From molecule to man: The full Cimbi-36 story

Dysfunctions in the serotonin (5-HT or 5-hydroxytryptamine) neurotransmitter system are implicated in the pathophysiology of a variety of brain diseases such as depression, Alzheimer's disease, and schizophrenia, yet it is currently not possible to quantify serotonin levels in vivo in the human brain. Positron emission tomography (PET) has unsurpassed sensitivity and specificity for measuring neurotransmitter receptors in the living human brain and furthermore, with the appropriate PET radioligand, neuroreceptor binding is inversely correlated to extracellular levels of neurotransmitters with dopamine as the prime example. Thus, PET has the potential to measure serotonin levels in a non-invasive way in the human brain. However, this requires a PET radioligand which is sensitive to changes in serotonin levels and no such ligand has currently been evaluated for use in humans.

The Cimbi platform 1 project group has been engaged to develop such new probes for PET imaging. The work has mainly focused on the serotonin system that regulates numerous important psychophysiological behaviors in humans. 5-HT signals through a range of different receptor molecules, the 5-HT receptors. The most abundant 5-HT receptor in the human brain is the 5-HT_{2A} receptor – this is also the main receptor being activated by hallucinogenic drugs, such as LSD. One of the main Cimbi goals has been to develop a PET radioligand that could be used to image specific activation of the 5-HT_{2A} receptor, i.e. to develop a 5-HT_{2A} receptor agonist PET radioligand.

Identification of compound class

Development of PET radioligands is a complex and sequential process that draws on highly specialized knowledge from fields such as medicinal chemistry, radiochemistry, and in vivo pharmacology. The phases that a PET radioligand for a given target undergoes involve: 1) chemical synthesis of cold ligands and in vitro test of which receptors the molecules bind to, 2) radiolabeling to incorporate a positron emitting isotope into the molecule, 3) in vitro and in vivo evaluation by testing the radiolabeled compound in the living brain with PET. The vast majority of PET radioligands are based on chemical structures and compounds developed within industry. In Cimbi, we took the approach to develop our own compounds.

The radioligand development programme was initiated at the beginning of the center's first five year term (2006-2010). Here, the Medicinal Chemistry group headed by Mikael Begtrup, the PET- and Cyclotron Unit headed by Jacob Madsen and Nic Gillings, and the Neurobiology Research Unit headed by Gitte Moos Knudsen came together to lift the tasks to be carried out during the 3 phases of radioligand development, as described above.

During the early stages of Cimbi, an interesting class of compounds (the *N*-benzylated phenethylamines) was indentified as lead compounds, i.e. structural stating points that later were modified to their properties (Figure 1). These compounds were in the scientific literature reported to be super-potent agonists of the 5-HT_{2A} receptor, i.e. they were known to activate this receptor even at very low concentrations.

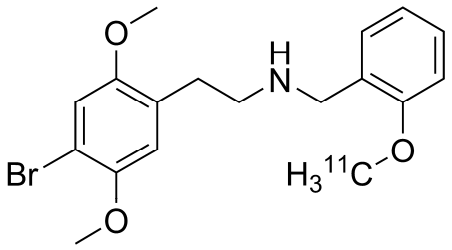

Figure 1. Chemical structure of [^{11}C]Cimbi-36. This compound is a *N*-benzylated phenethylamine and a member of the structural class of compounds that was identified in the literature as possible PET radioligands.

In the hooded benches of Mikael Begtrup's lab at the at that time Faculty of Pharmaceutical Sciences, the first compounds specifically designed to serve as 5-HT_{2A} receptor agonist PET radioligands were synthesized in 2007.

First Breakthrough – [^{11}C]Cimbi-5

The first lead compound, Cimbi-5, was synthesized along with starting material (precursor for the radiolabeling). Our final winner, Cimbi-36, is analogous to Cimbi-5 but it has an bromine (Br) atom instead of the iodine (I) atom in Cimbi-5. The radiolabeling of these structures is not trivial either since the methylation reaction that is required to incorporate C-11 into the molecule may occur both correctly at the oxygen (O) atom or incorrectly at the nitrogen (N) atom. To ensure that the radiolabeling occurred correctly, the N was protected with a so-called BOC-group which later can be removed with addition of acid, once the radiochemical reaction is completed. The first successful radiosynthesis of and PET scanning with [^{11}C]Cimbi-5 in a pig was then conducted in March 2008. The choice of pig as

experimental animal to test the in vivo properties of novel PET tracers was made very early. The Danish Landrace pig is a good alternative to monkeys, as a large animal species that resembles humans in many aspects. Specifically, large size of the pig brain favors PET imaging using standard equipment designed for human use (Figure 2). Compared to monkeys, pigs are also easier to house and handle, and they are readily available from industrial farms in Denmark.

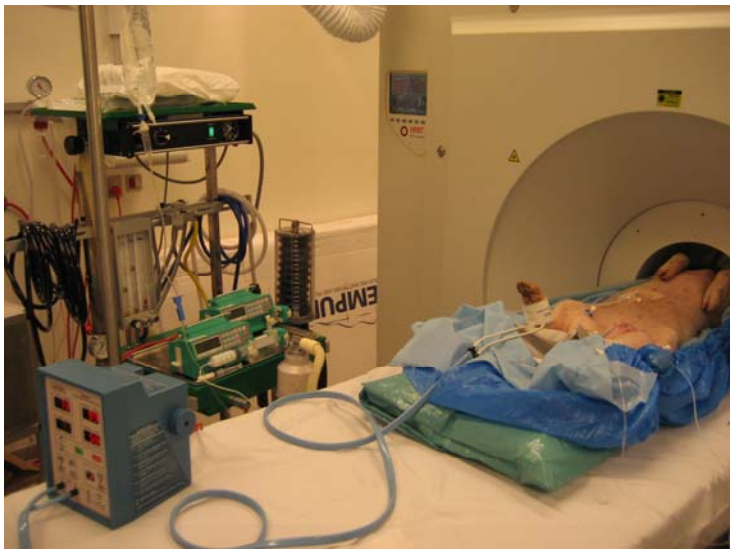

Figure 2. The experimental set-up in which novel Cimbi PET radioligands are tested in the living pig brain. Animal are anaesthetized, intubated and ventilated during all procedures. Vital signs are monitored throughout the duration of the PET scanning. Immediately after scanning, the animals are sacrificed under full anesthesia.

The in vivo outcome of the first [^{11}C]Cimbi-5 PET scans were immediately encouraging: This novel PET tracer entered the pig brain and biodistribution was in accordance with 5-HT$_{2A}$ receptors (i.e. radioligand uptake was highest in the cortical areas of the pig brain and low in cerebellum), and the signal could be blocked by pre-treatment with the 5-HT$_{2A}$ receptor ligand ketanserin. At this time [^{11}C]Cimbi-5 was the first successful 5-HT$_{2A}$ receptor agonist PET radioligand in vivo, and the results were published in the highly-esteemed Journal of Nuclear Medicine (Ettrup et al., 2010), thereby making the first by name mentioned "Cimbi-compound" in the scientific literature a reality.

Although the data for [^{11}C]Cimbi-5 looked promising, it would be preferable with a larger target-to-background signal. Specifically the magnitude of difference between [^{11}C]Cimbi-5 uptake in the cortex and in the cerebellum should ideally be greater. This prompted the project group to think more about how Cimbi-5 could be a good starting point for chemical modifications that could improve subsequent in vivo properties of the PET radioligand.

Systematic modification yielded success

In late 2008/early 2009, the first modified PET ligands using Cimbi-5 as a lead compound were synthesized and ready for testing in the pig brain. The first modifications aimed to reduce the lipophilicity in the hope that this would decrease the non-specific uptake of the tracer (measured by the uptake in the pig cerebellum) or that changing the labeling position in the Cimbi-5 molecule, where the positron emitter is placed, would change metabolism and thus impact the signal-to-noise ratio of the binding signal. However, the improvements in the compounds properties came through a different and unexpected way.

Cimbi-36 was prepared for radiolabeling and in vivo testing, not because it was predicted to be a better PET radioligand but because chemical reactions with compounds of this class are generally easier for structures containing bromine instead of iodine (on the left hand side of the molecule shown in Figure 1). In September 2009, [^{11}C]Cimbi-36 was tested for the first time in the living brain, and it became quickly clear that [^{11}C]Cimbi-36 showed large improvements in *in vivo* properties as compared to [^{11}C]Cimbi-5. As illustrated in Figure 3, [^{11}C]Cimbi-36 had the highest brain uptake of the 10 tested PET tracers, and it also displayed the highest ratio of cortex to cerebellum uptake indicating that the [^{11}C]Cimbi-36 had the best signal-to-noise ratio in its binding of all tested PET ligands.

After the discovery of the improved properties of [^{11}C]Cimbi-36 and identifying this as the most promising 5-HT$_{2A}$ receptor agonist PET radioligand, the decision to move forward with this compound was obvious. During 2009/2010, further studies of [^{11}C]Cimbi-36 in the pig brain were conducted. Through these it was found that binding of [^{11}C]Cimbi-36 to 5-HT$_{2A}$ receptors in the pig brain could be blocked by ketanserin, i.e. pre-treatment with the unlabelled 5-HT$_{2A}$ receptor antagonist, ketanserin, prevented the binding of [^{11}C]Cimbi-36 in the 5-HT$_{2A}$ receptor rich areas in the pig brain. Furthermore, we found that in the pig brain radioactivity was due to [^{11}C]Cimbi-36 only, and not its metabolites. These additional studies confirmed [^{11}C]Cimbi-36 as the most promising PET tracer for imaging cerebral 5-HT$_{2A}$ receptor agonist binding in the living brain (Ettrup et al., 2011), and thus for the center's second five-

year term (2011-2015), [^{11}C]Cimbi-36 was the prime candidate PET radioligand to take further through ultimate development targeting clinical applications.

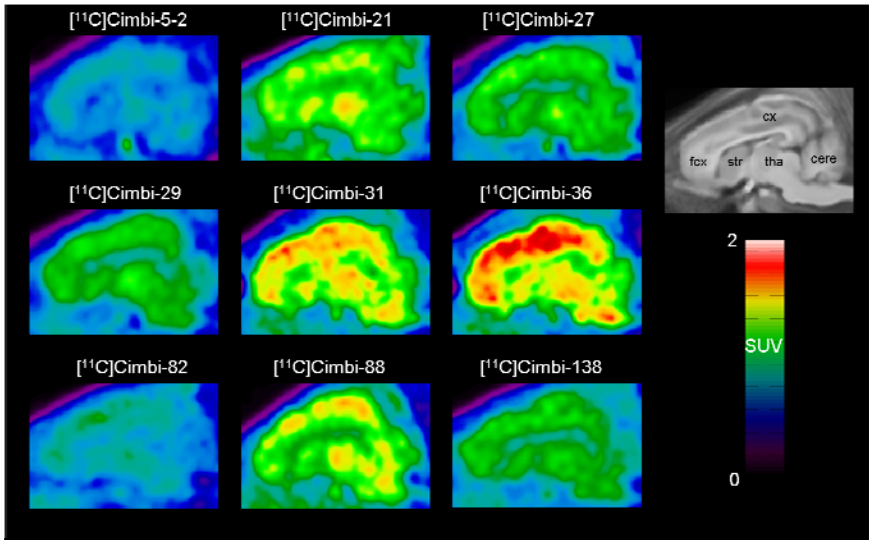

Figure 3. PET images showing the distribution of radioactivity in the pig brain after i.v. injection of tracers. Purple to blue represents low radioactive uptake while and yellow to red colors represents high concentrations of radioactivity. Right insert shows corresponding sagittal view of the MRI-based average atlas of the pig brain with structures labeled: fcx, frontal cortex; cx, cerebral cortex; tha, thalamus; cere, cerebellum; str, striatum. [^{11}C]Cimbi-36 showed the highest uptake of the tracers tested in the pig brain. Figure modified from (Ettrup et al., 2011).

Monkey business in Stockholm

With this very promising PET radioligand at hand, the project group in May 2010 approached Prof. Christer Halldin and Sjoerd Finnema at the Karolinska Institutet (KI) in Stockholm. The KI PET center has been performing PET studies on non-human primates for over 25 years and has developed a state-of-the-art methodology including the same HRRT PET scanner as used within Cimbi. Furthermore, their group had recent experience with 5-HT release studies with fenfluramine (a powerful serotonin releasing agent).

The group in Stockholm moved swiftly, and the first [^{11}C]Cimbi-36 PET experiments in non-human primates were conducted at KI already in August 2010. Immediately, it became clear that [^{11}C]Cimbi-36 also was a promising PET radioligand in monkeys, since here even better brain uptake and signal-to-noise ratios were found as compared to in pigs. Furthermore, pilot studies showed that [^{11}C]Cimbi-36 was sensitive to fenfluramine-induced increases in endogenous levels of 5-HT. These studies further elucidated the potential of [^{11}C]Cimbi-36 and also raised the interest and expectations for the subsequent clinical data with this radioligand.

Preparation for clinical trials

To ensure that injection of [^{11}C]Cimbi-36 in humans would not be associated with risk for study participants, and to aid the regulatory approval of clinical use, we investigated important safety aspects related to clinical use of [^{11}C]Cimbi-36 (Ettrup et al, 2013). The safety issues included in these investigations were dosimetry and in vivo pharmacology. The dosimetry estimation is important to ensure that the enrolled study participants would not be given prohibitively high radiation doses to any specific organs. Furthermore, since 5-HT$_{2A}$ receptor agonists, such as [^{11}C]Cimbi-36, when administered in sufficiently high doses can cause hallucinations in humans, we investigated the pharmacology of [^{11}C]Cimbi-36 after administration to mice. In mice, 5-HT$_{2A}$ receptor activation causes a distinct behavior with repetitive movements of the head and neck, and this behavior can be quantified and is also a marker for the hallucinogenic effects in humans. When given very large doses of [^{11}C]Cimbi-36, this behavior was indeed observed. However, when given at doses far larger than those given in connection with a PET scan no behavioural effects were seen. Hence, both in terms of dosimetry and pharmacology the use of [^{11}C]Cimbi-36 in micro-dosing seemed without any risk for adverse events.

At this point, [^{11}C]Cimbi-36 had thus been investigated in mice, rats, pigs, and monkeys without observing any adverse effects from microdosing injections, and with the promising data from the animal studies at hand, it was therefore decided to apply for regulatory approval to test [^{11}C]Cimbi-36 in humans. The pre-clinical data was submitted to the Danish Medicines Agency along with the technical specification on the [^{11}C]Cimbi-36 production. Additionally, a protocol was submitted to the medical ethical committee to approve two studies with [^{11}C]Cimbi-36: firstly the specificity of the binding of [^{11}C]Cimbi-36 in the human brain was sought to be evaluated with a blocking study using ketanserin, and secondly, in a proof-of-principle study, we sought to test the sensitivity of [^{11}C]Cimbi-36 binding towards changes in 5-HT levels in the human brain. Here, we would use a pharmacological combination of acute administration of selective serotonin reuptake inhibitors (SSRI) and pindolol to block the 5-HT$_{1A}$ autoreceptor response to increase the 5-HT levels. Conversely, the 5-HT levels would be decreased by administration of an amino acid drink

that is depleted of tryptophan, resulting in the so-called acute tryptophan depletion (ATD) in which the brain is depleted of its essential precursor for 5-HT synthesis thus decreasing brain levels of 5-HT acutely within hours. Twenty-four study participants would be randomized to one of the two active SSRI/ATD treatments or placebo in a double-blinded study to primarily investigate the effects of changed 5-HT levels on [^{11}C]Cimbi-36 binding in the human brain.

The first human images

To the great pleasure and excitement of the entire project group, the approvals to commence the human [^{11}C]Cimbi-36 studies were obtained in the summer of 2012. The "first-in-human" nature of the studies restricted the injection to be less than 1.5 µg cold dose of Cimbi-36, and it furthermore required collaboration with both a Good Clinical Practice (GCP) unit to monitor the studies and with an intensive care unit. Cimbi here profited from being rooted in the Capital Region of Denmark and collaborations with appropriate and experienced units were initiated. Hereafter, recruitment of study participants began rapidly, and the big day and important milestone was in sight: The first human PET scan with a Cimbi-radioligand that is developed in-house became a reality, a genuine "from molecule to man"-story all within this center (Figure 4).

Figure 4: Fractions of the proud team at the first clinical [^{11}C]Cimbi-36 PET scan.

For the first results from the [^{11}C]Cimbi-36 blocking study the reader is referred to the cover of this report for the PET images at baseline and after ketanserin pre-treatment. As noted, the initial results look indeed very promising, [^{11}C]Cimbi-36 definitely looks as a specific 5-HT$_{2A}$ receptor agonist PET radioligand in human, the first of this kind. Furthermore, most of the promising data obtained with the monkeys, i.e. biodistribution and signal-to-noise ratios, were replicated in humans. These first clinical data with a 5-HT$_{2A}$ receptor agonist PET radioligand was recently submitted in abstract form to the BrainPET 2013 conference. And the group very excitingly awaits the results of the large proof-of-principle study with SSRI/ATD. Interest for [^{11}C]Cimbi-36 has certainly grown in the global field with the clinical availability and many centers are expected to adopt and use this Cimbi-radioligand based on the positive results found at this stage.

Cross-disciplinary collaboration is the key

As is hopefully clear at this stage, the results described here have an inherited demand for cross-disciplinary collaboration in which essential contributions are made from many different and highly specialized fields. The type of development described herein also critically depends on focused and long-term commitments from all involved agents as the progress from bench to bedside is particularly time-consuming. Luckily, both well-functioning collaboration and commitments have been and still are present within Cimbi.

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