ALZHEIMER'S DISEASE

With special emphasis on *in vitro* and *in vivo* model systems and the effect of donepezil applied for treatment of the disease

Summary of Ph.D. Thesis

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Introduction

Alzheimer's disease (AD) is the most common form of senile dementia, affecting more than 20 million people worldwide. In Hungary the number of AD patients may be more than one thousand. Statistical data demonstrate that AD is the fourth leading cause of death after heart disease, cancer and stroke. It is the most common cause of dementia in the elderly, characteristically involving a progressive loss of cognition and changes in personality

So many hypotheses have been put forward to explain the aetiology of AD, that the situation is far from clear. Our attention turned first towards the *amyloid cascade hypothesis*, which postulates that it is the Aβ in the SPs which exerts a neurotoxic effect on the different types of neurons, and results in their degeneration.

A large number of neurochemical data draw attention to lesions of the cholinergic system. The amyloid hypothesis has also attempted to shed light on the causal relations of these changes. It is well documented that a correlation can be demonstrated between the increase in the amount of Aβ and the alterations in the elements of the cholinergic system. In consequence of the pathological accumulation of Aβ, a cholinergic hypofunction will develop in the CNS. Since the cholinergic system has been proved to be involved in the memory function, a *cholinergic hypothesis* was formulated with respect to the neuropathology of AD. The amyloid and the cholinergic hypotheses led to a need for further research to elucidate the role of Aβ in the pathogenesis of AD.

AChE inhibitors (donepezil, rivastigmin and galanthamine) have recently been introduced for the treatment of AD individuals in order to increase the efficacy of the remaining ACh and to ameliorate the memory problems of the patients. Although there have been a large number of neurochemical and clinical investigations on the effects of these drugs, there is no morphological evidence as to how the AChE inhibitors used for the treatment of AD affect the various areas of the CNS and within this the different cholinergic and cholinoceptive cells. This thesis summarizes our efforts to shed light on the aetiology and neuropathology of this terrible disease.

Objectives

The present study was designed to investigate the neuropathological alterations caused by Aβ1-42 in *in vitro* neuronal cultures and to study the effect in the CNS of donepezil applied for the treatment of AD.

The specific aims were:

1. To identify different transmitter-containing neurons in *in vitro* neuronal cultures.
2. To reveal the effects of human Aβ1-42 on different transmitter-containing neurons in tissue cultures (cholinoceptive, cholinergic and GABAergic).
3. To study the *in vivo* neurotoxic effects of human Aβ1-42 on the axonal transport of various proteins.
4. To demonstrate the intraaxonal transport of APP and PS-1.
5. To investigate: a) the regional selectivity of donepezil on AChE activity in the non-demented human brain; b) the AChE-inhibitory effects of donepezil in the non-demented human brain in comparison with those observed in the rat brain; c) the AChE-inhibitory effect on the NMJ.

Materials and methods

Materials: Autopsy samples from human brain
Central nervous system of rat
In vitro tissue cultures from embryonic rat brain
Sciatic nerve and spinal ganglia of rat

Methods: Acetylcholinesterase (AChE) histochemistry
Amyloid-β peptide (Aβ) immunohistochemistry
Amyloid precursor protein (APP) immunohistochemistry
Presenilin-1 (PS-1) immunohistochemistry
Vesicular acetylcholine transporter (VAChT) immunohistochemistry
Synaptophysin (SYN) immunohistochemistry
GABA immunohistochemistry
Western immunoblot
Image analysis

Results and discussion

In vitro experiments:

Our study for the first time identified the cholinergic neurons in *in vitro* tissue cultures and revealed that Aβ1-42 and its fragments (Aβ31-35 and Aβ34-39) are toxic not only to cholinergic neurons, but also to cholinoceptive and GABAergic neurons in basal forebrain cultures. The toxic effect predominated mainly on small, bipolar neurons, while the large multipolar neurons displayed more resistance to Aβ. We could demonstrate that the number of SYN-positive axon varicosities is reduced better than that of the VAChT-positive axon varicosities. Our results are in contradiction with the data of Pike and Cotman, who found that GABAergic neurons are resistant to Aβ treatment. We hypothesize that the apparent controversy may arise from differences in the solvent and in the concentrations of the peptides applied in the different experiments. Another difference lies in the age and origin of the cultures used by our group and by Pike and Cotman.

Our *in vitro* tissue culture data are therefore in close relationship with data found on AD patients: besides the cholinergic neurons, other neurotransmitter systems may be involved in the aetiopathology of the disease.

In vivo experiments:

APP and PS-1 are not only of importance for the normal functioning of the various neurons, but also play central roles in the pathogenesis of AD. First, we studied the axonal transport of PS-1 and APP in a spinal cord-sciatic nerve–NMJ model system in rat. In the double-ligated samples, the proteins were accumulated above the upper ligature and below the lower ligature after 6, 12 and 24 h. The results of immunohistochemistry were verified by semiquantitative Western blot studies. Our experiments clearly demonstrate that these proteins are transported in both the anterograde and the retrograde direction in the sciatic nerve of rat. Thereinafter, we provide experimental evidence that such an effect of Aβ *in vivo* may be due to the inhibition of axonal transport in the neurons.

Our results demonstrated that exogenously applied human Aβ1-42 could disturb the axonal transport of AChE, APP, VAChT and SYN in the sciatic nerve of rat The accumulation of immunoreactivity was restricted to the area where Aβ1-42 was present.

Effect of donepezil on acetylcholinesterase activity in central and peripheral nervous tissues:

Although the use of AChE inhibitors is the most highly developed approach for the treatment of AD, the precise morphological sites of their action have as yet not been demonstrated. Nor is it known how AChE inhibitors with known central effects influence the enzyme activities in the PNS.

The AChE-positive structures in the different areas of the human brain proved to be sensitive to donepezil in a dose-dependent manner. The most important finding in this experiment was that donepezil inhibited the AChE activity in the postsynaptic cholinoceptive neurons more effectively than that in the presynaptic cholinergic axons.

Similarly as in the human brain, various concentrations of donepezil ($5x10^{-9}$ M, $2x10^{-8}$ M, $5x10^{-8}$ M, $5x10^{-7}$ M and $1x10^{-6}$ M) dose-dependently inhibited the AChE staining in the various areas of the rat brain.

For quantitative analysis of the inhibitory effect of donepezil, we measured the changes in AChE histochemical staining in the AD brain and rat brain. The results revealed a dose-dependent inhibitory effect of donepezil in the various areas of the cortex, the hippocampus, the caudate-putamen and the NBM of the human brain

We studied the inhibitory effect of donepezil on AChE activity at the neuromuscular junction (NMJ). The results showed that donepezil has not only an AChE-inhibitory effect in the various areas and in the various neuronal structures in the CNS, but also an undesired effect at the NMJ. These histochemical results provide the first morphological evidence that, under *in vitro* circumstances, donepezil is not a general AChE inhibitor in the CNS, but rather selectively affects the different brain areas and, within these, the cholinergic and cholinoceptive structures.

Summary and conclusion

Experiments in *in vitro* basal forebrain neuronal cultures:

1. We have provided the first identification of the cholinergic and cholinoceptive neurons and their synaptic contacts in an *in vitro* basal forebrain neuronal culture system.
2. We have demonstrated that Aβ1–42 and its fragments Aβ25-35 and Aβ31-35 are toxic not only to cholinergic, but also to the cholinoceptive and GABAergic neurons.
3. Characteristic morphological changes have been revealed in the different transmitter-containing neurons after Aβ treatment:

4. The VAChT-positive axon varicosities proved more sensitive than the SYN-immuno-reactive neuronal structures to Aβ treatment.

Conclusion: The use of embryonic basal forebrain neuronal tissue cultures and their treatment with the neurotoxic Aβ provides a good *in vitro* cellular model with which to investigate the effects of various chemical agents on the cholinergic neurons, and also to study the pathomechanism relating to AD.

***In vivo* experiments on the axonal transport in the sciatic nerve of rat:**

1. PS-1 is present in the axons and can be transported in both the anterograde and the retrograde direction in the motoric and sensory axons of the sciatic nerve of rat.
2. We have discovered that not only the C-terminal and N-terminal fragments of PS-1, but also the holoprotein may be transported bidirectionally in the axons.
3. By means of Western blot studies, we have presented evidence that APP is conveyed bidirectionally and may carry information both from the cell body to the nerve terminal and from the axonal terminal to the neuronal perikarya.
4. It has been discovered that Aβ can disturb the fast axonal transport of AChE, APP, VAChT and SYN.

Conclusion: The in vivo neurotoxic effect of Aβ1–42 on the axonal transport of various proteins has been demonstrated.

***In vitro* effect of donepezil in autopsy human and rat brain:**

1. It has been demonstrated in *in vitro* experiments that donepezil (used for the treatment of AD patients) exerts a dose-dependent inhibitory effect on AChE activity both in the autopsy human brain and in rat brain samples.
2. Donepezil selectively affects the different brain areas and, within these, the cholinoceptive and cholinergic structures.
3. The most sensitive areas are the cortex and the hippocampal formation.
4. Within the different layers of the cortex, the cholinoceptive AChE-positive postsynaptic pyramidal cell bodies proved more sensitive than the presynaptic cholinergic axonal processes.
5. The most resistant cholinergic fibres are present in the putamen, the basolateral nucleus of the amygdala, the olfactory tubercle and the lateral habenular nucleus.

6. In the rat brain, the postsynaptic cholinoceptive and presynaptic cholinergic structures are inhibited by nearly the same dose of donepezil as in the human brain.
7. Donepezil can not only affect the AChE activity in the CNS, but also exert an inhibitory effect in the PNS tissue. It effectively inhibits the AChE activity at the NMJ and in the intra- and extracerebral blood vessel innervation.

Conclusion: Donepezil used for the treatment of AD individuals may inhibit the AChE activity not only in the brain, but also in the PNS tissue.

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Cumulative impact factor of related publications: 11,288

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