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Synthesis of ^{11}C-labelled acamprosate for PET studies

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Summary

A method for labelling acamprosate (calcium *N*-acetyl homotaurinate), an anti-craving compound for ethanol, with ^{11}C, has been developed for *in vivo* studies with positron emission tomography (PET). The synthesis was based on the acylation of homotaurinate using [^{11}C]acetyl chloride as the labelling agent. Reversed-phase HPLC in highly aqueous mobile phase conditions was used for product purification. The radiochemical yield achieved was about 1.14 GBq (31 mCi) with a specific radioactivity of 8.14 GBq/μmol (220 mCi/μmol). For the identification of ^{11}C-radioactivity and determination of specific radioactivities, HPLC and ^{1}H-NMR were used. Copyright © 2001 John Wiley & Sons, Ltd.

Key Words: [^{11}C]acamprosate; [^{11}C]*N*-acetyl homotaurinate; PET; biodistribution; alcohol

Introduction

Although acamprosate is used therapeutically in Europe to reduce relapse in weaned alcoholics, the mechanisms by which acamprosate

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decreases alcohol ingestion and relapse rates in alcoholics are still not clear. It is suggested that acamprosate acts by reducing neuronal hyperexcitability, responsible for the acute physical alcohol withdrawal syndrome on the level of both excitatory glutamate type and inhibitory GABA-type neurotransmitter pathways.

Acamprosate was initially believed to act via GABA receptors. It was shown that acamprosate binds preferentially to $GABA_B$ receptors[1,2] and administration of the drug enhanced synaptosomal [^{3}H]GABA uptake.[3] Results of electrophysiological studies and the finding that acamprosate restores the GABA basal concentration in alcohol-dependent rats support the GABA hypothesis.[4,5] Nevertheless, recent findings emphasize the role of the excitatory amino acid system for the action of acamprosate. Electrophysiological studies show that acamprosate decreases postsynaptic potentials in the rat's brain[6] and binding studies indicate that acamprosate binds to a specific spermidine-sensitive site that modulates the NMDA receptor.[7] Since it was suggested that 'craving', i.e. the irresistible desire to drink alcohol reported by alcoholic patients after withdrawal, is mediated, at least in part, by the excitatory amino acid system, the excitatory amino acid antagonism of acamprosate could also provide a biochemical explanation of the reducing action on alcohol craving observed in humans.[8]

Other observations show that acamprosate also acts via voltage-operated calcium channels and via a modulation of taurine levels in the central nervous system.[5] Although recent evidence suggests that the systems mentioned above are responsible for acamprosates action, the effect of acamprosate on other neurotransmitter systems cannot be excluded.[1,5]

An interesting method of investigation of the pharmacodynamic properties of acamprosate is to look for the drug action site. Acamprosate appears to be present in all areas of the rat's brain after crossing the blood–brain barrier[9] and there are also reports that it binds on specific sites in the hippocampus.[10] Since positron emission tomography (PET) has made it possible to examine the biodistribution of certain compounds *in vivo*, this imaging technique might be a powerful instrument for the characterisation of these binding sites in the human brain. In contrast to the techniques used in previous studies (current- and voltage-clamp, microdialysis and NMR) PET offers the unique possibility to study the uptake and binding of the acamprosate molecule itself, the brain regions where it accumulates and the behaviour of acamprosate in normal and alcohol-addicted subjects.

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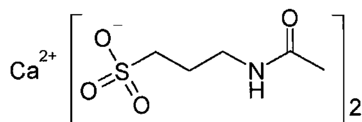

Figure 1. Structure of calcium *N*-acetyl homotaurinate (acamprosate)

For this application it is necessary to label acamprosate with a short-lived, positron-emitting radionuclide such as carbon-11 ($t_{1/2}$ = 20.4 min). From the structure of acamprosate, the acetyl group was considered to be the most accessible position for labelling with carbon-11 (see Figure 1). Therefore, the labelling was performed using [^{11}C]acetyl chloride, synthesised as previously reported.[11,12]

Experimental

Materials and apparatus

Reference acamprosate was obtained by crunching and dissolving Campral® tablets into water. Other chemicals and reference materials were purchased from commercial sources and were of analytical grade. Tetrahydrofuran (THF), sodium, benzophenone and methylmagnesium chloride were obtained from Merck (Darmstadt, Germany). Homotaurinate (3-aminopropane sulfonic acid), phthaloyl dichloride and triethylamine were obtained from Aldrich (Steinheim, Germany). A Grignard reagent solution (0.2 M methylmagnesium chloride in THF) was prepared by dilution of a commercially available 3 M methylmagnesium chloride/THF solution with freshly distilled THF (dried on sodium with benzophenone as an indicator). Dichloromethane (Lab Scan, Dublin, Ireland) and triethylamine, both dried on calcium hydride (Janssen Chemica, Geel, Belgium) were freshly distilled under argon before use.

HPLC was performed using columns and conditions noted below with a Waters 410 refractometer in series with a Canberra (Type 2007F) 2 × 2 inch NaI-detector or GM tube for the monitoring of the eluate.

Specific radioactivity was determined using a UV-spectrophotometer (Linear Instruments Corporation, Type UVIS 200; 202 nm). Traces of solvents were detected with a Philips PU 4500 GC-FID on a Porapak-Q column (Alltech) eluted with helium (column: 140°C; injector: 250°C;

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detector: 220°C). ^{1}H-NMR spectra were recorded in D_2O at 500 MHz (Bruker AN-500).

[^{11}C]carbon dioxide production

[^{11}C]carbon dioxide was produced by the ^{14}N(p, α)^{11}C nuclear reaction on nitrogen (Air Liquide N 60; 10.5 bar) using a CGR-MeV 520 cyclotron. Traces of oxygen present in the target gas convert nearly all the ^{11}C into [^{11}C]carbon dioxide. Irradiation was carried out for 20 min with a 15 μA beam of 18 MeV protons. At the end of bombardment (EOB) the target gas was vented into a stainless-steel tube coil at 2.0 l/min immersed in liquid argon (−186°C). Typically the radioactivity trapped in the coil was 34.4–39.2 GBq (930–1060 mCi), decay-corrected to EOB.

Preparation of [^{11}C]acetyl chloride

After warming up the steel coil to room temperature, the radioactivity was introduced in a helium gas stream over phosphorous pentoxide into the reaction vessel and trapped in 0.2 M methylmagnesium chloride in THF (200 μl, 70 μmol) at room temperature. The reaction was quenched by adding phthaloyl dichloride (200 μl, 1400 μmol) and the mixture was heated by a hot air blower. The generated [^{11}C]acetyl chloride was swept by increasing the flow rate of carrier helium up to 50 ml/min.

In preliminary experiments, the produced [^{11}C]acetyl chloride was trapped in cooled ethanol (−20°C, 500 μl) where it reacted to form [^{11}C]ethyl acetate. After distillation (5 min), the reaction mixture was transferred into a 500 μl HPLC loop. Separation was performed by reversed phase semi-preparative HPLC on a Bio-Rad Bio-Sil C_{18} column (25 × 1 cm, 10 μm) eluted with 35% MeOH in water at 3 ml/min. The eluate was monitored continuously with a GM tube. The radioactive fraction corresponding with [^{11}C]ethyl acetate (retention time: 13 min) was collected and measured for total radioactivity in a calibrated well-counter (Capintec).

Preparation of [^{11}C]N-acetyl homotaurinate

[^{11}C]acetyl chloride was produced as described above and collected in a vial cooled to −30°C containing homotaurinate (2.0 mg, 14 μmol), triethylamine (10 μl) and dichloromethane (500 μl). After distillation,

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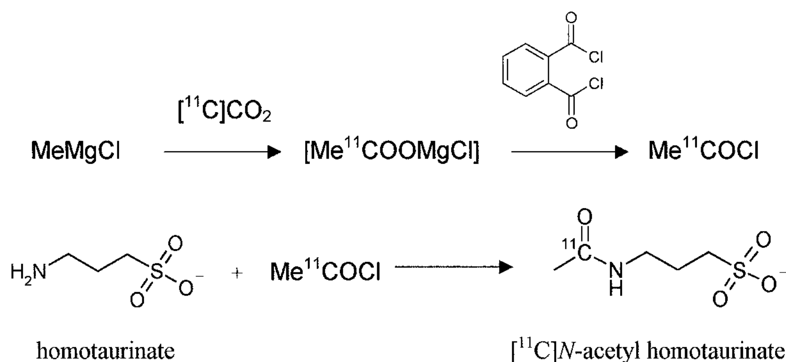

Figure 2. The radiosynthesis of [^{11}C]*N*-acetyl homotaurinate

the reaction vial was closed and the reaction mixture was heated to 60–70°C and stirred magnetically for 10 min. The solvent was then evaporated off with a helium-stream and HPLC mobile phase (500 µl) was added. Finally, the reaction mixture was transferred into a 500 µl HPLC loop.

Labelled compound purification was performed by reversed-phase semi-preparative HPLC on a Bio-Rad Bio-Sil C_{18} column (25 × 1 cm, 10 µm) eluted with 0.9% NaCl in water at 3 ml/min. The eluate was monitored continuously with a GM-tube and a refractometer. The radioactive fraction corresponding with [^{11}C]*N*-acetyl homotaurinate (retention time: 7.6 min) was collected. Finally, the eluate was sterilised by filtration through a sterile filter (0.22 µm pore size, Millipore®) (Figure 2).

Analysis of radiosynthesis

High-performance liquid chromatography. The radioactive product was analysed by HPLC, using a Bio-Rad Bio-Sil C_{18} column (25 × 1 cm, 10 μm) eluted with aqueous 0.001 M NaH_2PO_4 (adjusted to pH = 2 with phosphorous acid) at 3 ml/min (retention time for [^{11}C]*N*-acetyl homotaurinate: 10 min; Figure 3(B). The eluate was monitored for both radioactivity (NaI-detector) and refraction index. A single radioactive peak, with the same retention time as authentic *N*-acetyl homotaurinate was obtained. No radiochemical or chemical impurity was detected.

Nuclear magnetic resonance (NMR). Several preparations were carried out under identical conditions to the radiosynthesis but using small amounts (50–100 μmol) of stable carbon dioxide co-trapped with ^{11}C.

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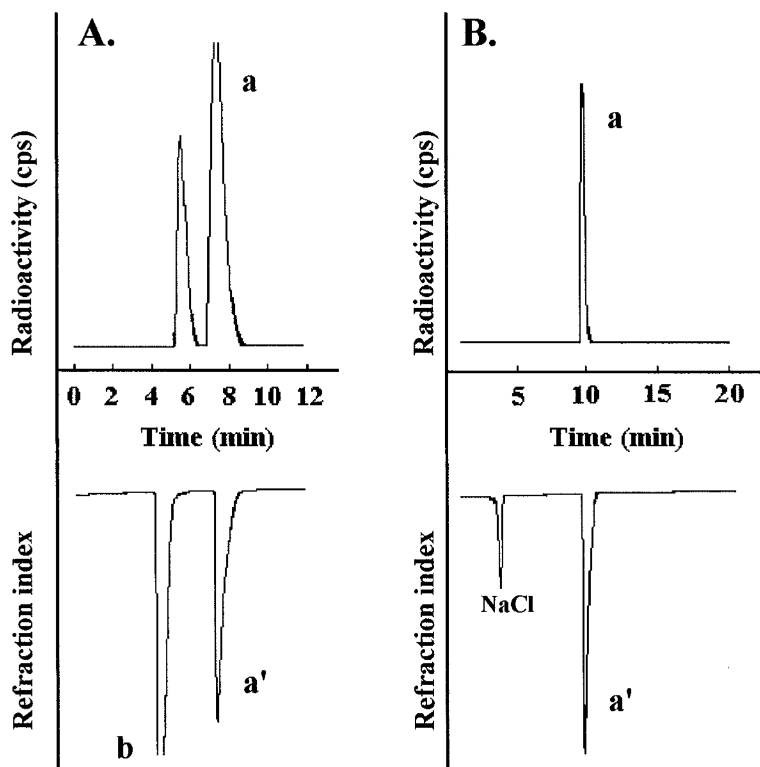

Figure 3. (A) Semi-preparative HPLC chromatogram from the purification of [^{11}C]*N*-acetyl homotaurinate: [^{11}C]*N*-acetyl homotaurinate (a), added standard *N*-acetyl homotaurinate (a′) and homotaurinate (b). (B) HPLC-chromatogram of purified [^{11}C]*N*-acetyl homotaurinate (a) and spiked reference *N*-acetyl homotaurinate (a′)

After decay, the collected fractions corresponding to the [^{11}C]*N*-acetyl homotaurinate peak were evaporated to dryness at 60°C under lowered pressure. The collected samples and standard *N*-acetyl homotaurinate were examined by ^{1}H-NMR spectroscopy.

Specific radioactivity. Since the detection limit of the refractometer for *N*-acetyl homotaurinate is to high (>1 μmol/ml), direct HPLC-determination of specific radioactivities was not possible. Therefore, the amount of cold carrier (*N*-acetyl homotaurinate) in the preparation was determined by HPLC with UV-absorption at 202 nm. Response of the spectrophotometer was found to be linear over the range 0–1 μmol/ml (0–200 μg/ml). For the calculation of the specific radioactivity, the

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collected fraction of [^{11}C]*N*-acetyl homotaurinate was measured for total radioactivity in a calibrated well-counter (Capintec). The average specific radioactivity achieved was more than 8.14 GBq/μmol (220 mCi/μmol) at the end of synthesis.

Gas chromatography. Preparations of [^{11}C]*N*-acetyl homotaurinate were also analysed by gas chromatography. No volatile compounds (dichloromethane <0.1 μg, THF <0.025 μg and triethylamine <0.2 μg) were detected.

Results and discussion

The synthesis of [^{11}C]*N*-acetyl homotaurinate via acetylation using no-carrier-added (NCA) [^{11}C]acetyl chloride is another application of this radiolabelling agent. The preparation involved the following major steps: (1) reaction of [^{11}C]carbon dioxide with methylmagnesium chloride forming [1-^{11}C]acetate and (2) conversion into [^{11}C]acetyl chloride with phthaloyl dichloride.

Formation of [^{11}C]acetyl chloride was inferred by detection and measurement of [^{11}C]ethyl acetate by HPLC. 95% of the injected radioactivity was converted to [^{11}C]ethyl acetate. Based on the radioactivity of [^{11}C]ethyl acetate and the radioactivity of initial [^{11}C]carbon dioxide, the yield of [^{11}C]acetyl chloride was estimated at 65–70%, decay-corrected to EOB. Only 11 min are required from the end of radionuclide production to provide an average of 25 GBq (670 mCi) of [^{11}C]acetyl chloride.

For the ^{11}C-acylation reaction several reaction parameters (reaction solvent and -time, temperature and concentration of starting material) were optimised. Dichloromethane was chosen for the reaction because it provided a higher reaction yield than other solvents (acetonitrile, THF, triethylamine). Triethylamine was used as the base. Best yields were achieved when the solution was heated for 10 min at 60–70°C after collection of [^{11}C]acetyl chloride at -30°C. Because the starting material was not dissolvable in the solvent, the reaction mixture was stirred continuously during the reaction. The radiochemical yield was about 1.14 GBq (31 mCi) and was 12% from the initial [^{11}C]carbon dioxide radioactivity, decay-corrected to EOB. The radiosynthesis took 35 min from the end of the radionuclide production to provide [^{11}C]*N*-acetyl homotaurinate ready for intravenous injection. As shown by the HPLC chromatogram (Figure 3(A)), the peak of [^{11}C]*N*-acetyl homotaurinate

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Table 1. Assignment of the ^{1}H-NMR spectrum (500 MHz) of the obtained product

Carbon atom	Chemical shift, δ_{TMS}
1	2.14 ppm, singlet, 3H
2	3.34 ppm, triplet, 2H
3	2.07 ppm, pentaplet, 2H
4	3.08 ppm, triplet, 2H

(same retention time as standard cold *N*-acetyl homotaurinate) is well separated from the other labelled or unlabelled products involved in the synthesis. Nevertheless, the (radio)chemical purity of the sample must be monitored strictly since the highly aqueous mobile phase conditions, needed for the product purification, can cause unstable retention times. Parallel synthesis with cold carbon dioxide followed by ^{1}H-NMR spectroscopy clearly demonstrates the identity of the product (see Table 1). UV-absorption at 202 nm shows that specific radio-activities > 8 GBq/μmol are achievable.

Conclusion

The ability to produce radiochemically pure [^{11}C]*N*-acetyl homotaurinate with a reasonable specific radioactivity enables a biodistribution study of acamprosate (*N*-acetyl homotaurinate) with positron emission tomography. Although our product satisfies the demands of a PET-tracer, a possible solution for the rather low yields and unstable HPLC-separation of the product could be the labelling of a sulphonate ester of the used homotaurinate-precursor, followed by hydrolysis of the ester. This possibility is currently under investigation.

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