# Innovation in cholinergic enhancement for Alzheimer's Disease

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CHAPTER 8

# Summary and discussion

# SUMMARY AND DISCUSSION

This thesis describes studies of the effects on cognition of drugs that stimulate or inhibit the cholinergic system by direct or indirect mechanisms. All study designs included extensive pharmacodynamic testing in various phases of drug development. Consequently, the study designs and study populations differed, depending on the aim of the study. In general we performed extensive studies of pharmacodynamics effects in subjects with normal or impaired cognition, or in healthy volunteers with a previously pharmacologically impaired cognitive system (challenge studies).

**Chapter 2** describes a study of the effects of several doses of the α7 nAChR partial agonist EVP-6124, alone and in combination with two doses of the cholinesterase inhibitor donepezil in healthy elderly subjects, receiving a scopolamine challenge prior to administration of EVP-6124 and/or donepezil. As preclinical studies showed a complete reversal of the scopolamine effect, the expectations were high. However, efficacy of EVP-6124, alone or in combination with donepezil, could not be shown. Despite these negative results, the compound was tested in patients with AD and provided some indications for therapeutic efficacy.[1] Subsequent clinical trials were less positive, and the compound was withdrawn from further development. The lack of effect reversal of scopolamine effects by EVP-6124 in Chapter 2 is therefore in line with the equivocal clinical effects of the compound. However, there are other possible explanations for the negative results of the scopolamine study. First, the results may have been obscured by the strong sedative effect of scopolamine in the healthy elderly subjects. Another explanation is that scopolamine is a muscarinic antagonist, which may not be antagonized by an α7 nAChR partial agonist. This highlights some problems of pharmacological challenge studies. The validation of such an intervention may require more work to get an optimal representation of the precise pharmacological effect or the real clinical condition that is mimicked.

The limitations of scopolamine as an anticholinergic challenge model were addressed in **Chapter 3.** This describes an extensive exploration of another anticholinergic challenge model with the nAChR specific antagonist mecamylamine. Although this challenge model has been used before, its PD and PK characteristics were largely unknown and a detailed comparison with scopolamine was never done. The pharmacokinetics of mecamylamine are described in **Chapter 4**. In contrast to scopolamine, mecamylamine did not have any sedative effects, but it did cause measurable cognitive decline. From a pharmacological point of view, it seems more logical to use this nicotinic challenge model for proof of concept studies with nAChR agonists. Clearly more work needs to be done to fully validate this model a challenge for drug development, but this study demonstrates the need for a systematic approach for such challenge models.

In **Chapter 5,** Gln-1062, a prodrug of the cholinesterase inhibitor galantamine was tested in an adaptive design. The reason of this was that although the active moiety was well known and safe, the prodrug was designed to have an improved brain penetration and hence increased cholinesterase inhibition in the brain. Consequently, equimolar dosage could not *a priori* be considered safe. The trial started with the standard starting dose of 10% of the level of no adverse effects (NOAEL) in animal studies. Since no PD effects were expected at the two lowest doses to be administered, these were given to healthy young male volunteers and PK, PD and safety were measured. Unexpectedly, even these low doses induced some measurable effects on attention and memory. The study continued in three cohorts of healthy elderly male volunteers, as they are expected to have a slight, age-related cholinergic deficiency, which was expected to increase the possibility of finding any pharmacodynamic effects, without the necessity of administration of an anticholinergic challenge. MMSE score was included in the exclusion criteria to prevent inclusion of demented patients in the study. The increasing doses of Gln-1062 in these cohorts were compared to donepezil and galantamine. Galantamine did not induce any measurable effects, while donepezil improved the performance on the adaptive tracking, comparable with the effect of the 33 mg and 44 mg dose of Gln-1062. While improvements were demonstrated on the adaptive tracking test and the VVLT, ceiling effects may well have limited the extent to which positive effects could be demonstrated in healthy younger subjects. Therefore in this study we introduced a novel approach to the evaluation of compounds for AD, namely to perform the study in 'physiologically impaired subjects', elderly without overt cognitive symptoms but with likely some incipient cholinergic neuronal dysfunction in contrast to the pharmacologically challenged subject.

In Chapter 5 we showed that the effects of galantamine can be measured acutely in healthy young and elderly subjects, even after a single dose. In **Chapter 6** we proceeded to a study in AD patients. Here we asked the question if the acute effects of a single dose of an anticholinergic medicine, galantamine, could predict a response to chronic treatment at 6 months. In this study, there was no significant effect on the adaptive tracking (primary endpoint), but the effect on EEG parameters after a single dose predicted the treatment response to galantamine at 6 months. Such an approach could lead to a more personalized approach to treatment. In case of CEI treatment, this could spare +/- 70% of the current medication prescriptions. Rather than directly affecting the cholinergic system, other approaches that affect cholinergic and cognitive systems indirectly are studied

and we applied our system of development to a histamine 3 receptor ($H_3R$) inverse agonist (CEP-26401) in **Chapter 7**. As histamine has an indirect effect on several neurotransmitter systems, including the cholinergic system, this was considered a target for procognitive medication. Based on previous studies with this compound, low doses were administered and its effects were compared to placebo, donepezil and modafinil. The population of healthy volunteers was chosen as initial target, because a comparison with positive controls was incorporated in the design, which included two very extensive test battery with sensitive tests (CANTAB and NeuroCart). In this study, the primary (cognitive) endpoint was not met, but there was a positive effect on subjective feelings, which was strongest at the 25 µg dose. There was no improvement on cognitive testing and even some worsening on the spatial working memory test (SWM 10 boxes; primary endpoint of the study) and paired associate learning test after administration of the highest dose of CEP-26401. As donepezil did not induce any improvement either, ceiling effects might still have influenced the outcome of this study, despite the chosen test battery. This remains a problem of the study of such drugs on a healthy population with optimal cognitive functioning that is difficult to improve. In our approach, but outside the scope of this thesis, the compound also requires testing in physiologically impaired or pharmacologically challenged subjects before any definite conclusions can be drawn about its clinical value. The positive effects of CEP-26401 on subjective feelings may also indicate that its mechanism of action (histamine 3 receptor ($H_3R$) inverse agonism) renders this class of compounds more suitable for the treatment of mood disorders than for the treatment of cognitive disorders.

Even though the primary endpoints were not met in some of the studies, the profile of cholinergic intervention on the used biomarkers seems to be quite consistent. Based on the function and localisation of acetylcholine receptors in the brain, cholinergic drugs are expected to influence mainly memory and attention. In all studies in this thesis, both with procholinergic and anticholinergic compounds, effects were mostly observed on the adaptive tracker (attention), N-back (working memory), visual verbal learning test (working memory and recall) and EEG parameters (table 1).

With regard to the effects on memory, it is remarkable that the direct recall is more often influenced than the delayed recall and recognition. This is inherent to the function of acetylcholine in learning. Memorising is a complicated process, consisting of an encoding phase, when information is received and comprehended, a consolidation phase to 'store' memories for a longer time and a retrieval phase to reproduce the previously learned information. Both preclinical and clinical research suggest that the encoding phase requires high acetylcholine levels in the brain, while for the consolidation phase lower levels of acetylcholine are sufficient.

**TABLE 1** **Effects of pro- and anti-cholinergic compounds on N-back, VVLT, EEG and adaptive tracking**

| | | N-back | VVLT | EEG | Tracker |
|---|---|---|---|---|---|
| PRO-CHLINERGIC COMPOUNDS | donepezil | improvement RT 0-back (5 mg); deterioration RT 2-back (5 mg) | | | improvement (2,5 mg and 10 mg) |
| | galantamine | | | decrease in relative frontal theta power; decrease in absolute alpha, beta and theta power (16 mg) | trend to improvement (16 mg) |
| | EVP-6124 | dose dependent improvement ACC 0-back (all doses); improvement ACC 1-back (2 mg) | | increase in absolute alpha power (16 mg) | |
| | GLN-1062 | | improvement IR (all doses compared to GAL 22 mg compared to placebo); improvement DR (5,5 and 11 mg compared to placebo) | | improvement (11, 33 and 44 mg) |
| | CEP-26401 | deterioration ACC 2-back (125 mcg)[1] | | | dose dependent improvement for all doses |
| ANTI-CHOLINERGIC COMPOUNDS | scopolamine | deterioration RT and ACC of all paradigms (0,5 mg) | deterioration on all parameters (0,5 mg) | decrease in absolute alpha and beta power (0,5 mg) | severe deterioration (0,5 mg) |
| | mecamylamine | | deterioration on IR and DR (20 mg) | | deterioration, but less severe than for scopolamine (20 mg) |

*VVLT: visual verbal learning test; EEG: electroencephalography; RT: reaction time; ACC: accuracy; IR: immediate recall; GAL: galantamine; DR: delayed recall.*

*1. Based on previous studies, the lower doses were expected to be more effective*

This is illustrated by a study by Miranda et al., where acetylcholine was measured in the rat brain and only in the encoding phase, a peak was registered.[2] In an fMRI study described by Kukolja et al., the cholinesterase inhibitor physostigmine was administrated during a memory task and was demonstrated to enhance activity related to neuronal encoding in the hippocampus, while it could not be shown to influence activity associated with memory consolidation or retrieval.[3] Gais et al. taught healthy subjects a paired associated learning task before the night and administered physostigmine during deep sleep.[4] They found that memory

retrieval was significantly worse after administration of physostigmine compared to placebo, and concluded that low cholinergic levels during deep sleep is essential for memory consolidation. Our results demonstrate that a relevant set of biomarkers used in a systematic approach, can be successfully applied during the development of new cholinergic interventions.

The EEG results of the study in patients with AD are especially interesting. At first glance, the increase in relative frontal theta frequency in responders to long-term galantamine treatment on the placebo occasion and the decrease on galantamine occasion seems to be counterintuitive, as theta activity is usually associated with worse cognitive functioning. However, the study of Wascher et al. reported an increase in frontal alpha and theta power over time in young healthy subjects, during a day filled with cognitive tasks.[5] They reasoned that this is a reflection of mental fatigue. Possibly, the cognitive tasks during the pharmacological challenge days result in less electroencephalographic signs of cognitive fatigue after administration of a single dose of galantamine in patients with AD, who are responders to long-term treatment with galantamine. One could even argue that the fact that there is no change in EEG activity during the pharmacological challenge study days in non-responders to long-term galantamine treatment is actually abnormal. This approach requires more attention, because the response rate of all cholinesterase inhibitors for AD is little higher than 30% and if a more personalized approach could be used considerable cost (and unnecessary side effects) could be saved.

The central question of this thesis is if the integration of pharmacokinetics (PK), CNS pharmacodynamics (PD) and clinical assessments in early phase drug development is feasible for drugs for Alzheimer's disease. At this moment, the only registered procholinergic drugs for Alzheimer's disease or Lewy Body dementia are cholinesterase inhibitors (galantamine, rivastigmine and donepezil). During the development trajectories of these drugs, the classical process of drug development was followed:

- Disease with a known or assumed pathophysiology, in this case AD.
- Possibility for pharmacological interference in the (assumed) cascade of pathophysiology, either in a curative or in a symptomatic manner. In this case the depletion of acetylcholine was targeted by acetylcholinesterase inhibitors and largely tested in animal models.
- This preclinical research leads to selection of a compound that is considered to be safe and effective.
- The compound is tested in healthy volunteers to evaluate safety, tolerability and PK.



In classic drug development, the possibility of physiologically or pharmacologically challenged subjects is skipped. Studies in large groups of patients are initiated, with largely questionnaire based outcomes. Such simple outcome measurements are inevitable due to the multinational, multicentre approach that is necessary in these classical Phase IIa and Phase III studies.

In the studies in this thesis, a different approach was chosen. In all studies, safety, tolerability, pharmacokinetic and pharmacodynamic measurements were included, regardless of the phase of drug development. This gave at least an impression of pharmacodynamics effects in an early stage of drug development. Our approach with quantitative and objective measurement of a central impaired function, memory and cognitive functioning in normal, physiologically impaired and pharmacologically challenged individuals before proceeding to large, risky and expensive trials may assist in a more economically and faster development.[6] This approach is clearly easier for interventions that have an immediate effect like cholinesterase inhibitors, but we also demonstrated that these short time effects predict long term clinical improvement to a certain extent. Additionally, a thorough systematic approach to drug development may demonstrate potential other beneficial effects that otherwise would not be detected as we demonstrated with the effects on mood of the $H_3$-antagonist in chapter 7 of this thesis.

Disease modifying treatments for Alzheimer's disease – if ever found – may require a different approach, but well validated quantitative measurements of clinical importance are still in short supply. Also, a wide variety of tests is used in clinical trials, which complicates mutual comparison.[7] Intermediate biomarkers of pathology like amyloid imaging or other functional imaging studies have value but do not always correlate with (lack of) clinical improvement. Clinical outcome measures are partly based on questionnaire evaluations by caregivers with a high interrater variability and often designed to diagnose dementia instead of measuring a relevant (reduction of) progression of dementia over time.[8] New biomarkers may be found in more specific, validated questionnaires, for example the Amsterdam iADL scale, or continuous wearable measurements.[9–12] The use of more specific and sensitive biomarkers in a carefully selected population will lead to a more efficient drug development process and probably faster availability of either disease modifying or more effective symptomatic treatment. This could eventually lead to an enormous reduction of drug development costs, and, even more important, health care costs.[6]

# CONCLUSION

Clearly the work presented in this thesis does not produce the final answer to all problems associated with the development of treatments for cognitive decline, which is until now largely unresolved, despite an enormous burden on healthcare. Cognitive decline is a complex process with many potential pathophysiological mechanism that allow many approaches, and we have only studied the cholinergic system. However for all interventions it would be ideal if there were good biomarkers of the severity of the disease that were shown to respond to interventions. Finding useful and disease modifying treatments for cognitive decline does not appear to be in close reach, but assuming that this will eventually occur, it is obvious that more efficient development paradigms are necessary to keep the pharmacological development trajectories economically feasible. Rapid evaluation of the most promising treatments in the right dose requires preclinical and early development, already directed towards the final clinical value based endpoint. Rapid elimination of interventions that do not work will of course help to focus limited resources on the more hopeful ones.

Thus, the road between 'working' (on a particular mechanism) and 'helping' (the patient) needs to be paved by improved selection and composition of subject populations to be maximally informative. Necessarily this may involve challenges to induce cognitive dysfunction. The cement between the paving stones is a set of biomarkers that are clinically practical, physiologically relevant and –not to forget– well-validated in a systematic manner. This road is by no means finished but this thesis has hopefully produced some building material.



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