

## Iodine-123 labelled Z-(*R,R*)-IQNP: a potential radioligand for visualization of M<sub>1</sub> and M<sub>2</sub> muscarinic acetylcholine receptors in Alzheimer's disease

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**Abstract.** Z-(*R*)-1-Azabicyclo[2.2.2]oct-3-yl (*R*)- $\alpha$ -hydroxy- $\alpha$ -(1-iodo-1-propen-3-yl)- $\alpha$ -phenylacetate (Z-IQNP) has high affinity to the M<sub>1</sub> and M<sub>2</sub> muscarinic acetylcholine receptor (mAChR) subtypes according to previous *in vitro* and *in vivo* studies in rats. In the present study iodine-123 labelled Z-IQNP was prepared for *in vivo* single-photon emission tomography (SPET) studies in cynomolgus monkeys. SPET studies with Z-[<sup>123</sup>I]IQNP demonstrated high accumulation in monkey brain (>5% of injected dose at 70 min p.i.) and marked accumulation in brain regions such as the thalamus, the neocortex, the striatum and the cerebellum. Pretreatment with the non-selective mAChR antagonist scopolamine (0.2 mg/kg) inhibited Z-[<sup>123</sup>I]IQNP binding in all these regions. The percentage of unchanged Z-[<sup>123</sup>I]IQNP measured in plasma was less than 10% at 10 min after injection, which may be due to rapid hydrolysis, as has been demonstrated previously with the *E*-isomer of IQNP. Z-[<sup>123</sup>I]IQNP showed higher uptake in M<sub>2</sub>-rich regions, compared with previously obtained results with *E*-[<sup>123</sup>I]IQNP. In conclusion, the radioactivity distribution from Z-[<sup>123</sup>I]IQNP in monkey brain indicates that Z-[<sup>123</sup>I]IQNP binds to the M<sub>1</sub>- and M<sub>2</sub>-rich areas and provides a high signal for specific binding, and is thus a potential ligand for mAChR imaging with SPET.

**Key words:** Z-(*R,R*)-IQNP – Muscarinic acetylcholine receptors – Brain – Single-photon emission tomography

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### Introduction

Reduced density of muscarinic cholinergic receptors (mAChR) might serve as a marker for the degeneration of the cholinergic system that has been reported in Alzheimer's disease [1]. This potential has motivated the development of radioligands for brain imaging of mAChR with single-photon emission tomography (SPET) and positron emission tomography (PET).

Iodine-123 labelled quinuclidinyl benzilate (QNB) and iododexetimide have been used to visualize mAChR distribution in the brain of healthy controls and patients with dementia and Alzheimer's disease [2–4]. None of these <sup>123</sup>I-labelled SPET radioligands or carbon-11 labelled PET ligands have shown ability to give high signal to noise ratios in the brain or to have high selectivity for subtypes of mAChR. 1-Azabicyclo[2.2.2]oct-3-yl  $\alpha$ -hydroxy- $\alpha$ -(1-iodo-1-propen-3-yl)- $\alpha$ -phenylacetate (IQNP) is a recently synthesized QNB analogue [5, 6]. We have recently reported that the <sup>123</sup>I-labelled *E*-isomer is a potential ligand for imaging of mAChR in the primate brain [7]. Furthermore, bromine analogues of IQNP labelled with bromine-76 have been used in preliminary PET investigations in primates to investigate central mAChR and have shown potential for the study M<sub>2</sub> receptors [8]. The *E*-(*R,R*)-isomer of IQNP (*E*-IQNP) has selectivity for the M<sub>1</sub> mAChR subtype whereas the Z-(*R,R*)-isomer (Z-IQNP) binds with subnanomolar affinity to both the M<sub>1</sub> and M<sub>2</sub> subtypes according to *in vitro* and *in vivo* studies in rats [5, 6]. The presynaptic M<sub>2</sub> mAChR subtype is of particular interest with respect to the degeneration of the cholinergic system in Alzheimer's disease.

In the present study <sup>123</sup>I-labelled Z-IQNP was prepared and used for SPET studies in cynomolgus mon-

keys. The metabolism of Z-[<sup>123</sup>I]IQNP was measured in monkey plasma using gradient high-performance liquid chromatography (HPLC).

## Materials and methods

### Chemistry

**General.** All chemicals were of analytical grade and were used without further purification. Z-IQNP and the tributylstannyl precursor for labelling Z-IQNP with <sup>123</sup>I were synthesized according to procedures described elsewhere [5, 6].

**Preparation of Z-[<sup>123</sup>I]IQNP.** Radiosynthesis of Z-[<sup>123</sup>I]IQNP was performed by MAP Medical Technologies Oy (Tikkakoski, Finland) with slight modification of a method reported previously [7]. To the reaction vial (1 ml) containing (100 µg) of tributylstannyl precursor of Z-IQNP was added 100 µl of ethanol, 65 µl of [<sup>123</sup>I]NaI (950 MBq) in 0.01 M NaOH, 65 µl of 0.1 M HCl and 50 µl of chloramine-T in water (1 mg/ml). The reaction mixture was gently stirred and kept at room temperature for 5 min. To this solution 500 µl of 40% acetonitrile in 10 mM phosphoric acid was added. The solution was injected into the semi-preparative reverse phase HPLC system (mobile phase 40/60 acetonitrile/10 mM phosphoric acid; flow rate 3 ml/min; column size 300×7.8 mm) and a 3 ml fraction containing Z-[<sup>123</sup>I]IQNP eluting at 8.5 min was collected. The fraction was evaporated and then diluted to 0.18 M phosphate buffer. A further purification step was performed as described previously [7] using a Sep-Pak C18 cartridge. The product was collected by eluting the cartridge with a small volume (2 ml) of ethanol, diluted by addition of phosphate-buffered saline (PBS, pH 7.4), and then sterile filtrated. The purification step in order to separate all [<sup>123</sup>I]I<sup>-</sup> from the product was necessary due to a slight deiodination during the evaporation process.

### SPET

A three-headed SPET system (Trionix Research Laboratory Inc., Twinsburg, Ohio, US) with low-energy collimators was used. This system has a spatial resolution of about 10 mm full-width at half-maximum at the centre of the field of view. The energy window (20%) was centered around the photopeak of <sup>123</sup>I. During a 360° rotation, 90 views/head were collected in a 128×128 matrix mode (voxel size of 2.2×2.2×2.2 mm<sup>3</sup>). Correction for scattered photons was not performed.

Two male cynomolgus monkeys with a weight of 3–4 kg were supplied by the National Laboratory for Bacteriology (Solna, Sweden). Ketamine (Ketalar, 15–20 mg kg<sup>-1</sup> h<sup>-1</sup>) was used for anaesthesia by intramuscular injections. A rapid bolus of 92–114 MBq of Z-[<sup>123</sup>I]IQNP was injected into the sural vein. A head fixation system was used to maintain the position of the monkey head, which was positioned so that the imaging plane was parallel to the plane defined by the canthomeatal lines. Radioactivity was measured by dynamic SPET imaging in nine or ten scans for two monkeys in the baseline study and in the pretreatment study with scopolamine lasting 140 min altogether.

### Metabolite studies in monkey plasma

A Kontron (Kontron Instruments Inc., Everett, Mass., USA) gradient HPLC system was used with a UV detector operated at 254 nm followed by a Packard (Packard Instrument Company, CT, USA) radioisotope detector (a PET cell) with computer data acquisition. The analysis was performed on a Waters (Milford, Mass., USA) µBondapak C18 300×7.8 mm (10 µm) column. Blood samples (2 ml) were obtained from the monkeys at 2, 8, 14, 22, 47, 60, 91 and 135 min after injection of Z-[<sup>123</sup>I]IQNP. Plasma was separated from the whole blood by centrifugation for 1 min at 2000 g [7]. After centrifugation, plasma (0.5 ml) was removed and mixed with acetonitrile (0.7 ml). The mixture was centrifuged and the supernatant (>1 ml) was removed and 1 ml used for the gradient HPLC separation. In the gradient HPLC system the Waters µBondapak C18 column was eluted with a mixture of acetonitrile in phosphoric acid (10 mM) from 25% acetonitrile to 60% in 5.5 min and back to 25% in 1 min, with the end of run at 7.5 min. The flow was 6 ml/min and a 1-ml sample loop was used.

### Calculations

Regions of interest (ROIs) were drawn onto the SPET images by delineation of ROIs for the whole brain, the thalamus, the occipital cortex, the frontal cortex, the temporal cortex, the striatum and the cerebellum using an atlas based on a cryosectioned cynomolgus monkey brain *in situ* [9]. The known radioactivity of <sup>123</sup>I (a reference standard) in a phantom of monkey brain was measured with the SPET system in order to obtain the radioactivity concentration.

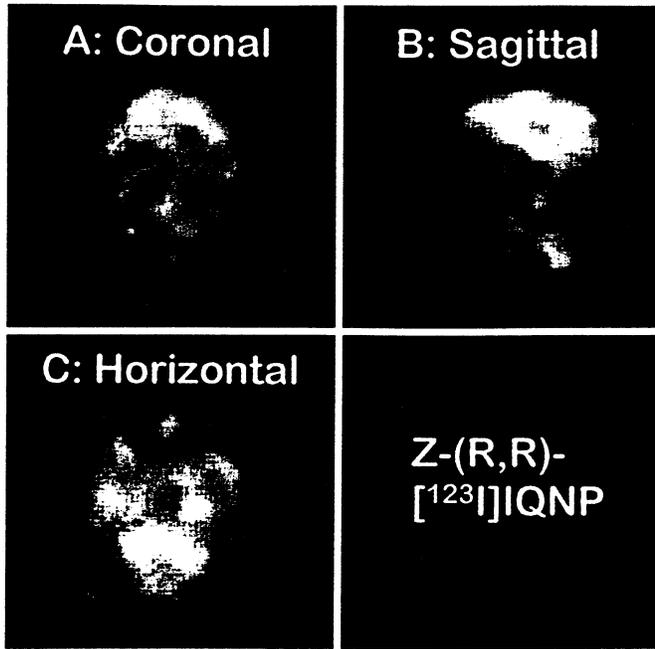
## Results

### Chemistry

The incorporation of [<sup>123</sup>I]NaI into Z-[<sup>123</sup>I]IQNP was 80% as measured with analytic HPLC after the labelling reaction. After purification the radiochemical purity of the final product representing the radioactivity of <sup>123</sup>I bound to the real compound in the injection fluid, was higher than 99%. The specific radioactivity was higher than 70 GBq/µmol.

### SPET

After *i.v.* injection of Z-[<sup>123</sup>I]IQNP in a cynomolgus monkey, there was a rapid accumulation of radioactivity in the brain. At 70 min after injection >5% of the total radioactivity injected was present in the monkey brain (Fig. 1). Dynamic SPET imaging with Z-[<sup>123</sup>I]IQNP demonstrated an increase in radioactivity in brain regions such as the thalamus, the neocortex and the striatum. In the cerebellum Z-[<sup>123</sup>I]IQNP demonstrated lower binding compared with the other regions (Fig. 2A). At 60–80 min after injection the radioactivity ratios of the thalamus to the cerebellum and of the occipital cortex to the cerebellum were 1.8 and 1.9 respectively. Radioac-



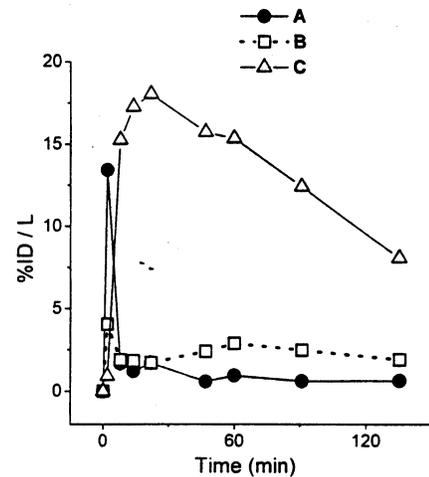
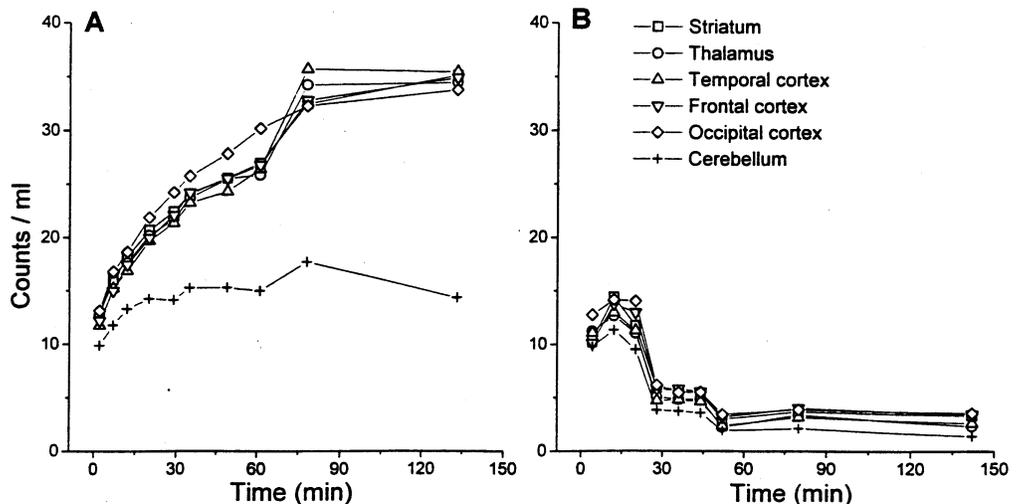
**Fig. 1.** SPET study showing the radioactivity distribution in a coronal (A), a sagittal (B) and a horizontal (C) section through the cynomolgus monkey brain after i.v. injection of 114 MBq of Z-[<sup>123</sup>I]IQNP. The SPET study was performed between 60 and 80 min after injection. Levels of radioactivity are colour encoded from low to high (black-yellow-white)

tivity seemed to approach a plateau at 90–140 min. The binding was markedly reduced after pretreatment with the non-selective mAChR antagonist scopolamine (0.2 mg/kg), which inhibited Z-[<sup>123</sup>I]IQNP binding in all these regions as well as in the cerebellum (Fig. 2B).

#### Metabolite studies

The relative composition of plasma radioactivity is shown in Fig. 3. The percentage of unchanged Z-

**Fig. 2A, B.** Time course of regional radioactivity, measured by dynamic SPET imaging in the brain of cynomolgus monkeys after i.v. injection 92–114 MBq of Z-[<sup>123</sup>I]IQNP. A Control and B following pretreatment with scopolamine (0.2 mg/kg) 30 min prior to injection of the radioligand



**Fig. 3.** The percentage of injected dose of radioactivity per liter (%ID/L) of the parent compound (A), polar metabolites (B) and the lipophilic metabolite (C) measured in monkey plasma using HPLC vs time after intravenous injection of Z-(R,R)-[<sup>123</sup>I]IQNP

[<sup>123</sup>I]IQNP measured in plasma was less than 10% at 10 min after injection. A major radioactive metabolite that eluted after the parent compound appeared in plasma rapidly after injection.

#### Discussion

The purpose of the study was to evaluate Z-[<sup>123</sup>I]IQNP uptake in the primate brain in order to develop a SPET method to visualize M<sub>1</sub> and M<sub>2</sub> mAChR changes in Alzheimer's disease. Dynamic SPET imaging showed high brain uptake of Z-[<sup>123</sup>I]IQNP in a cynomolgus monkey. The specificity of the binding to mAChR was confirmed by pretreatment with the non-selective mAChR antagonist scopolamine (0.2 mg/kg), which markedly inhibited the binding (Fig. 2). Compared with previously obtained results with E-[<sup>123</sup>I]IQNP [7], the Z-isomer has higher

uptake in brain regions representing the M<sub>2</sub> mAChR subtype.

According to autoradiographic studies on human whole-brain cryosections and SPET studies in monkeys, the E-isomer of IQNP has selectivity for the M<sub>1</sub> mAChR subtype. In vitro autoradiography with E-[<sup>125</sup>I]IQNP demonstrated binding in M<sub>1</sub> subtype-rich regions such as the neocortex and the striatum [7]. Post-mortem human brain studies have shown that the M<sub>1</sub> subtype is found in striatum, cortex and hippocampus [10]. Dynamic SPET imaging with Z-[<sup>123</sup>I]IQNP demonstrated high accumulation in brain regions such as the thalamus, the frontal cortex, and the temporal cortex as well as in the striatum and the cerebellum. Post-mortem human brain studies have shown that the M<sub>2</sub> subtype is found in high concentrations in the thalamus, medulla, pons and cerebellum [10]. Z-[<sup>123</sup>I]IQNP showed higher uptake in M<sub>2</sub> subtype-rich regions such as the thalamus and the cerebellum as compared with results previously obtained with E-[<sup>123</sup>I]IQNP [7]. This is consistent with the recent biodistribution studies with Z-[<sup>125</sup>I]IQNP, which have shown high uptake of radioactivity in both M<sub>1</sub>- and M<sub>2</sub>-rich areas in brain [11]. High accumulation of Z-[<sup>123</sup>I]IQNP in regions with high M<sub>2</sub> density supports the proposition that this ligand binds to both M<sub>1</sub> and M<sub>2</sub>.

Using gradient HPLC analysis, the major radioactive metabolite in plasma (Fig. 3) was found to be more lipophilic than the parent compound. This result is similar to that observed previously for E-IQNP, with the lipophilic metabolite demonstrating the same retention time in the HPLC as the E-acid [7]. The radioactivity of the major metabolite in plasma, which is assumed to be Z-acid, decreased after 30 min post injection (Fig. 3). Gradient HPLC analysis of radioactive metabolites suggests that rapid hydrolysis may be a major biotransformation route for Z-[<sup>123</sup>I]IQNP in the cynomolgus monkey and that radioactive metabolites of Z-[<sup>123</sup>I]IQNP account for a major part of the radioactivity in the vascular space.

In conclusion, the demonstrated radioactivity distribution of Z-[<sup>123</sup>I]IQNP in brain regions with a high density of M<sub>1</sub> and M<sub>2</sub> mAChR indicates that this radioligand binds to both subtypes and has potential as a ligand for mAChR imaging in Alzheimer's disease using SPET.

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