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High-performance liquid chromatographic determination of [^{11}C]1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinoline carboxamide in mouse plasma and tissue and in human plasma

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Abstract

The high-performance liquid chromatographic determination of 1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinoline carboxamide ([^{11}C]PK 11195) is described. The method was successfully applied for plasma and tissue analysis after i.v. injection of [^{11}C]PK 11195 in mice and for plasma analysis after administration of [^{11}C]PK 11195 to humans. Separation is effected on a RP-C_{18} column, using a mixture of acetonitrile–water–triethylamine (65:35:0.5, v/v). Quantitative measurements of radioactivity are performed on a one-channel γ-ray spectrometer equipped with a 2×2 in. NaI(Tl) detector. For humans rapid metabolisation of [^{11}C]PK 11195 was observed. At 5, 20 and 35 min post injection 5%, 22% and 32%, respectively, of the plasma activity consisted of at least two more polar metabolites. Despite the extensive metabolisation rate in mice (up to 42% at 10 min post injection of [^{11}C]PK 11195), no ^{11}C-labelled metabolites could be detected in the extracts of brain and heart. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Peripheral benzodiazepine receptor; PK 11195; 1-(2-Chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinoline carboxamide

1. Introduction

The peripheral benzodiazepine receptor (PBZR) is distinct from the central benzodiazepine receptor which is associated with the $GABA_A$ receptor complex. The PBZR is located on the mitochondrial membrane and is present in high concentrations in peripheral organs such as heart, lungs and adrenal glands [1] as well as in the brain, where it is associated with glial cells [2]. The PBZR is also found in several cell types associated with the immune system, such as mast cells and macrophages [3].

PK 11195 [1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinoline carboxamide, CAS registry No. 85532-75-8] (Fig. 1, **I**) is an isoquinoline derivative with high specificity, affinity and selectivity for the PBZR [4]. It has been reported by in vitro

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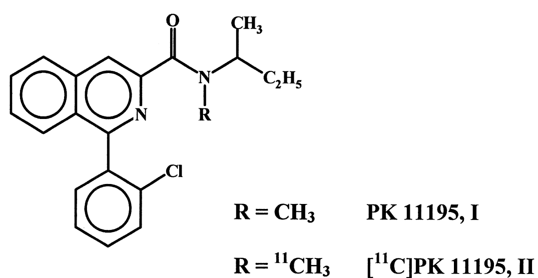

Fig. 1. Structures of **I** and **II**.

autoradiography that the density of PBZR is increased in different brain disorders such as stroke, malignant glioma, multiple sclerosis and Alzheimer's disease [5,6]. Moreover, a correlation has been observed between the increased PBZR density and the infiltration of macrophages in ischaemic lesions [7]. Therefore **II** is a useful tracer for the visualisation of neuronal damage associated with macrophage infiltration and glial proliferation by positron emission tomography (PET).

In the heart the PBZR is associated with Ca^{2+} channels. Antagonists of the PBZR decrease the contractility of the heart muscle, an effect that was antagonised by Ca^{2+} [8].

Different PET studies of the heart and the brain demonstrate the utility of **II** in the above mentioned heart [9] and brain pathologies [10–13]. However, absolute quantification of this receptor is difficult due the lack of knowledge on the amount of metabolites in plasma and target organs. In order to construct a mathematical model for the quantitative determination of the PBZR present in the brain, the clinical physician needs information on the relative amount unchanged **II** in plasma and brain tissue.

Once administered, radiopharmaceuticals like any other compounds may undergo rapid metabolisation. Considering the short half-life of ^{11}C ($t_{1/2}$=20.4 min, β^+ annihilation) a fast and reproducible specific assay for the determination of the relative amount **II** in plasma and organ tissue is required. Ferry et al. [14] demonstrated in healthy volunteers that **II** was rapidly distributed in the body with an elimination half-life of 3.7 h [14]. For **II** the amide link can be broken down in vivo to release [^{11}C]-*N*-methyl-*sec*-butylamine. Both [^{11}C]-*N*-methyl-*sec*-butylamine and **II** itself could also be demethylated with the release of [^{11}C]CH_2O, which easily penetrates the blood–brain barrier (Fig. 2). This paper describes a rapid high-performance liquid chromatography

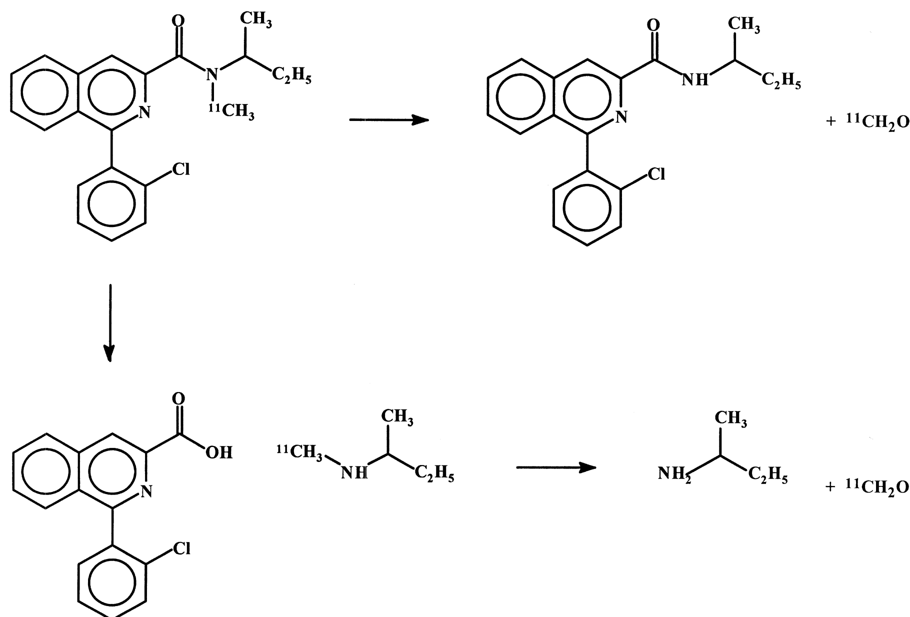

Fig. 2. Possible metabolisation pattern of **II**.

(HPLC) procedure for the quantitative determination of **II**. The developed method was successfully applied to plasma and organ tissue analysis for **II**.

2. Experimental

2.1. Chemicals

I was obtained from RBI (Natick, MA, USA). [^{11}C]**II** was synthesised in our laboratory according to a modified procedure described elsewhere [15]. Acetonitrile (HPLC quality) was purchased by Labscan (Dublin, Ireland). Triethylamine (analytical-reagent grade) was obtained from Sigma (Bornem, Belgium). All other chemicals were of analytical grade and purchased by Aldrich (Bornem, Belgium).

2.2. Equipment

The isocratic HPLC equipment consisted of a Waters Model 590 pump (Millipore, Waters, Milford, MA, USA), a UV detector (Pye Unicam, Cambridge, UK), a syringe injector equipped with a 750-μl loop (Valco Instruments, Eke, Belgium) and a CR-5A automatic integrator (Shimadzu, Tokyo, Japan). The eluate was collected in fractions with a Redifrac fraction collector (Pharmacia Biotech, Brussels, Belgium). All radioactivity counting was done with a one-channel γ-ray spectrometer equipped with a 2×2 in. NaI(Tl) detector (Canberra, Meriden, CO, USA) (1 in.=2.54 cm). All radioactivity measurements were corrected for physical decay.

2.3. Chromatographic conditions

An Alltima 5-μm RP-C_{18} column (250×10.0 mm I.D.) (Alltech, Laarne, Belgium) was used. The mobile phase consisted of a mixture of acetonitrile–water–triethylamine (65:35:0.5, v/v). The flow-rate was set at 5.0 ml/min. UV detection was achieved at 254 nm. The sensitivity of the UV detector was set at 1.0 AUFS. The analyses were performed at ambient temperature. The stock standard solution of **I** (0.5 μmol/750 μl) was prepared in ethanol and stored at ambient temperature.

2.4. Human plasma analysis

There was informed consent from all participants, and the Medical Ethics Committee of the University Hospital of Gent approved all human experiments. Two healthy controls and three stroke patients (50–75 years) were injected i.v. with 370 MBq **II**. At three different time points (5, 20 and 35 min post injection) arterial blood samples were taken. Plasma was obtained by centrifugation at 1000 *g* for 3 min. A 500-μl plasma sample was pipetted into an Eppendorf cup and 750 μl of acetonitrile was added. The mixture was vigorously shaken on a vortex mixer for 30 s. After centrifugation (3000 *g*, 3 min) the supernatant was separated from the pellet. Radioactivity was determined in the pellet and the supernatant. A 750-μl volume of the supernatant was injected immediately into the HPLC system. The eluate was collected with a fraction collector at time intervals of 1 min. The collected fractions were counted for radioactivity. Blank plasma samples were spiked with 37 kBq **II** and treated the same way.

2.5. Mice plasma and organ tissue analysis

All animal experiments were performed according to the ethical rules requested by the Belgian Government. A 10.1 MBq amount of **II** was administered i.v. to three white mice (strain NMRI). Animals were anaesthetised to death at 10 min post injection. Blood samples were taken and the heart and brain were rapidly removed and washed in physiological saline. Plasma was obtained by centrifugation of the collected blood at 3000 *g* for 3 min. The plasma samples were further treated as described under human plasma analysis. The organ tissues were washed in physiological saline and dried on tissue paper. The whole organ was transferred to a conical polypropylene tube of 15 ml and 1.5 ml of acetonitrile was added. The mixture was mixed on a polytron mixer for 30 s and centrifuged at 1000 *g* for 3 min. The supernatant was separated from the pellet. Radioactivity was determined in the pellet and the supernatant. A 750-μl volume of the supernatant was injected immediately into the HPLC system. The eluate was collected with a fraction collector at time intervals of 1 min. The collected fractions were

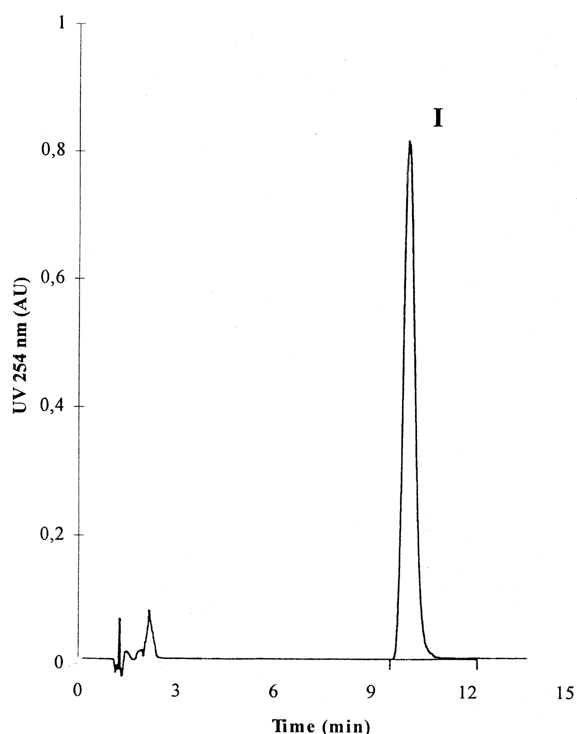

Fig. 3. UV chromatogram of a solution of 0.5 μmol **I** in 0.75 ml ethanol. Chromatographic conditions are described in the text.

counted for radioactivity. Blank plasma and organs were spiked with 37 kBq **II** and treated the same way.

3. Results and discussion

3.1. Chromatographic conditions

The retention time of **I** was 10.5 min ($k'=4.0$; hold-up time was determined with water) (Fig. 3). The chromatographic conditions provide a good resolution and allow the separation of **II** from its ^{11}C-labelled metabolites for radioactivity counting in a γ-ray spectrometer.

3.2. Validation of the method

The extraction yield of **II** from blank plasma and organs spiked with 37 kBq [^{11}C]**II** is shown in Table 1. For all samples the extraction yield was >90%. The stability of **II** during the extraction was controlled by HPLC. The radiochromatograms of the spiked blank plasma (human and mice) and spiked organ tissues, showed only one peak with the same retention time as **I**, indicating that no degradation of **II** took place during the extraction procedure. The relative proportions of unchanged **II** were calculated by measuring the ratio of the radioactivity corresponding to **II** to the total activity present in all collected fractions. The results are depicted in Table 1. In all cases >98% of the present activity still remains as **II**.

Control radioactivity measurements of the HPLC column demonstrated that no radioactivity was retained by the analytical apparatus.

3.3. Human plasma analyses for II

After i.v. injection of **II** into human volunteers, radioactivity rapidly cleared from blood and plasma. Fig. 4 shows a typical radiochromatogram (at 20 min after i.v. injection of **II**) for the separation of **II** and its radioactive metabolites (at 20 min after i.v. injection of **II**).

Two groups of more polar radioactive metabolites are revealed at all time points. The radioactivity in plasma, represented by unchanged **II** decreased from 95% (SD 1%, $n=5$) at 5 min post injection to 66% (SD 2.9%, $n=4$) at 35 min post injection (Table 2). The radiochromatograms of all patients showed the appearance of at least two polar metabolites, one set

Table 1
Extraction yield (expressed as % extraction) and stability of **II** during extraction (expressed as % unchanged **II**) in human plasma, mice plasma, heart and brain ($n=3$)

Sample	Extraction yield (%)	SD (%)	Unchanged **II** (%)	SD (%)
Human plasma	93	2.5	99	0.9
Mice plasma	92	1.9	99	0.8
Mice brain	94	2.7	99	0.9
Mice heart	94	2.4	98	0.7

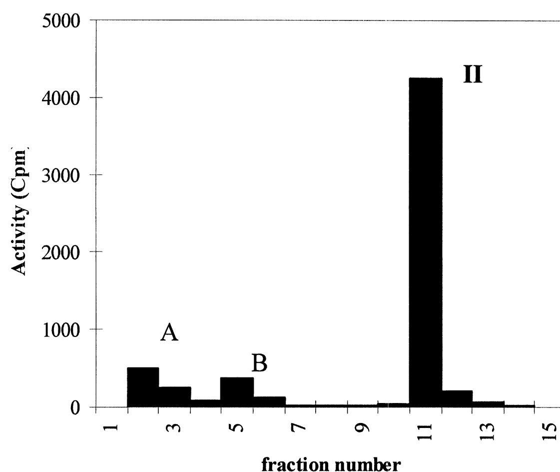

Fig. 4. Representative HPLC chromatogram of a human plasma sample at 20 min after i.v. injection of **II**. A and B are polar metabolites.

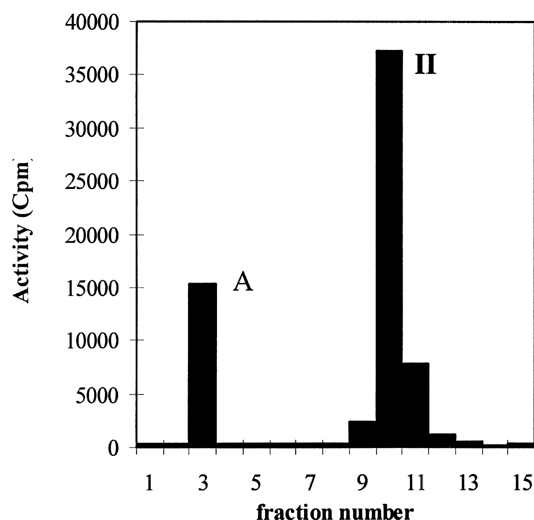

Fig. 5. Representative HPLC chromatogram of a mice plasma sample at 10 min after i.v. injection of **II**. A is a polar metabolite.

with the same retention time as the hold-up time and another one with a retention time of 5 min. The early metabolites might be identified as $[^{11}C]CH_2O$ and/or $[^{11}C]$*N*-methyl-*sec*-butylamine. A logarithmic correlation between the amount of authentic **II** versus time was observed [equation expressed as % authentic $\mathbf{II}=ae^{(-b\times \mathrm{time})}$; $a=100$, $b=0.012$, $r^2=0.9985$].

*3.4. Mice plasma and organ tissue analyses for **II***

Fig. 5 shows a typical radiochromatogram obtained after i.v. injection of **II** in mice. The radioactivity in plasma represented by unchanged **II** decreased rapidly, up to 58% (SD 17%, $n=3$) at 10 min post injection with the formation of one polar metabolite with the same retention time as the hold-up time. The more extensive metabolisation in mice according to humans could be based on the difference in body mass between the two species. In spite of the large amount of polar metabolite in plasma at 10 min post injection no other radioactive compound than authentic **II** could be detected in the extracts of brain and heart (97% SD 3% and 98% SD 1%, respectively, $n=3$). These values indicate that after i.v. administration no important penetration of ^{11}C-labelled metabolites of **II** occurred in the brain and heart of mice.

Table 2
Unchanged **II** (%) in human plasma at different time points after i.v. injection of 370 Mbq **II** ($n=5$ for all time points, except for 35 min where $n=4$)

Time (min)	Unchanged **II** (%)	SD (%)
5	95	1.0
20	78	3.3
35	66	2.9

4. Conclusions

A HPLC method for the separation of **II** from its ^{11}C-labelled metabolites has been developed. The method was successfully applied for plasma and organ tissue analyses for **II** after i.v. administration of **II** to humans and mice. The data from the human plasma analysis could be used as a correction factor in a compartmental model for calculation of the regional organ concentrations of PBZR. The mice data indicate that there is no need to correct measured brain activity for metabolite accumulation.

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