THE EFFECT OF P-GLYCOPROTEIN (Pgp) MODULATION ON SPECIFIC UPTAKE OF SUBSTITUTED [^{18}F]MPPF DERIVATIVES

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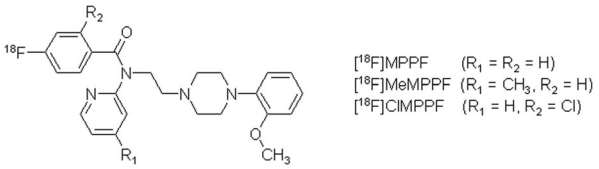
Objective: [^{18}F]MPPF has been applied successfully to image serotonin 5-HT$_{1a}$ receptors, although its cerebral uptake is low. [^{18}F]MPPF is actively transported by P-glycoprotein (Pgp), an ATP-driven efflux pump that forms part of the blood-brain-barrier. This pump is known to have a high affinity in particular for basic compounds with two planar aromatic domains. We hypothesized that the addition of substituents that distort the planarity of the aromatic amide moiety in MPPF might reduce the influence of Pgp on cerebral tracer uptake.

Methods: [^{18}F]MPPF, [^{18}F]MeMPPF and [^{18}F]ClMPPF were prepared by nucleophilic substitution of the corresponding nitro precursors with [^{18}F]fluoride. [^{18}F]MeMPPF and [^{18}F]ClMPPF were compared to [^{18}F]MPPF in biodistribution studies in male Wistar rats that were either untreated (controls), pretreated with 50 mg/kg cyclosporine A (Pgp modulator) or pretreated with NAN-190 (5-HT$_{1a}$ antagonist; not for [^{18}F]ClMPPF) prior to injection of the tracer (ca. 10 MBq). The percentage specific tracer uptake in the 5-HT$_{1a}$-rich hippocampus was calculated, using the intercept of the tracer uptake vs. receptor density plots as the amount of nonspecific binding.



Results: [^{18}F]MPPF, [^{18}F]MeMPPF and [^{18}F]ClMPPF were synthesized in 7, 10 and 2% radiochemical yields and with mean specific activities of >50, 30 and 3 MBq/nmol, respectively. Cerebral uptake of [^{18}F]ClMPPF in untreated animals was approximately 2-fold higher than that of [^{18}F]MPPF and [^{18}F]MeMPPF. As shown before, the regional distribution of [^{18}F]MPPF in the brain correlated well (r^2=0.87) with the distribution of 5-HT$_{1a}$ receptors as known from autoradiography studies. However, the distribution of [^{18}F]ClMPPF (r^2=0.11) and [^{18}F]MeMPPF (r^2=0.18) did not show any correlation. Pretreatment with NAN-190 significantly reduced [^{18}F]MPPF uptake in 5-HT$_{1a}$-rich brain regions, but did not affect [^{18}F]MeMPPF uptake. These results indicate that the chloro and methyl derivatives of MPPF do not display any specific binding in controls.

In cyclosporine A pretreated animals, cerebral uptake of the three tracers was 8 to 10-fold higher than in controls. Surprisingly, after modulation of Pgp, good correlations were obtained between the distribution of the 5-HT$_{1a}$ receptor and the regional distribution of [^{18}F]MPPF (r^2=0.97), [^{18}F]MeMPPF (r^2=0.95) and [^{18}F]ClMPPF (r^2=0.90), indicating specific binding of all these compounds to the 5-HT$_{1a}$ receptor. In Pgp modulated animals, the percentage specific uptake in the hippocampus was 75, 55 and 66% for [^{18}F]MPPF, [^{18}F]MeMPPF and [^{18}F]ClMPPF, respectively. For [^{18}F]MPPF, this specific uptake was comparable to that in controls (88%), but for [^{18}F]MeMPPF and [^{18}F]ClMPPF the specific uptake in Pgp modulated animals was substantially higher than in controls (9 and 0%, respectively).

Conclusion: These results show that the introduction of additional substituents in [^{18}F]MeMPPF and [^{18}F]ClMPPF does not reduce the influence of Pgp activity on cerebral tracer uptake. In fact, the introduction of the additional substituents leads to a complete loss of specific binding of these MPPF derivatives in controls. Remarkably, modulation of Pgp could restore specific binding of these MPPF analogs. To our knowledge this is the first time that this has been observed.

Keywords: 5-HT1a Receptor, MPPF Derivatives, P-Glycoprotein

DEVELOPMENT OF RADIOTRACERS FOR MEASURING THE FUNCTION OF MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN IN THE BRAIN *IN VIVO*

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Multidrug resistance-associated protein (MRP), energy-dependent efflux pump that belongs to the ATP-binding cassette superfamily of transporters,[1] is expressed widely in various tissues and serve as defense mechanisms limiting tissue accumulation of naturally occurring toxins, xenobiotics, and drugs. Since it is suggested that over-expression of MRP may be involved in resistance to various drugs such as antiepileptic drugs and anticancer agents, the development of radiopharmaceuticals that can measure the function of MRP non-invasively by PET or SPECT would be useful for elucidating pathological conditions or drug therapies of central nervous systems and cancers. We propose a new approach for measuring the function of MRP *in vivo*: a lipophilic tracer can enter the brain followed by the conversion to a GSH-conjugate which would be transported from the tissue to blood by MRP. The function of MRP can be estimated by tracing the radioactivity of GSH-conjugate in the brain. In this study, purine derivatives were designed, synthesized, and evaluated as a prototype of radiotracers leading to PET or SPECT tracers for measuring the function of MRP in the brain.

6-chloro-9-[^{14}C]methylpurine (9m6CP) and 6-chloro-7-[^{14}C]methylpurine (7m6CP) were prepared by the reaction of 6-chloropurine with [^{14}C]CH_3I. The radiochemical yields for 9m6CP and 7m6CP were 60% and 18%, respectively. Those radiochemical purities were more than 98%.

Radiotracers for measuring the function of MRP in the brain must meet the following criteria: 1) high permeability of BBB, 2) rapid conversion to GSH-conjugate, 3) extrusion of GSH-conjugate by MRP. When the reaction rates of these tracers with GSH in rat brain homogenate supplemented with 2 mM GSH were examined by TLC/BAS method, those of 9m6CP and 7m6CP were 0.0057 ($min^{-1}g^{-1}mL^{-1}$) and 0.33 ($min^{-1}g^{-1}mL^{-1}$), respectively. When 7m6CP with the high rate constant was injected into rats (Wistar, male, 8 weeks), the compound registered high uptake at 1 min (0.8% ID/g tissue) and a gradual decrease during 5-60 min. Furthermore, the chemical form of radioactivity in rat brain was analysed 15 min after the injection of 7m6CP by normal phase (NP) and reverse-phase (RP) TLC. The analysis by NP TLC confirmed that the radioactivity of the unaltered substance was hardly observed; the analysis by RP TLC (water:acetonitrile; 9:1 v/v) showed 35% of the radioactivity was identical with the authentic GSH-conjugate, but there were a radioactive peak adsorbed at the origin and an unknown peak. We also investigated the effect of probenecid (PRB), a well established MRP inhibitor,[2] on the efflux rate of metabolites including GSH-conjugate to examine if the conjugate was extruded by MRP. 7m6CP was injected into rats intravenously 15 min after the i.p. administration of PRB to measure the radioactivity in the brain at each time point (15, 30, 60 min). These data were fitted to exponential function by least-square method to obtain the efflux rate constant. As a result, the efflux rate in PRB-pretreated rats decrease significantly as compared to that of control, implying that 7m6CP could readily enter the brain across BBB, followed by the rapid conversion to GSH-conjugate, and then this conjugate would be extruded from the brain to blood by MRP.

The findings in this study suggested that this approach with 7m6CP would be useful for measuring the function of MRP in the brain *in vivo*.

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(1) *Pharmacol Rev* **2001***, 53*, 569-596.
(2) *J Pharmacol Exp Ther* **2003***, 306*, 124-131.

Keywords: Multidrug Resistance-Associated Protein, Purine, Brain

SYNTHESIS AND *IN VIVO* EVALUATION OF A NOVEL PET RADIOLIGAND FOR IMAGING THE PERIPHERAL BENZODIAZEPINE RECEPTOR

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Nearly all diseases of the brain reveal pronounced changes in functional states of glial cells. Most prominent is the presence of activated microglia in areas of progressive disease or tissue destruction. The glial expression of the peripheral benzodiazepine receptor (PBR) has been studied using the isoquinoline [^{11}C]PK11195 and PET, however, this radioligand is far from ideal. Specific PBR radioligands suitable for PET imaging remain limited. The pyrazolopyrimidine **1**, (N,N-diethyl-2-[2-(4-methoxyphenyl)-5,7-dimethylpyrazolo-[1,5-a]-pyrimidin-3-yl]-acetamide) has been reported as a selective PBR ligand (Ki=4.7 nM, compared with PK11195 Ki=9.3 nM) (1) and is suitable for radiolabelling using carbon-11. The present work describes the synthesis, radiolabelling and in vivo evaluation of [^{11}C]**1** using PET.

Compound **1** was synthesised in 4 steps from 4-methoxybenzoate and subsequently demethylated using hexadecyl tributyl phosphonium bromide in 45% HBr to form the phenolic precursor **2**. [^{11}C]**1** was prepared by O-alkylation of **2** with [^{11}C]CH_3I. The reaction was carried out in DMF with TBAH at RT for 3 min. Purification was performed using C-18 semi-preparative HPLC whilst radiochemical purity and specific activity (SA) were calculated using analytical HPLC. [^{11}C]**1** was imaged for 60 min on a Siemens/CTI Biograph PET/CT scanner in one male Papio hamadryas baboon in three separate experiments. Experiments were performed 4 weeks apart. The first experiment was a baseline study whilst the other two involved pre-treatment with either the PBR specific ligand, PK11195 (5 mg/kg), or the central benzodiazepine receptor ligand, flumazenil (1 mg/kg), 5 min prior to injection of [^{11}C]**1**.

Radiochemical yield of [^{11}C]**1** was 9% (non-decay corrected) with SA of 36 GBq/μmol at EOS. The radiochemical purity was > 98% with an average time of synthesis and formulation of 13.2 min (Scheme 1).

Following i.v. injection of [^{11}C]**1** (200 MBq), significant accumulation of radioactivity was seen in the baboon brain, particularly in cortical areas (Figure 1A). The radioactivity reached a maximum at 20 min and stayed at the same level for the remainder of the experiment. Compared to the baseline study, pre-treatment with PK11195 reduced [^{11}C]**1** uptake by about 70% in the whole brain (Figure 1B), whereas pre-treatment with flumazenil had no inhibitory effect (Figure 1C).

These results clearly demonstrate that the uptake and retention of the pyrazolopyrimidine [^{11}C]**1** in the baboon brain corresponds to specific PBR binding. Therefore, [^{11}C]**1** has the prerequisite pharmacological profile to be a useful radioligand for imaging the PBR using PET and warrants further investigation.

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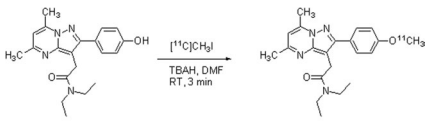
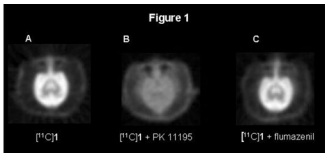


Scheme 1



Keywords: Peripheral Benzodiazepine Receptor, Pyrazolopyrimidine, Carbon-11

SYNTHESIS AND EVALUATION OF A NEW ^{18}F-LABELED LIGAND FOR PET IMAGING OF BRAIN PERIPHERAL BENZODIAZEPINE RECEPTORS

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Introduction. Some aryloxyanilides have recently provided promising radioligands for imaging brain peripheral benzodiazepine receptors (PBR) with PET (1). Effective ^{18}F-labeled PBR radioligands might have special utility, since they might be produced in very high activities and distributed to 'satellite' PET centers for wide application. So far promising aryloxyanilide PBR radioligands have required at least two-step labeling with fluorine-18. Here we have obtained a new promising ^{18}F-labeled PBR ligand (**3**) in a single radiosynthetic step.

Methods. Precursor **1** was synthesized by reductive alkylation of 2-phenoxyaniline with 2,5-dimethoxy-benzaldehyde followed by acylation with bromoacetyl bromide. **1** was converted into **2** by treatment with potassium fluoride. The affinity of **2** for PBR was assessed in a radioligand binding assay. Ligand **2** was radiolabeled by treating **1** with [^{18}F]fluoride-K$^+$-crown-6 in acetonitrile at 110 °C for 10 min, and purified by HPLC (2). Radioligand **3** was evaluated by i.v. injection into rhesus monkey with PET monitoring of regional brain radioactivity (baseline experiment) and in a similar experiment in which DAA 1106 (3 mg/kg i.v.) was administered at 24 min before radioligand (pre-block experiment). Radioligand metabolism was investigated by HPLC of monkey plasma and also investigated in rat. The in vitro profile of the radioligand in human whole blood/plasma was also measured.

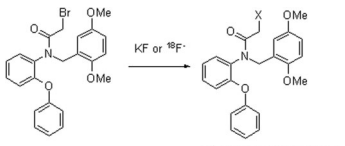

Figure 1. Syntheses of **2** and **3** from **1**.

Results. 1 and **2** were prepared in 44 and 42% overall yield, respectively. The IC_{50} of **2** for PBR was 0.2 nM. Radioligand **3** was obtained in up to 97% decay-corrected radiochemical yield (RCY) in experimental labeling reactions and in 10-20% isolated RCY (non-optimized) with a specific radioactivity of 0.5-0.7 Ci/μmol in production mode. In the baseline experiment radioactivity was avidly taken up into PBR-containing regions over 30 min (Figure 2) and well retained thereafter. In the pre-block experiment, radioactivity was rapidly taken up into PBR-containing regions and to a greater extent than in the baseline experiment (Figure 2). However, radioactivity declined to a very low level after 2 h (Figure 2).

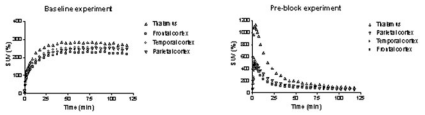

Figure 2. Monkey brain region time-activity curves in baseline and pre-block experiments with **3** [SUV (%) = % injected dose per g brain x g body weight].

3 was stable in whole human blood and *ex vivo* rat brain for 1 h. The human plasma-free fraction was 0.4%. **3** was distributed at 94% into cellular blood components. After administration of **3** into monkey the percentage of radioactivity in plasma represented by parent radioligand was 35% at 30 min reducing to 10% at 90 min after injection. Analysis of rat brain revealed a single polar radiometabolite that has a peripheral origin.

Discussion. 3 is easily prepared in a single step from the bromo precursor, **1,** and cyclotron-produced [^{18}F]fluoride**. 3** exhibits good entry into rhesus monkey brain and a high ratio of PBR-specific binding to non-specific binding.

Conclusion. 3 is easily prepared and is a promising ^{18}F-labeled ligand for PET imaging of brain PBR.

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2. Briard E et al. *J Label Compd Radiopharm* 2004; **47**: 217-232.

Keywords: Fluorine-18, Peripheral benzodiazepine receptor, Radioligand

RADIOSYNTHESIS AND EVALUATION OF (+)[^{11}C]PHNO AS RADIOTRACER FOR *IN VIVO* IMAGING OF THE DOPAMINE D2 HIGH AFFINITY STATE WITH POSITRON EMISSION TOMOGRAPHY (PET)

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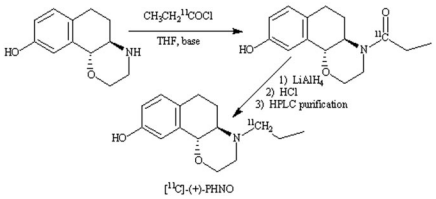
In vivo imaging of dopamine D2 receptors with agonist, as opposed to the more commonly employed antagonist, radiotracers could provide important information on the high affinity (functional) state of the D2 receptor in illnesses such as Schizophrenia or Movement Disorders. We report here the radiosynthesis and evaluation, in rat, of the potent D2 agonist, (+)-PHNO, labeled with carbon-11, as a potential radiotracer for imaging the high affinity state of dopamine D2 receptors with positron emission tomography (PET). Our methodology allows for the production of [^{11}C]-(+)-PHNO in the quantities, specific activities, and radiochemical purities required for human PET studies.

The synthesis of [^{11}C]-(+)-PHNO was described at this meeting several years ago (Luthra et al.,1997) but no further reports have emerged. [^{11}C]-(+)-PHNO was reliably synthesized by acylation of the norpropyl precursor with [^{11}C]-propionyl choride followed by reduction with $LiAlH_4$. Key points in the radiosynthesis included reduction of the intermediate amide at low temperatures, co-distillaton of the [^{11}C]-propionyl chloride wih THF, and, for purification, removal of all THF prior to HPLC column loading. Starting with 950 mCi (35 GBq) of [^{11}C]-CO_2, between 50 and 100 mCi (190-370 MBq) of the final formulated product was obtained in a synthesis time of 40 min. Specific activities (at end-of-synthesis) ranged from 700-1500 mCi/μmol (26-56 GBq/μmol) and radiochemical purities of [^{11}C]-(+)-PHNO were greater than 99%. No oxidation or radiolysis of the formulated radiotracer was observed.



Ex vivo biodistribution studies in rat brain demonstrated that [^{11}C]-(+)-PHNO crossed the blood-brain barrier readily and had an appropriate regional brain distribution for a radiotracer which maps dopamine D2 receptors i.e. high levels of radioactiivty in the striatum and low levels in all other brain regions examined including the D2-free cerebellum. [^{11}C]-(+)-PHNO binding was saturable, and demonstrated an excellent signal to noise as measured by its striatum to cerebellum ratio of 5.6, sixty min post injection. The binding was reversible and highly stereospecific. Blocking and displacement studies were consistent with selective and specific binding to dopamine D2 receptors e.g. haldol and raclopride effected complete blockade of specific binding while SCH 23390 had no effect. Physico-chemical measurements (lipophilicity, protein binding, phospholipophilicity) and brain radioactive metabolite measurements in rats will be described and are in full accord with the desired properties of a neuroreceptor imaging agent for PET.

All of the above, coupled with the documented full D2 agonistic properties of (+)-PHNO strongly indicates that [^{11}C]-(+)-PHNO is a leading candidate radiotracer for the imaging of the dopamine D2 high affinity state using positron emission tomography. Pilot studies in human subjects are underway.

Keywords: Dopamine Agonist, Carbon-11, Acylation

RADIOLABELED PHENETHYLGUANIDINES: NOVEL MARKERS OF CARDIAC SYMPATHETIC NEURONS AND ADRENERGIC TUMORS

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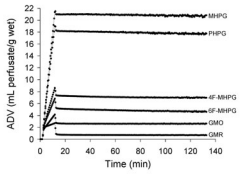
Radioiodinated *meta*-iodobenzylguanidine (MIBG), [^{11}C]*meta*-hydroxyephedrine (HED) and [^{11}C]epinephrine (EPI) are used clinically as markers of cardiac sympathetic neurons and adrenergic tumors (pheochromocytoma, neuroblastoma). In the heart, they are rapidly taken up into sympathetic neurons by the norepinephrine transporter (NET) and stored in vesicles by the vesicular monoamine transporter (VMAT). However, their rapid neuronal uptake rate prevents rigorous kinetic modeling, limiting our ability to obtain accurate and sensitive measures of nerve density. We hypothesize that a radiolabeled NET substrate must possess two kinetic properties to be 'ideal' for quantitative analyses: (1) a slower neuronal uptake rate than MIBG, HED and EPI; and (2) a long neuronal retention time, through efficient vesicular storage. Since many phenethylguanidines are known to be potent depleters of norepinephrine due to their high vesicular uptake, 18 different ^{11}C-labeled phenethylguanidines were synthesized and tested as potential sympathetic nerve tracers with ideal kinetic properties. Uptake into adrenergic tumors was also assessed for one compound.



Methods. [^{11}C]phenethylguanidines (PG) were prepared using a radiosynthetic method for incorporating ^{11}C into guanidines developed at Uppsala University[1]. Neuronal uptake and retention kinetics were measured in the isolated rat heart by infusing tracer for 10 min followed by a 2 hr washout study. Biodistribution studies of 5 promising agents were performed in rats. MicroPET imaging was used to test the ability of one compound with rapid uptake and long retention to localize pheochromocytomas in BALB/c *nu/nu* mice bearing PC12 pheochromocytoma xenografts (n = 2).

Results. Radiochemical yields were 10-50%, with specific activities >500 Ci/mmol EOS and radiochemical purities >98%. In isolated rat heart, 8 [^{11}C]PG's had the desired long neuronal retention (clearance half-times > 20 hr), reflecting efficient vesicular storage (6 are shown in the **Figure**). Neuronal uptake rates (ml/min/g wet) ranged from 0.08 for *N*-[^{11}C]guanyl-metaraminol (GMR) to 2.12 for [^{11}C]*meta*-hydroxy-PG (MHPG). For cardiac studies, *N*-[^{11}C]guanyl-*meta*-octopamine (GMO) is the most promising agent so far. Its neuronal uptake rate of 0.28 ml/min/g wet is well below those of MIBG (3.65), HED (2.34), and EPI (0.87). GMO biodistribution in rats showed liver and lung uptake comparable to HED and EPI, suggesting favorable heart imaging properties in humans. In microPET studies, [^{11}C]*para*-hydroxy-PG (PHPG) was avidly taken up by pheochromocytoma, reaching peak levels within 10 min and staying constant out to 60 min. Thus, a radiolabeled PG with rapid NET transport and long retention may be useful for localizing adrenergic tumors. The long neuronal retention of two fluoro derivatives, [^{11}C]*para*-fluoro-MHPG (4F-MHPG) and [^{11}C]*ortho*-fluoro-MHPG (6F-MHPG) suggests that a ^{18}F-labeled PG with kinetic properties tailored for either heart or tumor imaging is an achievable goal.

Conclusion. Phenethylguanidines are a promising class of compounds for developing radiotracers capable of quantifying cardiac sympathetic nerve density. In addition, they offer a novel approach to the design of diagnostic and radiotherapeutic agents for adrenergic tumors. (Supported by NIH HL079540).

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Keywords: Norepinephrine Transporter, Sympathetic Nervous System, Meta-Iodobenzylguanidine

RADIOLABELLING AND *IN VIVO* EVALUATION OF [^{11}C]GSK215083 AS A POTENTIAL 5-HT$_6$ PET RADIOLIGAND IN THE PORCINE BRAIN

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The 5-hydroxytryptamine-6 (5-HT$_6$) receptor is one of 14 distinct mammalian 5-HT (serotonin) receptors expressed in the central nervous system through which 5-HT is involved in the regulation of a number of biological processes (1). The 5-HT$_6$ receptor has been implicated in diverse CNS pathophysiologies, and several atypical antipsychotics have high affinity for it. A GSK internal PET radioligand development programme is investigating the feasibility of imaging the 5-HT$_6$ receptor in the living brain. A new generation of 5-HT$_6$ receptor antagonists based on the 3-benzenesulfonyl-8-piperazine-1-yl-quinoline scaffold have recently been developed (2). These compounds in general and the N-methyl derivative GSK215083 in particular have very high binding affinity for 5-HT$_6$ (pKi=9.8), lower affinity for 5-HT$_{2a}$ (pKi=9.1) and good CNS penetration. GSK215083 has been evaluated as a 5-HT$_6$ PET radioligand. [^{11}C]GSK215083 was prepared by N-methylation of the corresponding desmethyl precursor with [^{11}C]MeOTf in presence of TMP, followed by HPLC purification. [^{11}C]GSK215083 readily enters the brain of the anesthetized Yorkshire pigs (40kg). Peak regional tissue concentrations were reached at approximately 20min post injection followed by a slow washout from brain regions known to be rich in 5-HT$_6$ receptors with highest uptake and retention observed in striatum. The observed rank order of regional brain concentrations was striatum>cortical regions> cerebellum, consistent with reported 5-HT$_6$ receptor densities and localisation determined by tissue section autoradiography in animals and man (3,4). Radio-HPLC analysis revealed that [^{11}C]GSK215083 is rapidly metabolised in arterial plasma, the parent representing approximately 60% of the total radioactivity 30min post injection. Self-blocking studies with escalating doses of GSK215083 have demonstrated a saturable dose dependent signal in the striatum and cortical regions. Treatment of pigs with clozapine (6.25mg/kg), a compound known to have high affinity for the 5-HT$_6$ receptor, significantly reduced the specific binding in striatum as compared to cerebellum. No significant effect on [^{11}C]GSK215083 signal in striatum was observed following treatment with ketanserine (0.3mg/kg), a selective 5-HT$_{2a}$ receptor antagonist, in contrast the same treatment reduced >90% specific binding in cortical regions. [^{11}C]GSK215083 shows properties suitable for studies probing 5-HT$_6$ receptor with PET (5).

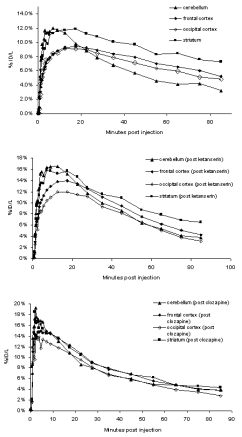

Fig. 1. Selected tissue TAC's for [^{11}C]GSK215083 in the pig brain pre (top), post 0.3 mg/kg ketanserin (middle) and post 6.25mg/kg clozapine (bottom)

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Keywords: 5-HT6, Radioligand, [^{11}C]GSK215083

AN INDIUM-LABELED DO3A CONJUGATE OF NALTRINDOLE WITH HIGH AFFINITY AND SELECTIVITY FOR DELTA OPIOID RECEPTORS: SYNTHESIS, BINDING STUDIES AND BIODISTRIBUTION

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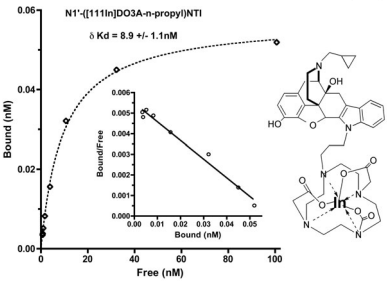
Opioid receptors (OR) are over-expressed by several human cancers with respect to normal tissues. Radioligands for non-invasive characterization of such cancers may have value as diagnostic tools. For instance, N1-([^{11}C]methyl)naltrindole, designed for brain imaging, also allows PET imaging of delta opioid receptors (DOR) on primary tumors of lung and breast cancer patients. We have been interested in development of OR ligands, such as iodoallyl analogs of naltrindole (NTI), for complementary SPECT imaging. We are extending our work to small molecule conjugates of relatively large, pendant radiometal complexes that might maintain high OR affinity despite significant structural perturbation. Here we report the synthesis and evaluation of an indium-labeled DO3A conjugate of NTI. NTI, with the phenol protected by a tosyl moiety, was selectively alkylated at the indole nitrogen with 3-bromopropionaldehyde dimethylacetal in 73% yield using NaH/DMF. Deprotection with NaOH/*i*-PrOH gave a stable adduct (85%) that was converted to the aldehyde by *p*-TsOH/acetone. The key step, reductive amination with DO3A (1,4,7,10-tetraazacyclododecane-1,4,7-trisacetic acid) was accomplished in 59% yield using sodium triacetoxyborohydride. DO3A was prepared from the tris-*t*-butyl ester by TFA cleavage. Complexation of cold indium (III) by the NTI-macrocycle in acetate buffer (pH 6.5) at 100 degrees was complete in 30 min. Incorporation of [^{111}In] was 91% under similar conditions. The radioactive complex was isolated (65%) in high purity as an EtOH solution after reversed-phase HPLC and solid-phase extraction. Thus, N1-(In/[^{111}In]DO3A-*n*-propyl)NTI is available in a concise 6 step synthesis. *In vitro*, the cold indium complex showed 38-fold selectivity for delta (Ki 11.9 nM) over mu (Ki 451 nM) sites, and high selectivity over kappa sites (IC50 >3,000 nM). Thus, the complex displays high affinity and selectivity for DOR although affinity is reduced >100-fold compared to NTI (Ki *ca.* 0.1 nM). Cold saturation binding studies in mouse brain membranes at a specific activity of 1500 mCi/μmol gave a Kd of 8.9 nM and an appropriate Bmax of 101 fmol/mg protein. In competition assays, hot complex was displaced potently by DOR ligands (NTI Ki 0.28 nM; DPDPE Ki 5.6 nM) but not by DAMGO (mu) or U69593 (kappa) at high concentrations (>1000 nM). *In vivo* in CD1 mice, N1-([^{111}In]DO3A-*n*-propyl)NTI did not penetrate the brain. Little bone uptake was observed, indicating good stability. Renal and hepatobiliary clearance were noted for this hydrophilic radioligand (log D pH 7.4, -0.73). Uptake in small intestine at 1 h could be partially blocked (42%) by naltrexone and saturated (52%) by cold complex at 10 μmol/kg (p<0.05). Interestingly, no effect gut uptake was observed with selective blockers (cyprodime, mu; U50488, kappa; NTI, delta) at 5 μmol/ Although NTI did not inhibit binding, the data set favors specific binding to sites with OR characteristics. Thus, ([^{111}In]DO3A-*n*-propyl)NTI has potential for localization of DOR on peripheral tumors. Support: NIH NCI P50 CA 103130.



Keywords: Opioid, Naltrindole, Indium - III

PET IMAGING AGENTS FOR THALAMIC AND EXTRATHALAMIC α4β2 NICOTINIC RECEPTORS

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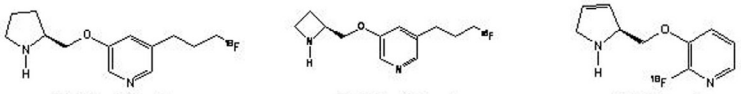
Objectives: Several neurological and psychiatric disorders (Alzheimers disease, Parkinson's disease, schizophrenia and substance abuse) have been thought to involve the nicotine α4β2 receptor. In order to optimize *in vivo* imaging properties (enhance kinetics and improve visualization of extrathalamic receptor sites), we have synthesized and evaluated replacement of the pyrrolidine ring in nifrolidine (Chattopadhyay et al., J.Nucl. Med., 46:130-140, 1995) with other ring systems. These new derivatives are based on the azetidine ring and 3,4-dehydropyrrolidine rings which has led to the development of agonists and antagonists.

Methods: Multistep syntheses to obtain nifzetidine and nifene were optimized in order to prepare tosylate and nitro-precursors and standards. The radiosyntheses of ^{18}F-nifzetidine, Fig-**2**, was carried out by reaction of the BOC-protected tosylate with ^{18}F in acetonitrile (ACN) at 96 °C for 30 mins. Radiosynthesis of ^{18}F-nifene, Fig-**3**, was carried out by using nitro-precursor, in DMSO-ACN at 126 °C for 30 min. Deprotection of radiolabeled N-BOC derivatives was carried out with TFA at 80 °C. The mixture purified by HPLC with 0.1 M NH_4CO_2H (40%) and ACN (60%), flow rate of 2.5 mL/min. The retention time of **2** and **3** was found to be 17 and 11 min. Brain slices were incubated with either ^{18}F-nifzetidine or ^{18}F-nifene, approx 2.5-4 μCi/cc final concentration in Tris at 37 °C for 1 hour in the absence/presence of nicotine. Slices were exposed to phosphor films. PET images were acquired on rhesus monkeys using an ECAT HR$^+$ scanner.

Results: Tosylate to ^{18}F-fluoride exchange for **2** and nitro to ^{18}F-fluoride exchange for **3**, both proceeded in high radiochemical yields. The radiosynthesis was accomplished in 2.5 hrs with an overall radiochemical yield 20-40%, decay corrected. A single HPLC separation was carried out after deprotection. The specific activity was estimated to be above 5000 Ci/mmol. Both ^{18}F-nifzetidine and ^{18}F-nifene are capable of identifying the α4β2 receptors rich regions, similar to that previously reported for ^{18}F-nifrolidine. *In vitro* study with the putative antagonists, **1** and **2** indicated selective high binding in the thalamus (Th) as well as binding in extrathalamus regions such as cortex (Cor), striatum, subiculum (Sub), lateral geniculate and other regions. **2** yielded higher region to cerebellum (Cer) ratios compared to **1** (^{18}F-nifrolidine: Th/Cer = 7; Sub/Cer = 4; Cor/Cer = 3; ^{18}F-nifzetidine: Th/Cer = 12; Sub/Cer = 5; Cor/Cer = 4). PET imaging study in rhesus monkey revealed Th to Cer ratio was about 1.7 for **1** at 2 hours post-injection whereas Th to Cer was about 2.1 for **2**. *In vitro* study with the agonist, ^{18}F-nifene in rat brain slices also revealed exceptional binding in receptor-rich regions (Th/Cer = 11; Sub/Cer = 6; Cor/Cer = 4). Nicotine at various concentrations was able to displace >90% of specific binding of all the three radiotracers.

Conclusions: Design and radiosynthesis of two new radiotracers, ^{18}F-nifzetidine and ^{18}F-nifene has been successfully carried out. Anteroventral thalamus and Thalamus exhibited the highest binding followed by regions of the temporal and frontal cortex as well as the striata. Comparative *in vivo* binding studies of **1, 2** and **3** is currently underway.



^{18}F-Nifrolidine **1** ^{18}F-Nifzetidine **2** ^{18}F-Nifene **3**

Keywords: Nicotinic Acetylcholine Receptors, PET Nonhuman Primate, F-18 Nifzetidine, F-18-Nifene

CHARACTERIZATION AND ^{111}In RADIOLABELING OF ErbB-2 RECEPTOR-TARGETING PEPTIDES

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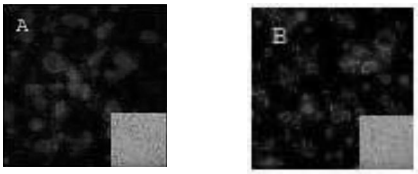
Introduction: The overexpression of the ErbB-2 receptor as well as its extracellular localization make it an attractive target for cancer imaging studies. It is hypothesized that the use of molecules that specifically target ErbB-2 receptors on carcinomas and metastases will lead to improved cancer detection and treatment modalities. Previously, we isolated the ErbB-2-binding peptide KCCYSL from a bacteriophage display library. The peptide bound to purified extracellular domain (ECD) of the ErbB-2 receptor protein and to human breast and prostate carcinoma cell lines, but not to normal cells. Using alanine substitutions in the core peptide sequence of KCCYSL, the binding ability of the peptide to the ErbB-2-ECD was evaluated in order to define those residues crucial for binding. Moreover, in order to improve the avidity and specificity of the binding, multiple antigenic peptide (MAP) constructs bearing four copies of KCCYSL were synthesized and characterized. The bifunctional-chelator DOTA was attached to the linear KCCYSL peptide or MAP and radiolabeled with 111In. Binding properties of the radiolabeled peptides to cultured carcinoma cells were evaluated, with anticipation that these peptides could be used as a cancer imaging agent in vivo.

Methods: Linear KCCYSL peptide or MAP constructs displaying four copies of the KCCYSL peptide were prepared using Fmoc chemistry and solid phase synthesis. For radiolabeling, the peptide(s) was chemically modified with the bifunctional chelator DOTA. The resulting DOTA coupled peptide was then radiolabeled with ^{111}In. A 60-70 % radiolabeling efficiency was achieved in ammonium acetate pH 4.5 buffer with 5 μCi ^{111}In at 90oC for 30 minutes. Cell binding studies with radiolabeled linear peptide and MAP were performed on cultured human breast cancer cells (MDA-MB-435) at different time intervals.

Results: Analysis of different alanine substituted peptides suggested that the original KCCYSL peptide had the best affinity (295 nM) for ErbB-2-ECD. While substituting alanine for the tyrosine residue significantly decreased the affinity, removal of lysine from the peptide completely abolished binding. As expected, the MAP version of the linear sequence showed improved affinity for ErbB-2-ECD in the low nanomolar (3 nM) range, improving the affinity 100 fold. Binding of linear KCCYSL (30 μM) or MAP (5μM) to MDA-MB-435, as analyzed by confocal microscopy, is shown in Figure-1. The cell binding capability of the ^{111}In-radiolabeled MAP indicated that the peptide conjugate bound MDA-MB-435 cells in the absence of competing nonlabeled MAP. Moreover, ^{111}In-labeled MAP binding was competed off in the presence of nonlabeled MAP.



A. KCCYSL B. MAP-KCCYSL

Conclusions: Our studies indicate that the MAP is far more superior to the linear peptide in binding the purified ErbB-2 protein as well as ErbB-2-expressing human carcinoma cells. Radiolabeling and cell binding studies indicate the potential of the radiolabeled MAP for ErbB-2 receptor-targeted imaging and treatment of cancer.

Keywords: ErbB-2 Receptor, Receptor Targeting, Antigenic Peptides

TARGETING EPIDERMAL GROWTH FACTOR (EGF) RECEPTOR-POSITIVE TUMORS WITH ^{64}Cu AND ^{86}Y-LABELED MONOCLONAL ANTIBODY ERBITUX

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Introduction: The epidermal growth factor receptor (EGFR) and its ligands have been recognized as critical factors in the pathophysiology of tumorigenesis and have been studied extensively. Over-expression of the EGFR plays a significant role in the tumor progression of a wide variety of solid human cancers. Therefore, the EGFR represents an attractive target for the design of novel anticancer diagnostic and therapeutic agents, including monoclonal antibodies and small molecule inhibitors. The capacity of anti-EFGR agents, such as Erbitux (Cetuximab), have been well established to slow tumor cell proliferation and modulate cell cycle phase distribution as well as cause significant growth inhibition in tumor cell lines and xenografts. The effective use of Erbitux will depend on the ability of physicians to detect and characterize EGFR-expressing lesions before and after the initiation of EGFR-directed treatments. Our goal is to image the over-expression of EGFR in tumors by Positron Emission Tomography (PET) with ^{64}Cu- and ^{86}Y-labeled Erbitux. PET imaging based on the binding, internalization, and retention of the radiolabeled, anti-EGFR antibody in intracellular compartments will provide a much-needed, quantitative, non-invasive *in vivo* method of evaluating EGFR. This allows for a better prediction of both efficacy and toxicity of the anti-EGFR antibody treatment. **Methods:** Erbitux was conjugated with either the bifunctional chelator DOTA (1,4,7,10-tetraazacyclotetradecane-N,N′,N″,N‴-tetraacetic acid) for Cu-64 [$T_{1/2}$ = 12.7 h] labeling, or CHX-A″-DTPA (N-[(R)-2-amino-3-(p-isothiocyanatophenyl)propyl]-trans-(S,S)-cyclohexane-1,2-diamine-N,N-N′,N′,N″,N″-pentaacetic acid) for labeling with Y-86 [$T_{1/2}$ = 14.7 h]. Binding specificity of radiolabeled Erbitux conjugate ^{64}Cu-DOTA-Erbitux, was evaluated in two tumor cell lines expressing different levels of EGFR: human epidermoid carcinoma EGFR-positive tumor cell line A431 and human breast cancer EGFR-negative tumor cell line MDA-MB-435. Biodistribution and microPET imaging studies of ^{64}Cu-DOTA-Erbitux were carried out in A431 human epidermoid carcinoma and MDA-MB-435 human breast tumor xenografts. **Results:** ^{64}Cu-DOTA-Erbitux showed high binding affinity to EGFR-positive A 431 cells (Kd of 6.23 nM). Both biodistribution and microPET imaging studies with ^{64}Cu-DOTA-Erbitux demonstrated that greater radioactivity accumulation was observed in EGFR-positive A431 tumors (19.24 ± 2.52 % ID/g at 24 h p.i.) compared to EGFR-negative MDA-MB-435 tumors (3.15 ± 0.43 % ID/g at 24 h p.i). This tumor uptake in A431 tumor-bearing mice was retained to 48 h post-injection (24.95 ± 7.72 % ID/g). Reduction of tumor uptake was observed in the blocking group (12.68 ± 1.13 %ID/g, 24 h p.i.), suggesting that the tumor uptake was receptor-mediated and ^{64}Cu was residualized in the tumor cells. Binding specificity, biodistribution and microPET imaging studies of ^{86}Y-CHX-A"-DTPA-Erbitux will be performed in A431 and MDA-MB-435 tumor xenografts. It is anticipated that improved tumor uptake and non-target organ clearance will be observed with ^{86}Y-CHX-A"-DTPA-Erbitux. **Conclusions:** These positive data suggest the potential of non-invasive imaging EGFR over-expression in tumors with ^{64}Cu or ^{86}Y radiolabeled Erbitux conjugates. **Acknowledgements:** Erbitux was kindly provided by ImClone Systems Incorporated (New York, NY). This work was supported in part by NCI grants CA064475 and R24 CA086037 (for ^{64}Cu and ^{86}Y production).

Keywords: Epidermal Growth Factor Receptor (EGFR), Copper-64, Yttrium-86, PET