RESEARCH HIGHLIGHT

The antagonist SPECT tracer ^{123}I-iododexetimide binds preferentially to the muscarinic M_1 receptor in-vivo, but is it also a potential tool to assess the occupancy of muscarinic M_1 receptors by agonists?

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Cognitive deterioration in neuropsychiatric disorders is associated with high attrition rates giving an urgent need to develop better pharmaceutical therapies. The underlying mechanisms of cognitive impairments are unclear but research has shown that the muscarinic receptor subtype 1 (M_1 receptor) plays a critical role. Blocking the M_1 receptor gives rise to profound cognitive deficits, while the administration of M_1 agonist drugs improves cognitive functioning. In this research highlight we will outline supporting data that the radiotracer ^{123}I-iododexetimide preferentially binds to the M_1 receptor in-vivo and can be used to assess changes in M_1 receptor expression in-vivo associated with cognitive decline. These findings come from a previously published paper extensively examining binding characteristics of $^{123/127}$I-iododexetimide to muscarinic receptors. Results of biodistribution studies also has shown that acute administration of the $M_1/_4$ receptor agonist xanomeline could inhibit ^{127}I-iododexetimide binding in M_1-rich brain areas in rats, suggesting that ^{123}I-iododexetimide may also be used to evaluate the occupancy of M_1 receptors by M_1 agonists in-vivo. This may be of clinical relevance considering the efficacy of M_1 agonist drugs in the treatment of cognitive deficts. Here we show the results from new biodistribution experiments in rats conducted to test the hypothesis that ^{123}I-iododexetimide may be a useful radiotracer to evaluate the M_1 receptor occupancy by M_1 agonists in-vivo. Contrary to our expectations, no significant change in ^{123}I-iododexetimide ex-vivo binding was observed after acute administration of xanomeline in M_1 receptor-rich brain areas, whereas significantly decreased ^{123}I-iododexetimide binding was found after chronic treatment with xanomeline. ^{123}I-iododexetimide single photon emission computed tomography (SPECT) may therefore be a useful imaging tool to further evaluate M_1 receptor changes in neuropsychiatric disorders, as a potential stratifying biomarker, to assess the occupancy of M_1 receptors after M_1 antagonist treatment, or after chronic treatment with M_1 agonists, although it may be less suited to evaluate the M_1 receptor occupancy after acute treatment with M_1 agonists. Future studies should concentrate efforts towards finding also an M_1 agonist radiotracer for positron emission tomography (PET) or SPECT to assess the working mechanism of M_1 agonists.

Keywords: ^{123}I-iododexetimide; SPECT; muscarinic M_1 receptor agonist; xanomeline; cognition; rat

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Introduction

There are longstanding implications that the muscarinic system, as part of the cholinergic system, plays a critical role in cognition [1]. Administration of cholinergic receptor antagonists give profound cognitive deficits, and cholinesterase inhibitors are broadly prescribed to maintain and improve cognition in neuropsychiatric disorders like Alzheimer's disease [2]. It is thought these deficits are mediated, at least partly, by the muscarinic receptor subtype 1 (M_1 receptor) due to its high expression in prefrontal cortex, hippocampus, and striatum, which are brain regions critical for cognition [3-5]. Moreover, muscarinic M_1 receptor knock out animals showed deficits in learning and memory [6-8]. There is also preliminary evidence that changes in M_1 expression may be the underlying pathophysiology of cognitive deterioration in schizophrenia and related disorders. Importantly, a hallmark post mortem study found 75% reduction in M_1 receptor density in a frontal brain area in a subgroup of patients with schizophrenia compared to healthy controls, which they termed muscarinic receptor deficiency schizophrenia or MRDS [9, 10]. Also, it has been suggested that particularly this subgroup of schizophrenic patients may suffer from cognitive deficits. In addition, clinical pilot studies examining effects of M_1 agonists and positive allosteric modulators (PAM) show improved scores on cognitive test batteries [11-13]. However, measuring the M_1 receptor selectively over the other 4 subtypes of muscarinic receptors in-vivo has proven challenging due to a lack of a well characterised selective radio ligand and the stereotactic homogeneity of the 5 muscarinic receptor subtypes (for a review see [14]).

Dexetimide is a candidate compound to image the muscarinergic system as it is a muscarinic antagonist prescribed in clinical practice for neuroleptic-induced Parkinsonism. Already back in the 1990's, two studies were published investigating radiolabelled dexetimide as a radio ligand to image muscarinic receptors in-vivo [15, 16]. These studies showed promising results with high binding of ^{123}I-iododexetimide in M_1-rich brain areas and a high brain uptake (7-8% of injected dose) [16], however it is unclear whether this was specific binding to the M_1 receptor. Consequently, we conducted a series of experiments to determine selectivity and binding profile of ^{123}I-iododexetimide to all muscarinic receptor subtypes. The current research highlight will address the key findings of these experiments previously published [17] together with ongoing experiments assessing ^{123}I-iododexetimide as single photon emission computed tomography (SPECT) tracer to measure occupancy of the M_1 receptor by M_1 agonists. These additional experiments resulted from promising results of clinical pilot studies which demonstrated that M_1 agonists and PAMs may improve cognition [11-13].

Binding profile of $^{127/123}$I-iododexetimide to muscarinic receptor subtypes

Series of in-vitro competitive binding studies were conducted to assess binding affinity and functional antagonism of ^{127}I-iododexetimide for all five human muscarinic receptor subtypes overexpressed on Chinese hamster ovarian (CHO) cell membranes [17]. The affinity was determined by the displacement of ^{3}H-n-methylscopolamine, a highly selective M_1 antagonist [18], by ^{127}I-iododexetimide. Results revealed that the affinity of ^{127}I-iododexetimide of binding to the M_1 receptor subtype was in the Pico molar range. Regarding selectivity, the affinity of ^{127}I-iododexetimide towards the M_1 receptor was much higher compared to the other subtypes. In addition, ^{127}I-iododexetimide binding to the M_1 receptor showed the highest affinity to antagonize acetylcholine activated receptor subtypes. Bio distribution studies in rats corroborated these findings by showing that ^{127}I-iododexetimide could be displaced by the $M_{1/4}$ selective agonist xanomeline in a dose dependent manner. To validate binding selectivity of ^{127}I-iododexetimide to muscarinic receptors, further studies were conducted in control and KO mice for each muscarinic receptor subtype. Results showed that only in KO mice of the M_1 receptor the ^{127}I-iododexetimide binding was significantly decreased in the M_1 receptor-rich frontal cortex (Figure 1).

Finally, bio distribution studies in rats were performed to evaluate whether the antipsychotic olanzapine, which has a high affinity for M_1 receptors (K_i= 1.9 nM) [19] and acts as an antagonist, was able to block ^{123}I-iododexetimide binding in M_1-rich brain areas ex-vivo. Phosphor storage imaging was conducted to measure brain distribution of ^{123}I-iododexetimide concurrent with administration of olanzapine [20]. As expected, acute administration of the M_1 antagonist olanzapine resulted in a significant decrease of ^{123}I-iododexetimide binding in M_1-rich brain areas.

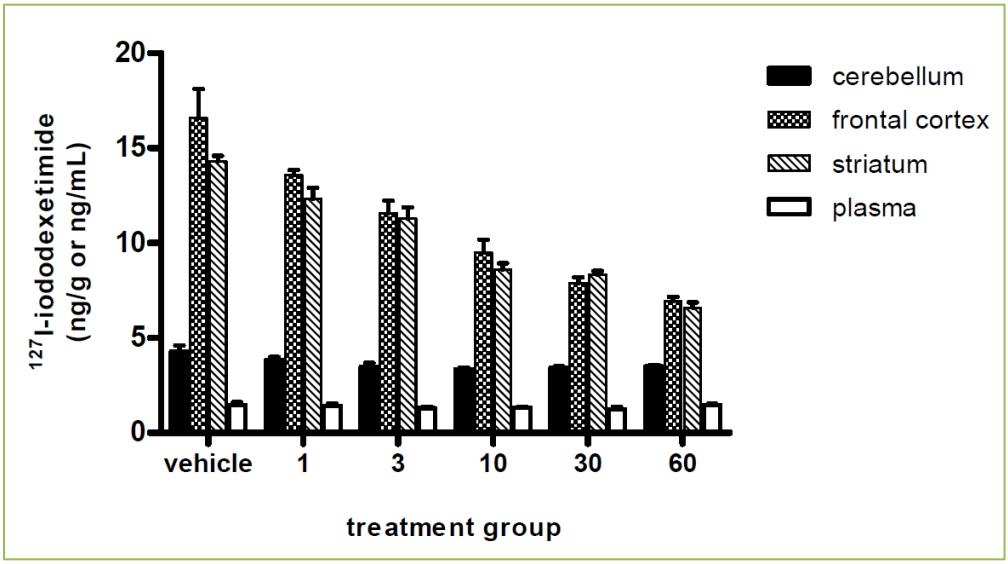

Figure 1. Validation of specific binding to M_1 receptors of ^{127}I-iododexetimide in M_1–M_5 receptor knock out mice, 40 min after injection. Binding potential was calculated as specific binding in frontal cortex (total binding minus nonspecific binding) divided by nonspecific binding. This research was originally published in J Nucl Med. Bakker et al. ^{123}I-Iododexetimide Preferentially Binds to the Muscarinic Receptor Subtype M_1 in Vivo. J Nucl Med 2015; 56:317-22. © By the Society of Nuclear Medicine and Molecular Imaging, Inc.

All things considered, it was concluded that ^{123}I-iododexetimide preferentially binds to M_1 receptors in-vivo as an antagonist SPECT tracer.

Current experiments: Can ^{123}I-iododexetimide SPECT be used to assess the occupancy of M_1 by agonist drugs?

Background

Since M_1 agonists and PAMs may improve cognition [11-13], we were interested to test whether the antagonist M_1 SPECT tracer ^{123}I-iododexetimide may be useful in future imaging studies to evaluate the occupancy of M_1 receptors by M_1 agonists, like xanomeline. As described earlier, we already showed that the acute administration of xanomeline was able to block ^{127}I-iododexetimide binding dose dependently ex-vivo in rats [17]. In these experiments, liquid chromatography-mass spectroscopy (LC-MS/MS) was used to assess ^{127}I-iododexetimide binding. An advantage of this technique is that the measurement is not influenced by metabolites that are formed after injection in rats. Contrary, using storage phosphor imaging, the formation of ^{123}I-labelled metabolites in rats could influence the outcome measurement. However, in clinical practice, ^{123}I-iododexetimide instead of ^{127}I-iododexetimide is used, and consequently results of studies using the SPECT tracer ^{123}I-iododexetimide may reflect clinical practice better than results obtained with ^{127}I-iododexetimide. Therefore, we conducted additional studies to evaluate whether acute and/or chronic treatment of xanomeline was able to reduce ^{123}I-iododexetimide binding in rat brains. Based on our previous results [17], we hypothesized that acute, but not chronic, administration of xanomeline would decrease ^{123}I-iododexetimide binding in M_1-rich brain areas.

Methods

We used storage phosphor imaging to study the effects of acute and chronic administration of xanomeline on ^{123}I-iododexetimide binding in M_1 receptor-rich brain areas. In brief, 16 male Wistar rats (average weight approximately 320 gram) received a single dose of xanomeline (n=8; dose 3 mg/kg body weight intraperitoneally) or placebo (0.3 ml saline; n=8) acutely, whereas 16 other male rats were pre-treated with xanomeline (twice a day 3 mg/kg) or placebo for 14 days. One hour after drug treatment in the acute group, and 24 h after the final injection in the chronic group the rats were anesthetized, injected intravenously in a tail vein with approximately 50 MBq ^{123}I-iododexetimide (synthesis, specific activity and radiochemical purity as previously described; [17, 21]) and sacrificed as previously described [17]. Then, binding of ^{123}I-iododexetimide was determined with storage phosphor imaging as earlier described [17]. For analysis, regions of interest (ROIs) were drawn manually for the prefrontal cortex, hippocampus and striatum, areas rich in M_1 receptors, as earlier described [3, 17]. Binding in the cerebellum was chosen as the non-specific region because of the low muscarinic acetylcholine receptor expression in this area. According to our previous study, the ratio of specific to non-specific binding was used as the

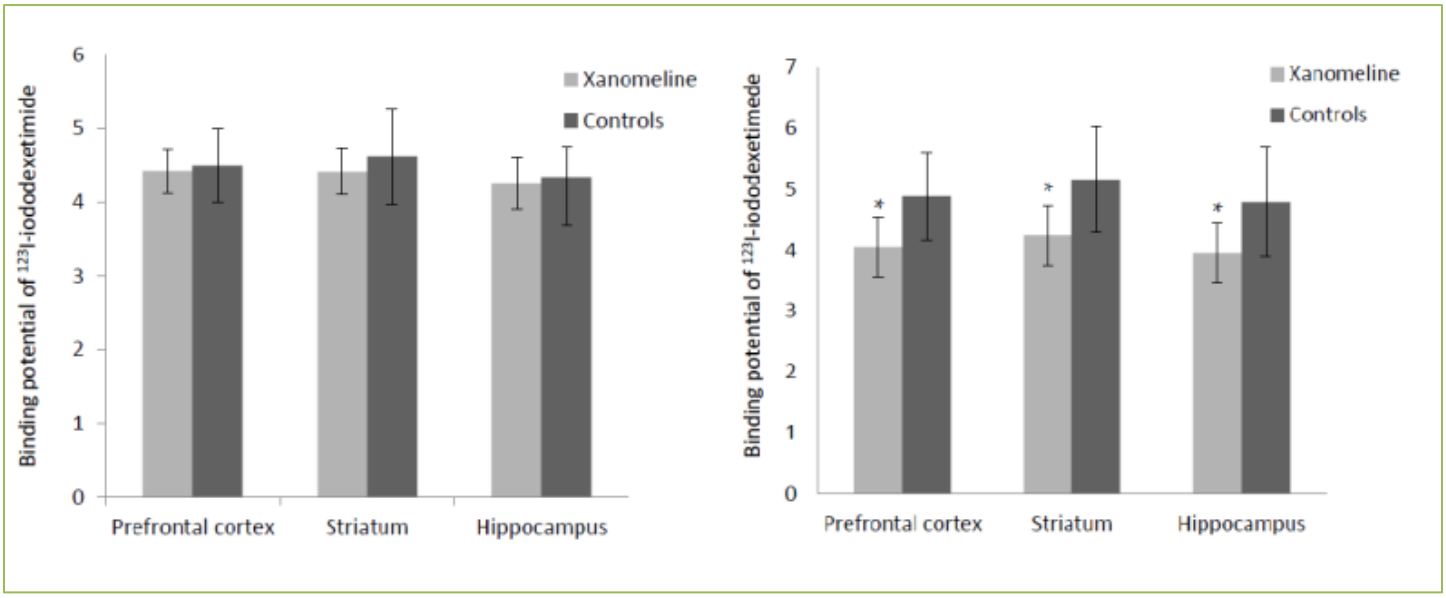

Figure 2. Binding potential of ^{123}I-iododexetimide in ROIs of prefrontal cortex, striatum and hippocampus. Binding potential was calculated as specific binding (total binding minus nonspecific binding) in ROI divided by nonspecific binding (measured in cerebellum). ^{123}I-iododexetimide binding was measured 2 h after intravenous injection of ^{123}I-iododexetimide. Upper panel (acute experiment): Rats (n= 8/group) were pre-treated with 1 dose of saline or xanomeline (3 mg/kg) 1 h before injection with radiotracer. Lower panel (chronic experiment): Rats (n= 8/group) were pre-treated for 14 days with 2 doses of saline or xanomeline (3 mg/kg) per day until 24 h before injection with radiotracer. *Statistically significantly lower as compared with control group.

outcome measure [17]. Differences in hippocampal, prefrontal and striatal ^{123}I-iododexetimide binding ratios for both the xanomeline and placebo treatment was analysed using a one-way multivariate analysis of variance (MANOVA).

Results

The acute group showed no significant decrease in ^{123}I-iododexetimide binding ratios for all M_1 receptor-rich brain areas examined, whereas the chronic group did show significantly lower binding ratios in all these brain areas (Figure 2).

Discussion

The current bio distribution studies in rats showed that ^{123}I-iododexetimide binding ratios were not significant lower after acute administration of xanomeline as compared to the placebo condition. This finding was unexpected, since we previously showed that the acute administration of xanomeline was able to block ^{127}I-iododexetimide binding dose dependently as assessed ex-vivo in rats. In more detail, a single dose of ^{127}I-iododexetimide decreased e.g., the specific to non-specific binding ratio (which is the outcome measure of our current storage phosphor imaging study) in the frontal binding by approximately 20% (Figure 3). In addition, in our previous study, ^{127}I-iododexetimide binding was determined 40 min after injection, while in the current study, the rats were killed 2 h after injection of ^{123}I-iododexetimide. Also, in our previous study LC-MS/MS was used (which measurement is not influenced by metabolites formed after injection in rats), while in the current study we used storage phosphor imaging. These factors might explain why we did not observe a decreased ^{123}I-iododexetimide binding ratio in our present study after an acute dose of 3 mg/kg xanomeline. We cannot exclude, however, that we will find reduced ^{123}I-iododexetimide binding ratios after administration of a higher dose than 3 mg/kg. However, the question then remains whether such results would be translatable to humans, because the dose used in this study was already high compared to doses used in human trials [13, 22]. So, since the storage phosphor measurements using the SPECT tracer ^{123}I-iododexetimide may reflect the clinical practice better than results obtained with ^{127}I-iododexetimide, we conclude that it is not likely that ^{123}I-iododexetimide SPECT is a useful tool to assess the occupancy of M_1 receptors after acute administration of an agonist like xanomeline.

Since ^{123}I-iododexetimide itself is a M_1 receptor antagonist, this might explain why ^{123}I-iododexetimide may not be the ideal radiotracer to assess occupancy of the M_1 receptor by M_1 agonists. Commonly, the occupancy of receptors is much higher when therapeutic doses of antagonists are used as compared to agonists. It is therefore possible that a M_1 receptor agonist radiotracer could better serve as a potential tracer to assess the occupancy of M_1 receptors by M_1 agonists, and further research is needed

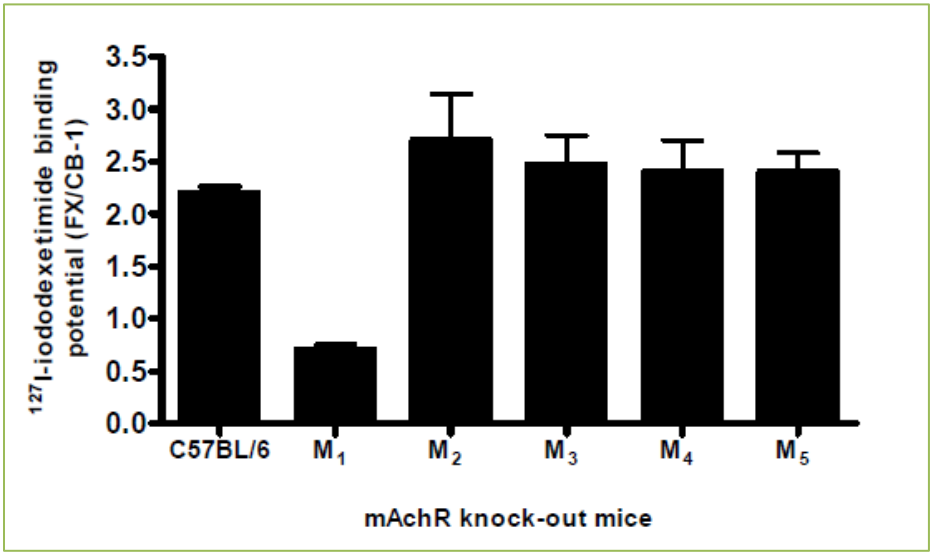

Figure 3. Distribution of ^{127}I-iododexetimide in cerebellum, frontal cortex, striatum, and plasma (n= 3–4) in response to dose increase of agonist xanomeline (1–60 mg/kg) 40 min after injection of ^{127}I-iododexetimide. Mice were pre-treated with vehicle (0) or xanomeline 30 min before injection of radiotracer. Data points represent mean specific binding ± SEM. This research was originally published in *J Nucl Med*. Bakker et al. ^{123}I-Iododexetimide Preferentially Binds to the Muscarinic Receptor Subtype M_1 In Vivo. *J Nucl Med* 2015; 56:317-22. © By the Society of Nuclear Medicine and Molecular Imaging, Inc.

to explore this possibility. Importantly, the development of M_1 agonist radiotracers for positron emission tomography (PET) imaging has started [23, 24].

Interestingly, (sub) chronic administration of xanomeline did induce significantly lower ^{123}I-iododexetimide binding ratios, possibly reflecting down-regulation of M_1 receptors. It is well known that agonists can induce down-regulation of receptors on the cell membrane [25,26]. Consequently, ^{123}I-iododexetimide SPECT might be a promising tool to assess the long-term effects of M_1 agonists on M_1 receptor expression.

Future directions

PET or SPECT imaging of M_1 receptors is highly important to fully understand the role of M_1 receptors in cognitive symptoms such as seen in schizophrenia. Cognitive deficits are the best established predictors of functional disability in this disorder [27]. In this regard, our data suggest that ^{123}I-iododexetimide SPECT may be a useful imaging tool to further evaluate M_1 receptor changes in neuropsychiatric disorders, as a potential stratifying biomarker, or to assess the occupancy of M_1 receptors of M_1 antagonists or after chronic treatment with M_1 agonists like xanomeline, although it may be less suited to evaluate efficacy of agonist drugs. Indeed, we recently started a clinical study in which we will examine the existence of MDRS using ^{123}I-iododexetimide SPECT. However cognitive deficits in schizophrenia as well as in other neuropsychiatric disorders have proven difficult to treat and therefore more research on M_1 agonists is needed. Future studies should concentrate efforts towards developing an adequate M_1 agonist radiotracer to get more insight into M_1 agonist functioning.

Finally, regarding studies on ^{123}I-iododexetimde, it may be of interest in future studies to evaluate whether also the acute administration of other M_1 agonists than xanomeline will influence ^{123}I-iododexetimide. Also, it may be of interest to test whether the ^{123}I-iododexetimide binding is sensitive to changes in acetylcholine concentrations e.g., induced by cholinesterase inhibitors.

Conclusions

In conclusion, extensive characterisation of ^{123}I-iododexetimide validates that its antagonistic in-vivo binding predominantly reflects binding to the M_1 receptor. Consequently ^{123}I-iododexetimide SPECT may a useful means to assess M_1 receptors in-vivo related to cognitive deterioration in neuro-psychiatric disorders, such as Parkinson's disease, Alzheimer's disease and psychotic disorders, and to assess occupancy of M_1 receptors by antagonist M_1 drugs, although it may be less suited to assess efficacy and occupancy of the M_1 receptor of M_1 agonist drugs.

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