Fundamental studies on radiotracers intended for receptor imaging in dementia

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Due to demographic ageing of the population, a substantial increase in the incidence of dementia is expected in the near future. The progressive course of the disease increasingly interferes with daily tasks in such patients, which puts a serious burden on caregivers and public healthcare. Dementia is therefore prone to develop into a major medical, social and economic problem. In the past decades, the etiology of dementia has increasingly gained attention in the scientific literature, and this has led to a better understanding of the neuropathological mechanisms that are involved in the various types of dementia.

Today, dementia is still a clinical diagnosis, but the available diagnostic criteria for the various types of dementia are frequently limited in terms of sensitivity and specificity. Complementary use of biomarkers may assist in the diagnostic process. A biomarker is being defined as a measurable indicator of disease activity, is based on a relevant pathophysiological aspect of the disease, is ideally measurable in all patients and is able to measure the effects of treatment. Data obtained from medical imaging studies are often suitable for use as a biomarker, and might be of interest for either clinical or scientific purposes. In this respect, biomarkers may contribute to the early diagnosis of dementia, the differentiation between the various types of dementia or the prediction of treatment response. Moreover, biomarkers may also be used to monitor disease activity or to evaluate the effectiveness of experimental treatments in clinical trials. At present, the main purpose of medical imaging studies in patients suffering from dementia, is to exclude potentially treatable causes of the condition. Additionally, in-vivo anatomical measurements of brain structure volumes are performed routinely and imaging studies that evaluate brain metabolism or perfusion are also carried out. Such imaging studies contribute to the clinical diagnostic process to some extent. Molecular imaging has the potential to selectively target a particular aspect of the pathophysiological mechanism of the disease that underlies the dementia. Therefore, data obtained from molecular imaging studies may be very suitable for use as biomarkers. In the past
decades, much effort has been put in the development of in-vivo imaging procedures that could differentiate between the various types of dementia, or reflect specific neuropathological mechanisms of the underlying disease, using nuclear medicine techniques. Although many (candidate) radiotracers have been developed and evaluated for this purpose, only few of these tracers are currently being used in the daily clinical care. Apart from compounds that image brain metabolism or brain perfusion, the only radiotracers that are used routinely to differentiate between types of dementia are dopamine transporter tracers. There are two reasons for this. The first reason is that most candidate tracers that have been developed, have proven to be unsuitable for use as radiotracers in patient care, usually due to low signal-to-noise ratios or other unfavorable in-vivo characteristics. However, tracers have been developed that would be, after decent evaluation in clinical trials, appropriate for use in patients that suffer from dementia. This applies to, for instance, recently developed tracers that target nicotinic receptors, muscarinic receptors, or β-amyloid containing plaques, although the exact value of such tracers as biomarkers for each of these categories is still under debate. The second reason seems to be at least of equal importance. Although every patient benefits from an accurate diagnosis, which is essential for optimizing all aspects of care, no effective therapy for dementia is available to date, and this may be a reason for the apparent lack of interest of clinicians for these new imaging strategies. As mentioned above, one exception is the SPECT or PET evaluation of the dopaminergic transporter status in patients that suffer from dementia with Lewy bodies (DLB), which is being performed to differentiate this type of dementia from Alzheimer’s disease (AD). In the case of DLB, an accurate diagnosis is important, since these patients may have very serious adverse effects, i.e. acute Parkinsonian crisis, when treated with neuroleptics. It is conceivable that this consideration stimulates clinicians to request a dopamine transporter scan. However, due to the present scientific interest for dementia and the increasing knowledge of its pathophysiological determinants, it is very likely that better therapies will be developed for the disease in the future. Many pharmaceuticals that may alter the course of dementia, are under investigation at present. In this respect, drugs are being developed that prevent the fibrillisation of β-amyloid or the formation of plaques, such as anti-β-amyloid vaccines. Other drugs that target the same pathophysiological mechanisms include β- and γ-secretase inhibitors, statines and selective muscarinic M1 receptor agonists. Another promising
group of drugs are the neuroprotective agents, such as anti-oxidants, actrocyte modulating substances, NMDA receptor antagonists, anti-inflammatory drugs, AMPA receptor modulators, nicotinic receptor agonists and drugs that target the synthesis of the tau protein. Prevention of dementia may become a very important issue once effective therapies have been developed. When pharmaceuticals are available that are able to slow down the progression of dementia, or can reverse specific pathophysiologic events of the disease (e.g. anti-Aβ-vaccine), or even medication that simply decrease the symptoms of the disease (such as more effective cholinergic medication), early detection of the disease becomes much more important. This will inevitably increase the clinical need for proper biomarkers, and these could be provided by molecular imaging. Therefore, it is of crucial importance to expand the molecular imaging toolbox with novel, well characterized radiotracers that are potentially suitable for imaging of specific neuropathological aspects of dementia. However, in scientific research, suitable radiotracers that could act as biomarkers or even surrogate markers (biomarkers that can be used as endpoint in clinical trials) for dementia are already highly requested. Thus, the synthesis of novel candidate radiotracers and in-vitro and in-vivo characterization of such compounds, and even the evaluation of new methods for neuropharmacologic research, with respect to imaging procedures for dementia, is important.

Most of the experiments that are reported in this thesis implicate the cholinergic neurotransmitter system. Disturbances of this system, which are present in dementia, are thought to be an important contributor to the cognitive dysfunction in these patients and this neurotransmitter system is therefore a potential target for novel radiotracers intended for imaging of dementia. Ever since the formulation of the cholinergic hypothesis for AD, much effort has been put in the development of tracers that selectively target a part of this system. The holy grail of such radiotracers could be a selective radiotracer for the muscarinic M2 receptor subtype, since multiple previous studies have shown that the density of this particular receptor is decreased in patients that suffer from AD. The most promising radiotracer that is being developed in this field is \([18F]FP-TZTP\), which at least displays selectivity for the M2 receptor in-vivo. To date, this PET tracer has not been introduced in clinical medicine, but it has been evaluated in normal elderly controls and subjects that genetically predisposed for AD. In this thesis, a study is reported in which we synthesized analogues of this tracer as potential SPECT tracers, and one of these candidate tracers
showed favorable in-vitro characteristics, although the selectivity for the M2 receptor was somewhat limited. Unfortunately, the candidate tracer proved to be metabolized very rapidly in-vivo and showed a high non-specific uptake in the brain, probably due to its high lipophilicity and thus, the tracer was considered unsuitable for use in humans. From the literature it can be told that such adverse in-vivo characteristics are observed frequently in the development of new radiotracers that are intended for use in brain imaging. Other attempts to synthesize a M2 selective radiotracer, were based on the earlier described, very M2 receptor selective compound 6β-acetoxynortropane\textsuperscript{13}, but our newly synthesized analogues failed in the in-vitro experiments, not only due to severely decreased affinity for muscarinic receptors as compared to the lead compound, but also due to a loss in selectivity for the M2 receptor. Both iodinated analogues of TZTP and 6β-acetoxynortropane, or similar analogues that could potentially be iodinated, resulted in compounds with inferior in-vitro or in-vivo binding characteristics than the original molecules. The synthesis of a potent, iodinated, selective and metabolically stable tracer for the M2 receptor proved to be difficult. Based on the knowledge of molecules that bind selectively to M2 receptors, it seems conceivable that the first appropriate tracer that will be developed for the M2 receptor, will be a \textsuperscript{18}F or \textsuperscript{11}C labeled PET-tracer. Reasons for this include the more favorable in-vivo characteristics of such tracers after radiolabeling, since no iodine atom is necessary in the molecule, but also because the imaging technique is gaining popularity rapidly and becomes increasingly available.

Whether a selective M2 receptor tracer has to be pursued is also debatable. Although a decline in M2 receptors is characteristic for AD, there is also proof for a decline of receptors of the nicotinergic neurotransmitter system, and these may precede the decline of M2 receptors, so targeting the nicotinic system may be more appropriate with respect to the early diagnosis of AD. Recent advances in the development of nicotinic receptor radiotracers\textsuperscript{14, 15} implicate that targeting of this neurotransmitter system may be an attractive alternative for the exploration of disturbances of the cholinergic system in AD. However, receptor selective muscarinic radiotracers may gain new attention due to the role of the muscarinic M1 receptor in the APP cleavage process\textsuperscript{16} and tracers for the M1 receptor may therefore be useful for the prediction of response to M1 agonists. Imaging of the M1 receptor may also be of value for the evaluation of other neuropsychiatric diseases. A subgroup of patients that suffer from schizophrenia that shows a vast decline of cortical muscarinic
M₁ receptor density, has recently been identified and this offers opportunities for both treatment and molecular imaging. All in-vivo experiments that are reported in this thesis use ¹²³I labeled radiotracers. Experiments in rats in which the biodistribution was investigated in small or complex brain structures, necessitated a more sensitive experimental method than the classical dissection technique, in which brain structures are dissected and counted for radioactivity separately. Conventional autoradiography is a very precise method, but could not be used due to the short half-life of ¹²³I. A similar method, storage phosphor imaging, proved to perform very good in this respect. Although this technique is used frequently in biodistribution experiments, the characteristics of the phosphor plates for short-living radioisotopes had never been evaluated systematically for a larger series of isotopes. The study that is reported in this thesis shows that the phosphor imaging technique is suitable for use with tracers that are labeled with short-living radioisotopes, such as ¹²³I, and that this method is valuable for pharmacologic experiments in the brains of small animals using radioactive tracers. Although the maximal resolution is much lower than that of conventional autoradiography, the storage phosphor imaging technique has some important advantages. Advantages are e.g. the short exposure periods that are necessary to obtain images and the high capacity of the plates which prevents over-exposure. For imaging of microscopic structures, the conventional autoradiography films remain to be the method of choice. As compared to dissection techniques, the storage phosphor imaging technique is more precise in measuring radioactivity in small and complex brain structures such as the hippocampus. However, the method is rather labor-intensive and when a fast screening in a large group of animals is to be performed, the dissection technique is more attractive. As compared to small animal SPECT procedures, the resolution of the phosphor imaging technique is superior, but whether this is also the case when compared with micro-PET, has to be established.

In this thesis, the phosphor imaging technique was used for an experiment in which the in-vivo characteristics of [¹²³I]CNS-1261, a candidate radiotracer that targets activated NMDA receptor, were explored. Activation of these receptors is a non-specific characteristic of neurodegenerative diseases and this mechanism is therefore potentially of interest for imaging and quantification of disease activity in patients with dementia. In this study, we showed that the binding of the tracer could partially be influenced, indicating specific binding of the tracer to
the NMDA receptor, but also that a high nonspecific uptake occurred in the brain, which may be the result of fast in-vivo metabolism of this tracer. Indeed, this has also been reported by another group, and this renders the value of this tracer for use in humans as limited.

The value of dopamine transporter tracers, such as \[^{123}I\]FP-CIT has been emphasized above, and this particular tracer is now used clinically for the differentiation between AD and DLB. Similar to patients that suffer from Parkinson’s disease (PD), DLB patients show a marked decrease of dopamine transporter density in the brain, which discriminates this type of dementia from AD. Knowledge about the effects of drugs on the outcomes of neuroimaging procedures is crucial, since patients may need to discontinue the medication before such scans are being performed. In this thesis, an animal experiment is presented in which the effects of AChEIs, which are used frequently by both AD and DLB patients, on the binding of \[^{123}I\]FP-CIT are evaluated. Although interactions between the dopaminergic and the cholinergic are well known, no effects of administration of the AChEIs donepezil and rivastigmine, on the binding of the tracer to the presynaptic dopaminergic nerve terminals were revealed. Effects of other AChEIs such as galantamine are also expected to have no effects on the \[^{123}I\]FP-CIT binding in the brain.

These results are in line with a recently performed retrospective study, in which data from human studies were evaluated, and which also showed no effects of concurrent use of AChEIs on obtained images that reflect the dopamine transporter status in DLB patients. However, the retrospective character of this particular study does not provide the ultimate proof, and a randomized trial should ideally be performed to prove that AChEIs do not interfere with \[^{123}I\]FP-CIT imaging. Until then, there is no need for discontinuation of AChEIs before a \[^{123}I\]FP-CIT scan is being performed.

Potentially interfering effects of medication on the binding of \[^{123}I\]Iododexetimide were also evaluated. This tracer binds non-selectively to all muscarinic receptor subtypes and has favorable in-vivo characteristics. Since the density of muscarinic M\(_1\) receptors in the human brain is much higher than the density of M\(_2\) receptors, this tracers reflects predominantly the muscarinic M\(_1\) receptor status, when used in imaging studies. The tracer may therefore be of clinical value for the SPECT imaging of the postsynaptic muscarinic neurotransmitter system, and may be useful for the prediction of therapy response to AChEIs in DLB, although an earlier report using a similar radiotracer, R,R \[^{123}I\]QNB, failed to distinguish responders from non-responders within a group of
AD patients. Non-subtype selective muscarinic receptor tracers may also prove to be useful for the group of schizophrenia patients, which exhibited a profound loss of cortical M1 receptors, according to the study by Scarr et al. Patients with either dementia or schizophrenia frequently use neuroleptics, and this has been the motivation to study the effects of subchronic administration of neuroleptics (olanzapine and risperidone) on the binding of [123I]Iododexetimide in the rat brain. The experiment revealed no effects of neuroleptics on the binding of the tracer in brain areas that are known to express a high density of M1 receptors. Thus, there seems to be no necessity to discontinue the use of neuroleptics before a [123I]Iododexetimide scan is being performed. Human data on potential influences of neuroleptics on the binding of [123I]Iododexetimide are not available yet, and this issue should be further studied before this tracer is used in patient groups that use this type of medication.

However in this thesis, alteration of the dopaminergic system has been shown to influence the binding of [123I]Iododexetimide in the brains of rats. To detect these small influences, we used the sensitive storage phosphor imaging technique. Unilateral damage to the dopaminergic neurotransmitter system due to an induced brain lesion, revealed a decrease of tracer binding in ipsilateral neocortical brain areas of lesioned rats. This is presumably the result of hyperactivation of the muscarinic neurotransmitter system, which is thought to occur due to the release from the inhibitory actions of dopaminergic system that had been damaged. It is hypothesized that the decreased binding of [123I]Iododexetimide is the result from either a downregulation of postsynaptic M1 receptors, or competition of acetylcholine on the binding of the tracer to the receptor. One potential limitation of this study is the fact that the animals served as their own control group, since binding of [123I]Iododexetimide was compared for the lesioned versus the unlesioned hemisphere. There are indications for minor effects of the unilaterally performed dopaminergic lesion on the contralateral dopaminergic system, and this can not be excluded in this study. In further studies using this animal model, a sham operated control group should therefore be included.

The effects of a decreased dopaminergic inhibition on the binding of [123I]Iododexetimide are likely to be small and presumably not detectable with SPECT, which is a less sensitive imaging technique as compared to phosphor imaging. For imaging of neurodegenerative diseases that are accompanied by a marked decrease in muscarinic receptor
density, hyperactivity of the cholinergic system will not be an important issue.

In the human SPECT study, in which [123I]Iododexetimide was used to detect differences in tracer binding between PDD and PD patients, potential effects of the release of the muscarinic system from the inhibition by the dopaminergic system were most likely non-existent. The Hoehn and Yahr scores of the patients of both groups were similar and showed no significant difference. The demonstrated decline of [123I]Iododexetimide binding in brain areas that are important in memory function, in PDD versus PD patients, may therefore be the result of degenerative changes in the muscarinic neurotransmitter system, as would be expected in PDD. This study suggests that non-subtype selective muscarinic receptor tracers may be of value for the detection of deficiencies in the cholinergic system, although it can not be excluded that differences in perfusion or regional atrophy account for the observed decreased tracer binding\textsuperscript{30}. In future studies, measurements on atrophy by MRI or CT techniques, or measurements on cerebral perfusion would be a valuable addition, although the acquisition of multiple scans may be difficult in this particular population. From a scientific point of view, it would be interesting to determine whether prediction of treatment response is feasible by performing [123I]Iododexetimide brain SPECTs in PDD patients. However, it has become clear that the observed small decreases in tracer binding do not allow prediction of treatment response in individual patients, although this verdict would be strengthened after data is also obtained from an age-matched control group. The homogenous distribution of muscarinic receptor subtypes in the human brain will complicate the visual interpretation of [123I]Iododexetimide SPECTs in diseases that only show small alterations of muscarinic receptor densities. In the subgroup of schizophrenia patients from the postmortem study by Scarr et al.\textsuperscript{17}, a 74\% decrease of [3H]pirenzepine binding to muscarinic M\textsubscript{1} receptors was detected, and in such patients, [123I]Iododexetimide SPECT should be perfectly adequate for the visual determination of the muscarinic receptor status in individual patients.

The aims of this thesis were to explore novel candidate radiotracers intended for imaging of dementia by means of molecular imaging techniques, and to evaluate the value of existing radiotracers that are currently being used, or could potentially be used for imaging of dementia. In conclusion, it can be stated that attempts to synthesize a selective
muscarinic M2 receptor radiotracer have led to disappointing results. All candidate radiotracers have failed either in-vitro or in-vivo. The experimental radiotracer intended for imaging of activated NMDA receptors also proved to be unsuitable for use in human imaging, due to unfavorable in-vivo characteristics of the compound. Existing neuroreceptor tracers seem to have more potential for use in clinical diagnostics. A good example is the dopamine transporter tracer [123I]FP-CIT, which is now used clinically for the differentiation between DLB and AD. In rats, we did not find evidence for interfering effects on the binding of this tracer to its target by AChEIs. Since similar results have been reported in humans, there is no reason to discontinue this type of medication before an [123I]FP-CIT SPECT is being performed. Also no effects of sub-chronic administration of neuroleptics were detected on the binding of [123I]Iododexetimide in the rat brain, and therefore there seems to be no reason for discontinuation of these pharmaceuticals before performing a [123I]Iododexetimide SPECT. However, this should be verified in humans before this tracer is being used clinically. Small effects of dopaminergic deficiency have been shown in rats, but are not likely to influence the interpretation [123I]Iododexetimide SPECTs in patients. A decrease of [123I]Iododexetimide binding in the brains of PDD patients has been shown as compared to PD patients, but is of a small magnitude and probably not visually detectable in individual patients. A non-subtype selective muscarinic receptor radiotracer such as [123I]Iododexetimide is likely to be of value for the evaluation of diseases of the central nervous system, that are accompanied by a larger decrease in muscarinic receptor density.

References


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