings using [^{11}C]methyl 1-[(1*R*)-1-phenylethyl]-1*H*-imidazole-5-carboxylate ([^{11}C]MTO, 1) show high uptake in lesions of adrenocortical origin, including adenomas, but low uptake in lesions of non-adrenocortical origin. In this paper the synthesis and preclinical evaluation of two new ^{11}C-labelled analogues of MTO, [^{11}C]methyl 1-[(1*R*)-1-(4-chlorophenyl)ethyl]-1*H*-imidazole-5-carboxylate ([^{11}C]CLM, 2) and [^{11}C]methyl 1-[(1*R*)-1-(4-bromophenyl)ethyl]-1*H*-imidazole-5-carboxylate ([^{11}C]BRM, 3), using frozen-section autoradiography, organ distribution and a metabolic study are presented.

Keywords: PET; [^{11}C]methylation; MTO; metomidate; adrenocortical tumours

Introduction

The 1-[(1*R*)-1-phenylethyl]-1*H*-imidazole-5-carboxylic acid esters are a class of compounds that interact selectively with the mitochondrial cytochrome P-450 species in the adrenal cortex.[1] Compounds labelled with β^+-emitting radionuclides such as ^{11}C ($t_{1/2}$ = 20.3 min) and ^{18}F ($t_{1/2}$ = 109.7 min) might serve as radiotracers for the diagnosis of adrenal cortical masses such as incidentalomas, adenomas and primary and metastatic adrenocortical carcinoma. Clinical findings with the radiotracer [^{11}C]methyl 1-[(1*R*)-1-phenylethyl]-1*H*-imidazole-5-carboxylate [^{11}C]MTO (**1**) have indicated high uptake in lesions of adrenocortical origin, including adenomas, but very low uptake in lesions of non-adrenocortical origin.[2,3] A study of the value of [^{11}C]MTO **1** in the diagnosis of gliomas, and in differentiation between malignant and benign ones, showed that tumours of all types had remarkable uptake of the positron emission tomography (PET)-tracer.[2] A retrospective study demonstrated that MTO-PET is very useful in the imaging work up of adrenal incidentalomas and may be beneficial for the examination of patients with primary aldosteronism or adrenocortical cancer.[4] Although [^{11}C]MTO **1** has good biological properties for visualization of the adrenals and their tumours, there remains interest in improved tracers with improved properties especially in regard to the adrenal-to-liver ratio. The assessment of the right adrenal is hampered by the large uptake in the liver.

The halogenated analogues of **1**, [^{11}C]CLM **(2)** and [^{11}C]BRM (**3**) were synthesized and some of their biological properties were explored.

Results and discussion

[^{11}C]MTO (**1**), which is a tracer binding to 11β-hydroxylase,[2] was developed in response to the clinical need for a PET imaging agent that could visualize and characterize lesions found incidentally around the adrenals during CT of the abdomen. This tracer could differentiate between malign and benign lesions found in incidentalomas.[2,3] In this report, two potential ^{11}C-analogues of MTO were synthesized and a biological assay with MTO was used to decrease liver uptake compared with the lead. The calculated log *P*-values for the MTO analogues using ACD-labs[5] were 2.73 ± 0.34 (**1**), 3.32 ± 0.35 (**2**) and 3.50 ± 0.43 (**3**).

1-[(1*R*)-1-Phenylethyl]-1*H*-imidazole-5-carboxylate analogues were synthesized from the corresponding enantiopure phenylethylamine as described in the literature,[6] and labelled as shown in Scheme 1.

Frozen-section autoradiography

As the first validation step, frozen-section autoradiography was used to show binding properties.[2,7–9] The incubation of frozen sections with [^{11}C]MTO and its analogues was used to show specific binding to the adrenal cortex and few other tissues (Figure 1). The degree of specific binding in rat adrenal was 83% (**1**), 62% (**2**) and 21% (**3**). In rhesus adrenal the specific binding was 76, 65 and 35%, respectively.

Organ distribution

Organ distribution was used to access the adrenal uptake as well as the adrenal-to-organ ratio for the different analogues. The

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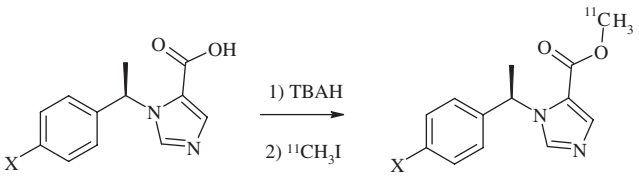

(**4**) X = Cl
(**5**) X = Br

(**1**) X = H
(**2**) X = Cl
(**3**) X = Br

Scheme 1. ^{11}C-labelled ester analogues.

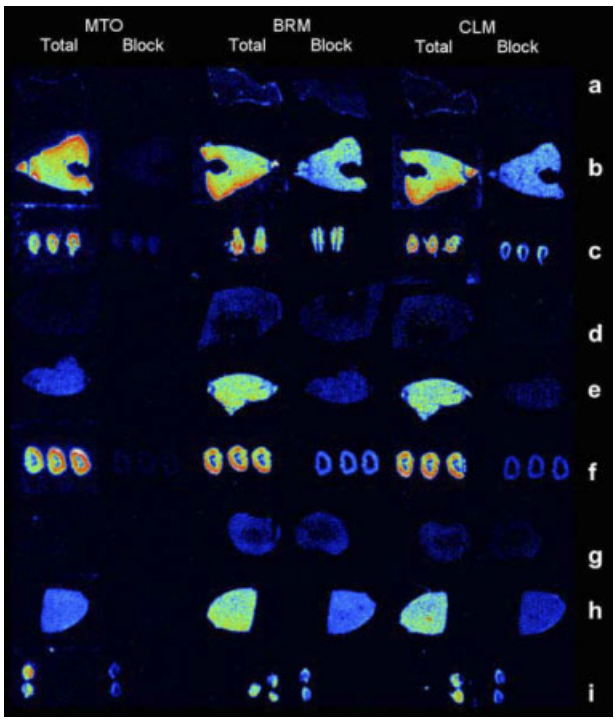

Figure 1. Frozen-section autoradiography of [^{11}C]MTO (**1**), [^{11}C]BRM (**3**), and [^{11}C]CLM (**2**). Total binding and non-specific binding (after blocking with 1 μM etomidate) are shown. Organs, from top: (a) rhesus monkey kidney, (b) rhesus monkey liver, (c) rhesus monkey adrenal, (d) pig kidney, (e) pig liver, (f) pig adrenal, (g) rat kidney, (h) rat liver and (i) rat adrenal.

standardized uptake values (SUVs) for all organs were lower for the analogues having a halogen in the para position on the phenyl group than for MTO (Table 1). However, the adrenal-to-organ ratios increased more rapidly for these analogues than for MTO itself (Figure 2). In liver the adrenal-to-liver and blood ratio remains unchanged at the time points studied, whereas those for the halogen analogues increases with time.

Analysis of radiolabelled metabolites in plasma

A metabolite study was performed on **2** and **3**. Blood samples were collected 5 and 30 min after injection to measure the amounts of unchanged tracer and radioactive metabolites in plasma. The metabolite analysis revealed that tracer **2** was 62 and 16% intact after 5 and 30 min of injection, respectively. The recovery was 69 ± 10%. One hydrophilic metabolite was observed. The metabolite analysis revealed that tracer **3** was 52 and 37% intact after 5 and 30 min of injection, respectively. The recovery was >99%. One hydrophilic metabolite was observed. The results from the metabolite study indicate that analogue **3** is more stable over time in rat, than analogue **2**.

Table 1. Organ distribution of (**1**), (**2**) and (**3**) after 15, 30 and 60 min in rat ($n = 2$, except for ∗ where $n = 1$)

	1		2		3	
	Mean	SD	Mean	SD	Mean	SD
15 min						
Blood	0.80	0.27	0.15	0.02	0.28	0.00
Heart	0.74	0.19	0.22	0.04	0.47	0.04
Lung	11.53	1.88	2.81	1.82	8.35	0.43
Liver	2.97	0.58	0.55	0.02	1.39	0.20
Pancreas	2.80	0.35	0.89	0.19	2.06	0.24
Adrenal	12.02	0.33	2.71	0.38	8.30	2.57
Kidney	8.13	2.19	0.43	0.06	0.94	0.08
Testis	0.64	0.54	0.21	0.05	0.53	0.08
Brain	0.77	0.18	0.20	0.03	0.40	0.04
30 min						
Blood	0.85	*	0.13	0.02	0.37	0.22
Heart	0.78	*	0.15	0.01	0.45	0.25
Lung	5.20	*	3.39	2.08	6.51	7.64
Liver	3.27	*	0.61	0.06	1.78	0.70
Pancreas	1.82	*	0.56	0.03	2.21	1.11
Adrenal	12.38	*	2.71	0.19	12.87	3.54
Kidney	4.14	*	0.29	0.02	0.86	0.46
Testis	0.79	*	0.18	0.01	0.53	0.21
Brain	0.73	*	0.12	0.01	0.34	0.19
60 min						
Blood	0.79	0.02	0.09	0.01	0.29	0.05
Heart	0.62	0.00	0.09	0.02	0.24	0.07
Lung	3.31	0.71	1.14	0.15	3.46	2.07
Liver	3.12	0.13	0.35	0.04	1.47	0.45
Pancreas	1.48	0.36	0.32	0.04	1.15	0.20
Adrenal	13.12	0.62	2.47	0.55	11.87	1.88
Kidney	3.18	0.35	0.17	0.05	0.48	0.11
Testis	0.55	0.04	0.07	0.02	0.21	0.05
Brain	0.56	0.02	0.06	0.01	0.18	0.08

Organ values are shown as SUV (mean ± SD).

Materials and methods

General

[^{11}C]Carbon dioxide was produced at Uppsala Imanet by the ^{14}N(p,α)^{11}C reaction in a gas target containing nitrogen (AGA, Nitrogen 6.0) and 0.1% oxygen (AGA, Oxygen 4.8), using 17 MeV protons produced from a Scanditronix MC-17 cyclotron. [^{11}C]Carbon dioxide was converted in two steps to [^{11}C]iodomethane via reduction with lithium aluminium hydride and subsequent treatment with hydroiodic acid.[10] Liquid chromatographic analysis was performed with a Beckman 126 gradient pump and a Beckman 166 variable wavelength UV-detector (Fullerton, CA, USA) in series with a β^+-flow detector. The following mobile phases were used: 50 mM ammonium formate pH 3.5 (A) and acetonitrile/water

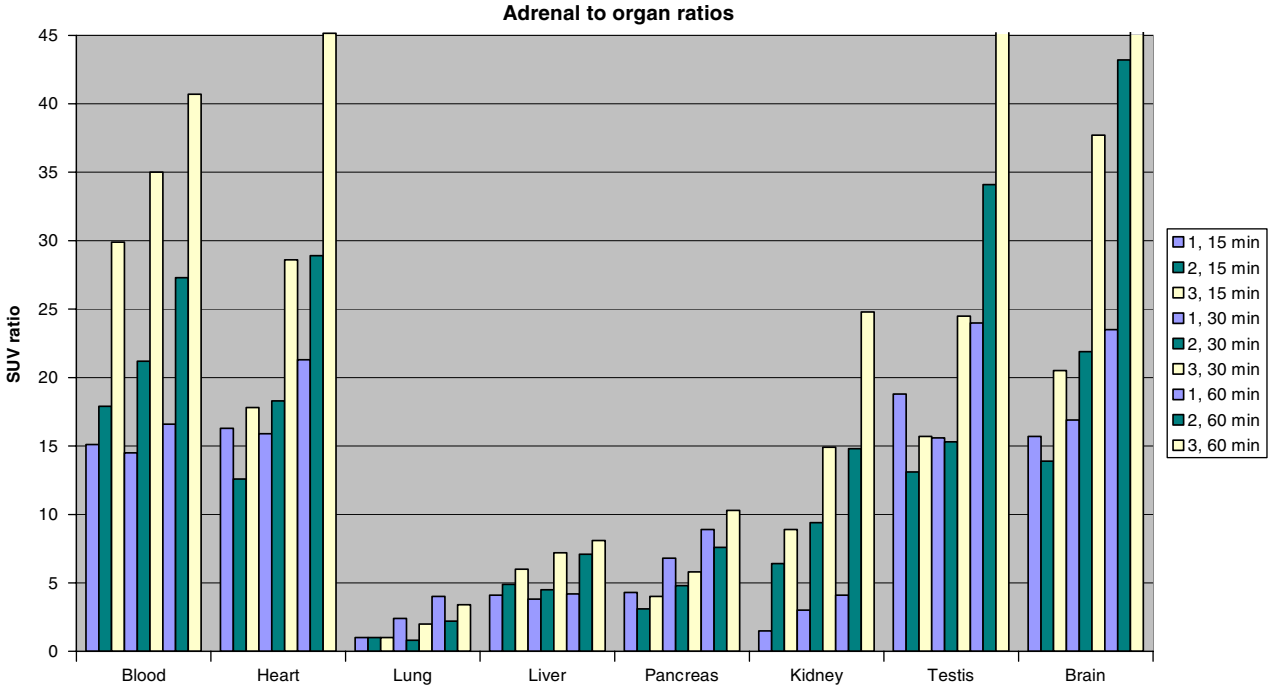

Figure 2. Adrenal-to-organ ratios with compound (**1**), (**2**) and (**3**) after 15, 30 and 60 min in rat (calculated from Table 1). This figure is available in colour online at www.interscience.wiley.com/journal/jlcr.

(50:7) (B). For analytical LC, a Jones chromatography genesis C_{18}, 4 μm × 250 mm × 4.6 mm column was used at a flow of 1.5 ml/min. For semi-preparative LC, a Jones chromatography genesis C_{18} column, 4 μm × 250 mm × 10 mm, was used at a flow of 5 ml/min. Synthia®, an automated synthesis system,[11] was used for LC injection and fraction collection. Data collection and LC control were performed with the use of a Beckman System Gold chromatography software package (USA). Radioactivity was measured in an ion chamber (Veenstra Instrumenten bv, VDC-202, Holland). For coarse estimations of radioactivity during synthesis, a portable dose-rate meter was used (Långenäs elektriska AB, Sweden). In the analysis of labelled substances, reference substances were used for comparison in LC runs. All syntheses were conducted under a nitrogen atmosphere using dried glassware and magnetic stirring. All chemicals and anhydrous solvents were bought from commercial suppliers. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ (7.26 ppm ^{1}H, 77.0 ppm ^{13}C) on a Varian Unity 400 spectrometer (400 MHz for ^{1}H and 100.6 MHz for ^{13}C nuclei). Liquid chromatography mass spectrometry (LC-MS) analyses were performed using a Gilson HPLC and Finnigan AQA mass spectrometer, in ESI mode.

General method for ^{11}C-labelling: [^{11}C]methyl 1-[(1*R*)-1-phenylethyl]-1*H*-imidazole-5-carboxylate analogues (1), (2) and (3)

The corresponding carboxylic acid (1.4 ± 0.1 mg) in dichloromethane (300 μl) was treated with tetrabutylammonium hydroxide (TBAH, 20%, 3 ± 1 μl). The solvent was then evaporated and co-evaporated with dichloromethane (2 × 300 μl). The activated carboxylate was dissolved in dimethyl formamide (300 μl). [1-^{11}C]Iodomethane[10] was trapped in the solution and heated at 130°C for 7 min. The crude product was injected on semi-preparative LC (**1**, **2** and **3**; t_R = 5.54, 6.80 and 7.47 min, respectively), flow 5 ml/min, mobile phase A:B (40:60). The organic solvent was evaporated from the collected fractions, and the residue was dissolved in phosphate buffer (pH = 7.5, 4 ml) containing ethanol (99.5%, 0.4 ml). Sterile filtration of the final product solution through a filter (Dynagard ME, 0.22 mm pore size) into a sterile vial was performed. Radiochemical purity (> 97%) and analytical radiochemical yield 60–80% were determined by analytical LC. Analytical LC (**1**, **2** and **3**; t_R = 5.50, 6.71 and 7.52 min, respectively), mobile phase A:B (40:60).

Precursors and references

Methyl 1-[(1*R*)-1-(4-*chlorophenyl*)*ethyl*]-1*H*-*imidazole*-5-*carboxylate* (**2**): ^{1}H NMR ($CDCl_3$): δ 7.66 (s, 1H), 7.60 (s, 1H), 7.08 (d, 2H), 6.92 (d, 2H), 6.14 (q, 1H), 3.58 (s, 3H), 1.64 (d, 3H). ^{13}C NMR ($CDCl_3$): δ 160.0, 139.5, 139.2, 137,6, 133.2, 128.2, 127.3, 121.8, 54.4, 51.0, 21.6. LC-MS (ESI^+), *m/z* 265 (75.8%), 267 (24.2%) $[M+H]^+$.

Methyl 1-[(1*R*)-1-(4-*bromophenyl*)*ethyl*]-1*H*-*imidazole*-5-*carboxylate* (**3**): ^{1}H NMR ($CDCl_3$): δ 9.17 (s, 1H), 7.95 (s, 1H), 7.55 (d, 2H), 7.22 (d, 2H), 6.49 (q, 1H), 3.93 (s, 3H), 2.01 (d, 3H). ^{13}C NMR ($CDCl_3$): δ 158.4, 150.4, 137.4, 132.8, 128.8, 127.4, 123.7, 111.9, 58.4, 53.2, 22.2. LC-MS (ESI^+), *m/z* 309 (50.7%), 311 (49.3%) $[M+H]^+$.

1-[(1*R*)-1-(4-*Chlorophenyl*)*ethyl*]-1*H*-*imidazole*-5-*carboxylic acid* (**4**): ^{1}H NMR ($CDCl_3$): δ 8.06 (s, 1H), 7.85 (s, 1H), 7.29 (d, 2H), 7.16 (d, 2H), 6.58 (q, 1H), 1.85 (d, 3H). ^{13}C NMR ($CDCl_3$): δ 176.1, 163.0, 138.9, 137.6, 134.0, 133.2, 129.0, 127.8, 55.0, 21.0. LC-MS (ESI^+), *m/z* 251 (75.8%), 253 (24.2%) $[M+H]^+$.

1-[(1*R*)-1-(4-*Bromophenyl*)*ethyl*]-1*H*-*imidazole*-5-*carboxylic acid* (**5**): ^{1}H NMR ($CDCl_3$): δ 12.97 (bs, OH), 8.11 (s, 1H), 7.87 (s, 1H), 7.45 (d, 2H), 7.10 (d, 2H), 6.53 (q, 1H), 1.85 (d, 3H). ^{13}C NMR ($CDCl_3$): δ 162.7, 138.9, 136.8, 131.9, 130.4, 128.1, 126.7, 122.2, 55.4, 21.5 LC-MS (ESI^+), *m/z* 295 (50.7%), 297 (49.3%) $[M+H]^+$.

Frozen-section autoradiography[12–14]

Frozen sections (20 μm) were prepared in a cryomicrotome and put on superfrost glass slides. Kidney, liver and adrenal from rhesus monkey, pig and rat were used. The slides were kept in a freezer (-20°C) before use. At the start of the experiment, the slides were pre-incubated for 10 min in TRIS (50 mM, pH = 7.4) buffer. The slides were then transferred to containers containing approximately 1 nM of compound (**1**), (**2**) or (**3**) in TRIS buffer. In a duplicate set of containers, 1 μM of etomidate was added to block specific binding. After incubation for 30 min, the slides were washed for 3 × 3 min in buffer. The slides were dried and then exposed to phosphor imaging plates (Molecular Dynamics, USA) for 40 min and scanned in a Phosphor Imager Model 400S (Molecular Dynamics, USA, purchased from Amersham Bioscience, Uppsala, Sweden).

Organ distribution[12–14]

The *in vivo* studies were carried out in adult male Sprague Dawley rats (300–700 g) (Möllegård, Denmark). All animals were handled according to the guidelines of the Swedish Animal Welfare Agency, and the experiments were approved by the local Ethics Committee for Animal Research, permit no: C46/3. In order to evaluate uptake in normal tissues, a biodistribution study was performed. Six rats were injected intravenously with 20 MBq/kg (**1**), (**2**) or (**3**) solution. At 15, 30 and 60-min post-injection, two animals/time point were sacrificed and their organs were dissected out, weighed and measured. Organ values were calculated as SUVs as follows:

$$\mathrm{SUV} = \frac{\mathrm{ACT(Bq/cm^3)} \times 1000(\mathrm{cm^3/kg})}{\mathrm{DOSE(Bq)/BW(kg)}}$$

where ACT is the measured concentration of radioactivity corrected for physical decay form start of camera, DOSE is the administered amount of radioactivity (Bq), BW is the body weight of the subject (kg) and 1000 is an approximate conversion of body weight to body volume. Ratios of adrenal uptake versus other organs were calculated.

Metabolite study

The metabolite analysis in plasma was performed in rat at the 5- and 30-min time points. At each time point, one male rat was injected via the tail vein with 50 MBq radioligand. The plasma was obtained by a 2-min centrifugation of the venous blood at 3082 × *g* at 4°C. Plasma proteins were precipitated by adding acetonitrile (600 μl) containing unlabelled reference to the plasma (600 μl). The resulting mixture was centrifuged at 16 100 × *g* for 1 min at 4°C. The supernatant was filtered through a 0.2 μm pore size membrane (Corning Incorporated, Corning, NY, USA) while centrifuging at 16,100 × *g* for 1 min at 4°C. The sample was then analysed by HPLC to separate the radioligand from radioactive metabolite. The sample (90 μl) was injected on an ACE 5 C18-HL 250 × 10 mm column and the tracer and its metabolite were eluted with MeCN–50 mM AMF (60:40 vol/vol) at a flow rate of 5 ml/min. The eluent was collected in two fractions. Observation of the UV peak from the unlabelled standard indicated that [^{11}C]CLM and [^{11}C]BRM eluted in the second fraction. The radioactivity of different fractions was measured in a γ-counter. Even if no detectable radiopeak co-eluted with the unlabelled standard, the fraction was collected and its radioactivity was measured.

Conclusion

MTO-PET is a specific and sensitive method for diagnosing adrenal incidentalomas and for the examination of patients with adrenocortical cancer. The addition of a halogen in compound **2** and **3**, two new ^{11}C-labelled analogues of **1**, showed increased adrenal-to-liver ratios in rats and primates compared with **1**. This may be advantageous in the further characterization of incidentalomas since assessment of the right adrenal can be made more readily. The possibility to detect adrenal metastases in the liver is also improved with an increased ratio. The adrenal-to-organ ratios indicate that analogue **3** has better properties with regard to ratio to liver and stability in blood over time.

Acknowledgement

This work was conducted in collaboration with Uppsala Imanet, GE Healthcare. We are grateful to Elisabeth Bergström-Pettermann for her assistance in the biological study.

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