Fundamental studies on radiotracers intended for receptor imaging in dementia

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CHAPTER 6

NO SIGNIFICANT EFFECTS OF SINGLE INTRAVENOUS, SINGLE ORAL AND SUB-CHRONIC ORAL ADMINISTRATION OF ACETYLCHOLINESTERASE INHIBITORS ON STRIATAL [123I]FP-CIT BINDING IN RATS

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Abstract

[123I]FP-CIT SPECT is a valuable diagnostic tool to discriminate Lewy body dementia from Alzheimer’s dementia. To date, however, it’s uncertain whether the frequently used acetylcholinesterase inhibitors (AChEIs) by demented patients, have an effect on [123I]FP-CIT binding to dopamine transporters (DATs). Earlier animal studies showed a decline of DAT-availability after acute intravenous injection of AChEIs. The aim of this study was to

Figure 6.1a. Specific striatum-to-cerebellum ratios of [123I]FP-CIT in the rat brain after intravenous administration of a single dose of either rivastigmine (2.5mg/kg), donepezil (0.5mg/kg) or methylphenidate (10mg/kg) at 10 min prior to injection of the radiotracer, as compared to controls (* p<0.05).

Figure 6.1b. Specific hypothalamus-to-cerebellum ratios of [123I]FP-CIT in the rat brain after intravenous administration of the same substances.

Figure 6.1c. Specific striatum-to-cerebellum ratios of [123I]FP-CIT after administration of a single oral dose of either rivastigmine (2.5mg/kg), donepezil (2.5mg/kg) or methylphenidate (10mg/kg) at 2h, h and 40 min prior to injection of the radiotracer, respectively, as compared to controls (* p<0.05).

Figure 6.1d. Specific hypothalamus-to-cerebellum ratios of [123I]FP-CIT in the rat brain after single oral administration of the same substances.

Figure 6.1e. Specific striatum-to-cerebellum ratios after sub-chronic administration of 1mg/kg rivastigmine, 1.5mg/kg donepezil or 2.5mg/kg methylphenidate during 14 consecutive days (before the day of the experiment), as compared to controls.

Figure 6.1f. Specific hypothalamus-to-cerebellum ratios of [123I]FP-CIT in the rat brain after sub-chronic administration of the same substances. All data are expressed as the mean ± 1 standard deviation. Abbreviations: Hyp; hypothalamus, Cer; cerebellum.
investigate effects of single intravenous, single oral and sub-chronic oral administration of AChEIs on DAT-availability in the rat brain, as measured by $^{[123]}$I-FP-CIT.

Biodistribution studies were performed in Wistar rats ($n=5-16$ per group). Prior to $^{[123]}$I-FP-CIT injection, rats were injected intravenously with a single dose of the AChEI rivastigmine (2.5mg/kg body weight) or donepezil (0.5mg/kg), the DAT-blocker methylphenidate (10mg/kg) or saline. A second group was orally treated with a single dose of rivastigmine or donepezil (2.5mg/kg), methylphenidate (10 mg/kg), or saline prior to injection of $^{[123]}$I-FP-CIT. Studies were also performed in rats that were orally treated during 14 consecutive days with either rivastigmine (1mg/kg daily), donepezil (1.5mg/kg daily), methylphenidate (2.5mg/kg) or saline. Brain parts were assayed in a gamma counter and specific striatum/cerebellum ratios were calculated for the $^{[123]}$I-FP-CIT binding to DATs.

No significant effects of either single intravenous, single oral or sub-chronic oral administration of AChEIs on striatal FP-CIT binding could be detected. Single pre-treatment with methylphenidate resulted in an expected significantly lower striatal FP-CIT binding.

We conclude that in rats, single intravenous and single or sub-chronic oral administration of the tested AChEIs does not lead to an important alteration of $^{[123]}$I-FP-CIT binding to striatal DATs. Therefore, it is unlikely that these drugs will induce large effects on the interpretation of $^{[123]}$I-FP-CIT SPECT scans in routine clinical studies.

Introduction

$^{[123]}$I-FP-CIT (DaTSCAN) is used nowadays in clinical practice to detect dopaminergic cell loss in e.g. Parkinson’s disease by means of brain SPECT. This radiotracer binds primarily to the dopamine transporter (DAT), which is located on the presynaptic terminal of dopaminergic neurons. Dementia of the Lewy Body type (DLB) is also characterized by pronounced dopaminergic cell loss, whereas in dementia of the Alzheimer type (AD) no or only a very mild dopaminergic degeneration could be detected. DLB is the second most common form of dementia after AD, and is often clinically misinterpreted as AD. Although a definitive diagnosis of DLB can only be established post-mortem, $^{[123]}$I-FP-CIT SPECT greatly improves the discrimination of DLB from AD during life.

It’s important to discriminate between DLB and AD. For example, in contrast to AD patients, DLB patients are prone to develop serious adverse effects to neuroleptics (i.e. acute parkinsonian crisis). The discrimination between AD and DLB also facilitates the prediction of response to AChEIs (acetylcholinesterase inhibitors) and leads to more accurate information to the patients and caregivers. Importantly, $^{[123]}$I-FP-CIT was recently registered to differentiate between DLB and AD.

Apart from dopaminergic deficits there is also a cholinergic deficit in both AD and DLB, and both forms of dementia show beneficial effects to early AChEI therapy. Due to this, patients that are referred by clinicians...
for a FP-CIT SPECT to differentiate DLB from AD, will often be on AChEIs. However, effects of cholinergic medication on the availability of DATs in animals have been shown after acute intravenous injection. In contrast, a recent retrospective FP-CIT SPECT study in AD and DLB patients showed no influences of AChEI-treatment on the ability of FP-CIT to distinguish between AD and DLB. In these patients, AChEIs were administered orally and chronically, whereas the conflicting results from the animal studies were based on changes in DAT-availability after intravenously injected AChEIs.

In this animal study, we therefore evaluated the effects of single intravenous, single oral and sub-chronic oral administration of AChEIs on FP-CIT binding in the rat brain.

Material and Methods
To study the effects AChEIs on the binding of [123I]FP-CIT in the rat brain, male Wistar rats (200-300g, obtained from Harlan, Horst, The Netherlands) were used. In three experiments the AChEIs were administered prior to injection of the radiotracer. Pre-medication was administered intravenously or orally as a single dose, or orally during 14 consecutive days, respectively. All performed experiments are in agreement with The Dutch Experiments on Animals Act (1977) and were approved by the Animal Ethics Committee (AMC, Amsterdam, The Netherlands).

For pre-medication that was administered intravenously, active pharmaceutical ingredients (APIs) were extracted from the required mass of the commercially available drug formulations by crushing the tablets and dissolving the content in H₂O or 5% aq. EtOH as indicated below. Donepezil (API: 2,3-Dihydro-5,6-dimethoxy-2-[1-(phenylmethyl)-4-piperidinyl]methyl-1H-inden-1-one, 5.25mg/3.75mg) was dissolved in H₂O (8.4mL/1.5mL). The insoluble solids were filtered off using a 0.22µm Millipore® filter. (TLC: EtOAc/MeOH 3:1, Rf=0.58). Rivastigmine (API: Ethylmethylcarbamic acid 3-[(1S)-1-(dimethylamino)ethylphenyl ester, 5.25mg/3.75mg) was dissolved in EtOH (99%, 5mL), the insoluble solid was filtered off using a 0.22µm Millipore® filter, and the solvent was evaporated under reduced pressure. The residue was dissolved in 5% EtOH/H₂O (8.4 mL/1.5mL) (TLC EtOAc/MeOH 2:1, Rf=0.37). Methylphenidate (API: α-Phenyl-2-piperidine-acetic acid methyl ester, 21mg/15mg) was dissolved in H₂O (8.4mL/1.5mL) and the residue was filtered off using a 0.22µm Milli-pore® filter.
EtOAc/MeOH 4:1, Rf=0.32).
Immediately before the medication was administered intravenously, it was dissolved in 0.4ml acetate-buffer (pH 4.7). The experiment was performed on one single day. Injections were given via a lateral tail vein under ketamine/xylazine (2:1, i.m.) anesthesia.

Pre-medication that was given orally was administered in approximately 1.2ml distilled water as a suspension. No anesthetics were used during oral administration. Both the biodistribution experiment after a single oral dose of pre-treatment medication and the experiment after sub-chronic pre-treatment were performed on two separate days. Since the methods were identical and the results of the experiments were not statistically significantly different between study days, data were pooled and thus experiments are presented as one experiment. In the first experiment, the effects of single intravenous injection of the AChEIs rivastigmine and donepezil on the binding of FP-CIT in the rat brain were studied. Five rats received a single dose of either rivastigmine (2.5mg/kg) or donepezil (0.5mg/kg) at 10 min prior to [123I]FP-CIT injection. A positive control group (n=5) received a single dose of the potent DAT-blocker methylphenidate (10mg/kg) intravenously, at 10 min prior to injection of [123I]FP-CIT [9]. A negative control group (n=5) received 0.4ml of saline at 10 min prior to injection of [123I]FP-CIT.

In the second experiment, rats received a single oral dose of either rivastigmine (2.5mg/kg, n=12) or donepezil (2.5mg/kg, n=10), at 2h prior to [123I]FP-CIT injection. A positive control group (n=11) received a single oral dose of methylphenidate (10mg/kg), at 40 min prior to injection of [123I]FP-CIT [9]. A negative control group (n=16) received 1.2ml of saline at 2h prior to injection of [123I]FP-CIT.

In the third experiment, groups of rats were pre-treated with either rivastigmine (1mg/kg, n=11), donepezil (1.5mg/kg, n=6), methylphenidate (2.5mg/kg, n=6) or placebo (1.2ml saline, n=11), for 14 consecutive days. On the 15th day, rats were injected with [123I]FP-CIT.

[123I]FP-CIT (DaTSCAN; specific activity \( \geq 185\) MBq/nmol; radiochemical purity \( \geq 98\% \)) was a generous gift from GE Healthcare, Eindhoven, The Netherlands. All rats received approximately 3.7MBq [123I]FP-CIT intravenously via a lateral tail vein, under ketamine/xylazine (2:1, i.m.) anesthesia, in order to evaluate the effects of pre-medication on the binding
of the tracer in the rat brain. All rats were sacrificed at 2h after injection of the tracer by bleeding via either heart or portal vein puncture under anesthesia. Brains were rapidly dissected and striatum (representing predominantly specific binding to DATs), hypothalamus (representing predominantly binding to serotonin transporters) and cerebellum (representing non-specific binding) were separately weighted and counted for each rat in a gamma counter (Minaxi \( \gamma \) A5530, Canberra Packard, Pangbourne, Berks, United Kingdom). Data were corrected for radioactive decay, brain structure weight and body weight and were eventually expressed as the percentage of the injected dose, multiplied by the body weight in grams, per gram tissue (%ID*g/g). Specific striatum-to-cerebellum and hypothalamus-to-cerebellum ratios were then calculated ((striatum-cerebellum)/cerebellum and (hypothalamus-cerebellum)/cerebellum, respectively).

**Statistical analysis**

Differences between specific striatum-to-cerebellum and hypothalamus-to-cerebellum ratios in all tested groups after both single and sub-chronic pre-medication were analyzed with the non-parametric Kruskal Wallis test using SPSS 12.01. Mann-Whitney U tests were performed to analyze differences between control groups and their specific subgroups. Probability values <0.05 were considered significant.

**Results**

In the first experiment, \([^{123}\text{I}]\text{FP-CIT}\) injection at 10 min after single intravenous administration of rivastigmine resulted in a specific striatum-to-cerebellum ratio of 2.78±0.71 (SD; Figure 6.1a.). At 10 min after injection of donepezil, a specific striatum-to-cerebellum ratio of 2.53±0.32 was calculated (Figure 6.1a.). No statistically significant differences were found as compared to the control group (2.63±0.36; Figure 6.1a).

In contrast, the group pretreated with methylphenidate did show a statistically significant decrease (1.33±0.43, P=0.009; Figure 6.1a.). The specific hypothalamus-to-cerebellum ratios for the rivastigmine, donepezil and methylphenidate group were 0.81±0.13, 0.69±0.13 and 0.76±0.30, respectively. No statistically significant differences were found as compared to the control group, which showed a specific hypothalamus-to-cerebellum ratio of 0.68±0.07 (Figure 6.1b.).
In the second experiment, [\(^{123}\)I]FP-CIT injection at 2h after single oral administration of rivastigmine resulted in specific striatum-to-cerebellum ratios of 2.67±0.71 (Figure 6.1c). 2h after administration of donepezil, the specific striatum-to-cerebellum ratio was 2.35±0.47 (Figure 4c). No statistically significant differences were found as compared to the control group (2.68±0.40; Figure 6.1c). However, [\(^{123}\)I]FP-CIT injection at 40 min after single oral administration of methylphenidate did show a statistically significant decrease (2.16±0.33, P=0.001; Figure 6.1c).

After single oral administration of pre-medication, the specific hypothalamus-to-cerebellum ratios for the rivastigmine, donepezil and methylphenidate group were 0.63±0.20, 0.67±0.23 and 0.74±0.12, respectively. No statistically significant differences were shown for specific hypothalamus-to-cerebellum ratios as compared to the control group, which showed a ratio of 0.63±0.34 (Figure 6.1d).

In the third experiment, injection of [\(^{123}\)I]FP-CIT on the day after sub-chronic oral administration during 14 consecutive days of either rivastigmine, donepezil or methylphenidate resulted in specific striatum-to-cerebellum ratios of 2.88±0.88, 2.44±0.45 and 2.54±0.42, respectively (Figure 6.1e). No statistically significant differences were found as compared to the control group, which showed specific ratios of 2.76±0.52 (Figure 6.1f).

Also no statistically significant differences were found in the specific hypothalamus-to-cerebellum ratios between the rivastigmine, donepezil, methylphenidate and the control group (0.70±0.36, 0.65±0.18, 0.74±0.26 and 0.62±0.24 respectively; Figure 6.1f).

**Discussion**

[\(^{123}\)I]FP-CIT SPECT is a valuable diagnostic tool to discriminate DLB from AD. To date, it’s uncertain whether the oral use of AChEIs by demented patients has an effect on the binding of FP-CIT to DATs. In this study we evaluated the influence of rivastigmine and donepezil, two commonly prescribed AChEIs for demented patients, on the binding of FP-CIT in the rat striatum and hypothalamus. These AChEIs were administered intravenously as a single dose, orally as a single dose and sub-chronically by oral administration.
Intravenous injection of rivastigmine or donepezil at 10 min prior to injection of $[^{123}\text{I}]$FP-CIT did not result in a decrease of the tracer in the rat striatum as compared to control rats. As a positive control, pre-injection of methylphenidate, did show the expected decrease of $[^{123}\text{I}]$FP-CIT binding in the rat striatum. Oral pre-administration of either rivastigmine or donepezil, at 2h prior to injection of the tracer, also did not result in a decrease of tracer binding in the striatum whereas pre-administration of methylphenidate did result in a decrease of tracer binding. Finally, neither rivastigmine nor donepezil or methylphenidate showed a decrease of tracer binding in the striatum after sub-chronic oral administration of the substances.

FP-CIT has a high affinity for the DAT but also a modest affinity for the serotonin transporter $^{15}$ (please note that in this particular paper FP-CIT is named RTI-313). The rat striatum was chosen as an area of specific uptake of $[^{123}\text{I}]$FP-CIT due to its high density of DATs. In contrast, the hypothalamus is known to express a high density of serotonin transporters but not DATs. Binding of $[^{123}\text{I}]$FP-CIT to this brain structure therefore represents predominantly specific binding to serotonin transporters. As expected, none of the tested substances (rivastigmine, donepezil and methylphenidate) resulted in alterations of FP-CIT binding in the hypothalamus in any of the administration protocols.

In line with this study, a recent cross-sectional retrospective study in 99 patients performed by Taylor et al. $^{6}$ also did not show significant influences of chronic oral administration of AChEIs on the ability of FP-CIT to distinguish DLB from AD. However, earlier studies in animals $^{4, 5}$ showed inhibitory effects on DAT-availability after injection with AChEIs. After intravenous pre-treatment of rats with the AChEI phenserine, Kilbourn reported a 24% decrease in $d$-threo-$[^3\text{H}]$methylphenidate binding in a preliminary, yet unpublished study $^{4}$. Moreover, Tsukada $^{5}$ showed that intravenous administration of the AChEI donepezil has a dose-dependent inhibitory effect on DAT-availability in the conscious monkey brain, as measured by $[^{11}\text{C}]\beta$-CFT PET.

We were not able to reproduce such a decrease in DAT-availability in our study, using FP-CIT after intravenous pre-injection of the AChEIs rivastigmine or donepezil. This may be due to differences between the administered type of AChEI, drug dose, pharmacological potency, pharmacodynamics, or anesthesia-related effects. Another explanation may be the difference in radiotracers.

In our study, we did not measure plasma or brain levels of the injected AChEIs or cholinesterase activity in the rat brain. Intravenous doses
were based on earlier reports\(^8,16\) and were well above the human doses (which are 1.5mg twice daily for rivastigmine and 5-10mg once daily for donepezil). Many studies showed that both donepezil and rivastigmine are centrally selective AChEIs, and donepezil is known to have even higher brain-to-plasma ratios in rats than rivastigmine\(^7\). Therefore, plasma levels of these centrally active AChEIs are not very indicative for the concentrations of the AChEIs in the rat brain. Determination of brain concentrations of the AChEIs was not considered useful due to the lack of reference data.

In our study, at 10 minutes after injection of 0.5mg/kg donepezil, fasciculations were clearly seen instantly in all animals (n=5), which is due to peripheral pharmacological actions of the drug\(^8\). Also, rectal temperature showed a larger decline as compared to control rats, suggesting a central pharmacological effect (maximum of -1.6°C in the first 15 min after injection; data not shown)\(^17\). Yawning, another central pharmacological effect\(^8\) was not seen in any of the rats, probably due to the use of anesthetics. After injection, fasciculations were not seen in the rivastigmine group (2.5mg/kg) and rectal temperature declined with a maximum of only -0.6°C in this study group, which can also be attributed to immobilization due to the anesthesia. However, Kosasa et al.\(^7\) showed that even after an oral dose of 2.5mg/kg rivastigmine, there is a significant reduction of cholinesterase inhibition (\(\approx 40\%\)) in the rat brain. Intravenous injection of this dose should therefore at least result in an inhibition of this magnitude.

Tsukada and co-workers used dosages up to 0.1mg/kg donepezil in the abovementioned \([^{11}\text{C}]\beta\text{-CFT PET study in conscious monkeys}\(^5\). In our study, we injected 0.5mg/kg donepezil in rats, however no effects on FP-CIT binding to DATs were detected. An explanation for this discrepancy may be the use of anesthetics in the present study. We used ketamine/xylazine 2:1 as the anesthetic agent. Although there may be effects of ketamine on the dopaminergic system, we consider such possible effects as systematic, since the same anesthesia was used for all study groups. As expected, injection of the potent DAT antagonist methylphenidate at 10 min prior to injection of \([^{123}\text{I}]\text{FP-CIT did show a statistically significant decrease of tracer binding in the DAT-rich rat striatum but not in the hypothalamus, which lacks the DAT. This indicates the feasibility of the model to detect changes in DAT-availability. Although not supported by the results from the present study, Kilbourn and co-workers hypothesized that acute intravenous administration of cholinergic medication may induce rapid trafficking of DATs across the brain.}
membrane of dopaminergic cells. Tsukada et al. suggested that modulation of receptor subtypes on the presynaptic dopaminergic nerve terminals or indirect modulation through transsynaptic effects may decrease DAT-availability after acute injection of AChEIs. Taylor et al. postulated that one explanation for the discrepancy between their retrospective study in patients and the monkey study performed by Tsukada, may be the difference in the radiotracers. In contrast to \([^{11}C]\beta-CFT\) PET, as mentioned by Taylor et al., \([^{123}I]\)FP-CIT binds not only to the DAT, but also to a lesser extent to the serotonin transporter. One can hypothesize that an inhibitory effect on DAT availability is counterbalanced by an increase in serotonin transporter availability, which would compensate the decrease of \([^{123}I]\)FP-CIT binding in the striatum. However in the present study, we did not find any statistically significant changes in serotonin transporter density as measured by specific binding of \([^{123}I]\)FP-CIT in the hypothalamus, and thus no indications for compensatory mechanisms in the serotonergic neurotransmitter system.

Furthermore, Taylor et al. also postulated that an explanation for the discrepancy between the results of their patient study and Tsukada’s monkey study may be the difference in species. Indeed, it cannot be ruled out that the discrepancy between the present study and the monkey study are also based on species-specific differences.

In clinical practice however, AChEIs are administered orally and chronically. Orally treated animals may therefore provide a more suitable model to simulate the human setting. Therefore, we also tested the availability of DATs in rat brain with \([^{123}I]\)FP-CIT after single oral administration of rivastigmine or donepezil. Additionally, we investigated the effects of sub-chronic oral administration of rivastigmine, which may be the most favorable model for the human situation. The timeframe between single oral administration of the AChEIs and the \([^{123}I]\)FP-CIT injection was based on previous studies. Matsui and coworkers showed that up to 4-8h after oral administration of 1mg/kg donepezil, there is a high concentration of the unmetabolized compound in the rat brain. Kosasa reported a significant inhibition of ChE-activity already at 1h after oral administration of 2.5mg/kg donepezil. Enz and Gentsch showed that, at 2h after oral administration of only 1.5 mg/kg rivastigmine, there is approximately 20% inhibition of AChE-activity in the brain of male Wistar rats. For the present study, we therefore considered an oral dose of 2.5mg/kg for both the
donepezil and the rivastigmine group, at 2h before the $[^{123}\text{I}]\text{FP-CIT}$ injection as adequate.

The dose of orally administered methylphenidate was based on previous work by Gerasimov et al.\textsuperscript{9}. At 40 min after oral administration of 10mg/kg methylphenidate in rats there is a peak in extracellular dopamine concentration in the brain, which indicates maximal occupancy of DATs by methylphenidate at this time point. Indeed, our present data show a significant occupancy of the DAT by methylphenidate when administered at such a dose. In line with the results of the present study using intravenous pre-administration of AChEIs, no significant effects could be detected on the striatal $[^{123}\text{I}]\text{FP-CIT}$ binding, and thus DAT availability, in the rat brain after single oral administration of rivastigmine or donepezil. Oral pre-administration of AChEIs is probably even less likely to induce rapid trafficking of DATs across the membrane\textsuperscript{4} or modulation of DATs\textsuperscript{5} because both proposed mechanisms may even be less pronounced during a gradual increase of AChEI concentration in the brain after oral administration as compared to bolus injection.

Effects of anesthesia are also less likely as compared to the present study in which AChEIs were administered intravenously. In the oral pre-treatment study, anesthetics were used only from the moment the radiotracer was injected. Moreover, also orally-pretreated rats that were given methylphenidate (10mg/kg) did show the expected decline of FP-CIT binding, which again indicates the feasibility of the model to detect changes in DAT availability.

Sub-chronic administration of either rivastigmine, donepezil or methylphenidate on 14 consecutive days before, but not on the day of the biodistribution experiment (to exclude acute pharmacological effects), did not show any influences on the $[^{123}\text{I}]\text{FP-CIT}$ binding in the rat striatum or hypothalamus. The doses of the medication were based on previous reports\textsuperscript{12, 13}. In the present study, also sub-chronic oral administration of methylphenidate did not result in a significant alteration of FP-CIT binding in the rat brain. Based on the findings of previous studies, one may expect a lower DAT expression even after 1 week of methylphenidate treatment\textsuperscript{19}. However, in their study, methylphenidate was administered continuously for one week, and DAT density was measured one day after the end of the experiment, which is at odds with ours. Moreover, a recent study\textsuperscript{20} showed that methylphenidate was unable to down-regulate the DAT.

Although the results of this rat study which showed no significant effects of sub-chronic oral administration of FP-CIT binding to DATs can not
be simply extrapolated to the human setting, the results of this substudy are in line with the previous report by Taylor et al. It is therefore unlikely that AChEIs will induce large effects on the interpretation of \([^{123}\text{I}]\text{FP-CIT SPECT} \) scans in humans. However to prove this, randomized, placebo controlled studies in humans by means of \([^{123}\text{I}]\text{FP-CIT brain-SPECT} \) after administration of AChEIs are necessary.

**Conclusion**

In this study, no significant differences in \([^{123}\text{I}]\text{FP-CIT binding} \) could be shown in the rat striatum after a single intravenous or single oral dose of either rivastigmine or donepezil. Also no effects were seen after sub-chronic oral administration of these AChEIs.

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**References**


6. Taylor JP, Colloby SJ, McKeith IG, Burn DJ, Williams D, Patterson J, et al. Cholinesterase inhibitor use does not significantly influence the ability of $^{123}$I-FP-CIT imaging to distinguish AD from DLB. J Neurol Neurosurg Psychiatry. 2007, Epub ahead of publication.


