An update on drug treatment options of Alzheimer's disease

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1. ABSTRACT

Alzheimer's disease (AD) is characterized by progressive decrease in cognitive function and loss of short-term memory known to be associated with a dysfunction of the cholinergic system. The pathological hallmarks of AD are beta-amyloid (Abeta) plaques and neurofibrillary tangles (NFTs) consisting of hyperphosphorylated tau. Hypercholesterolemia and disturbances in glucose metabolism are another risk factors. During the last two decades therapeutic strategies were mainly targeting the Abeta hypothesis. As this approach virtually failed to show a significant clinical benefit research on potential therapeutics has been shifted to tau pathology. However, also this approach has as yet not yielded in new therapeutics. Hence, rebalancing the cholinergic input to improve the cognitive symptoms of AD by inhibition of acetylcholine esterase (AChE) is still the only mechanistic target in addition to N-methyl-D-aspartate (NMDA) receptor blockade by memantine that can be addressed by currently approved medications. Despite the fact that the available AChE inhibitors are directed at an identical target they exhibit some pharmacodynamic and pharmacokinetic features that should be considered when used clinically.

2. INTRODUCTION

Alzheimer's disease (AD) is one of the most disabling conditions that affect the elderly, and is likely to become a major health problem since the proportion of the elderly population is continuously increasing. In 2006, AD affected more than 26 million people worldwide (1). On average, the life expectancy following diagnosis is approximately seven years (2). In the United States, about 5.3. million people currently suffer from AD (3-4), and more than half of these individuals are categorized as being moderately or severely affected (4). These advanced stages of AD may extend over a period of several years and are often most challenging for patients and caregivers (3).

AD that is characterized by a progressive decrease in cognitive function and loss of short-term memory is known for more than 30 years to be associated with a dysfunction of the cholinergic system (5). Early histologic studies showing loss of cholinergic activity as AD progresses have meanwhile been supplemented by advanced imaging techniques, including positron emission tomography and magnetic resonance imaging (MRI). Diminution in cortical acetylcholine esterase (AChE) activity in patients with mild to moderate AD (6-7) is

correlated with an increase in cognitive deficits (8). In addition, atrophy of the nucleus basalis of Meynert, the primary cholinergic input to the cortex, was observed in patients with AD using MRI (9).

Significant loss of acetylcholine, acetylcholine transferase and high-affinity choline uptake occurs in brain areas associated with memory and learning, i.e. the hippocampus and cortex. In addition, diminution of cholinergic activity in AD patients is associated with cognitive deficits as measured by dementia rating scales (4). Cholinergic dysfunction associated with AD is not considered the cause but a consequence of the still not completely understood pathophysiological mechanisms, and is one of the few mechanistic targets that can be addressed by currently approved treatment options. Accordingly, the strategy of rebalancing the cholinergic input to improve the cognitive symptoms of AD has been a primary but still on-going therapeutic approach. Enhancement of cognitive function occurs when the action of acetylcholine is increased by inhibition of its metabolizing enzymes, primarily AChE (10).

In addition to the degeneration of the cholinergic system, AD is associated with an increased loss of glutamatergic neurons (11) accompanied by disturbances in N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-receptor expression in the cerebral cortex and hippocampus (12). Moreover, there may be a dysfunction in remaining glutaminergic neurons, as the ability of glial cells to remove glutamate from the synaptic cleft was impaired in various brain regions including the temporal cortex (13). The raised background concentration („noise") of glutamate may enhance the depolarization frequency of the postsynaptic membrane, and as a consequence, may reduce the detection of physiological NMDA receptor-mediated signals. Accordingly, this dysfunction in glutamate neurotransmission may contribute to cognitive impairment in AD (13).

In 2002, the NMDA receptor antagonist memantine was approved by the European Medicines Agency (EMA) for the treatment of moderate to severe AD. Rather than simply providing a complete block of the NMDA receptor, memantine exhibits moderate voltage dependence and fast blocking/unblocking kinetics. There is evidence that these characteristics allow it to block the NMDA receptor channel during the constant background 'noise' of pathological glutamatergic activation (13). Although memantine could cause improvements in symptoms and cognition, as with AChE inhibitors its effectiveness diminishes as AD proceeds (14).

The present review provides a brief summary on the presently approved AD medication as well as on some other therapeutic approaches that could be promising based on currently discussed pathophysiological mechanisms. It hardly needs mentioning that the selection of new therapeutics is bound to be subjective and is not intended to be exhaustive as well.

3. MECHANISMS UNDERLYING DEMENTIA OF THE ALZHEIMER TYPE

AD is characterized by marked atrophy of the cerebral cortex and loss of cortical and subcortical neurons (15). The pathological hallmarks of AD are senile plaques, represented by spherical accumulations of beta-amyloid (Abeta) and neurofibrillary tangles (NFTs; also referred to as paired helical filaments) consisting of hyperphosphorylated tau (16, 17,18). However, also in about 30 percent of normal-aged individuals a similar amount of Abeta plaques is found as in AD patients (19). Obviously, both Abeta plaques and NFTs are of multifactorial origin and not unique to AD.

Early onset familial AD is an uncommon form of the disease accounting for only about 1 percent of all Alzheimer cases. It is diagnosed usually between the age of 50 and 65 and is due to autosomal dominant mutations in one of at least three genes encoding the transmembrane proteins, amyloid precursor protein (APP), presinilin 1, or presinilin 2. The presenilins represent the catalytic core of γ-secretase intramembrane protease (20).

In contrast, the primary cause of the common sporadic form of AD accounting for the remaining 99 percent of Alzheimer cases is as yet not fully understood. Complex interactions of genetic and environmental risk factors presumably contribute to the evolvement of the disease that normally affects people aged older than 65 years (16-17).

Abeta (39-42 amino acid peptide) is generated by proteolytic cleavage by beta- and gamma-secretase also in regular APP turnover. However, in particular, the 42 amino acid Abeta aggregates easily and forms extracellular senile plaques. Abeta deposits are considered to influence synaptic function and to lead to apoptosis of neuronal cells (20).

Abeta degradation is mediated through insulin-degrading enzyme and neprilysin. An imbalance in Abeta formation and clearance results in formation of senile Abeta plaques (21). Evidently next to the aggregated plaques soluble highly toxic and detrimental Abeta oligomers exist (22). Abeta oligomers are related to early memory loss by blocking long-term potentiation (23). Polyclonal antibodies directed against pathologic AD brain-specific Abeta oligomers (M94) showed in rat hippocampal neurons highly specific binding, characterized by clusters within neuritic arbors. In addition, an overexpression of morphology changing actin-binding protein (Arc) was shown in dendritic spines, possibly leading to receptor dysfunction (23). De Felice *et al.* (2008) (24) demonstrated that next to synthetic soluble Abeta oligomers also Abeta oligomer-containing soluble extracts from AD brains increased tau hyperphosphorylation. Apparently only neurons with trypsin-sensitive sites are targeted by these Abeta oligomers. More recent studies underline the detrimental effect of Abeta oligomer-induced tau phosphorylation on the microtubule structure resulting in loss of axonal transport and synaptic decomposition (25).

This selective neuronal vulnerability suggests the microtubule cytoskeleton as valid therapeutic target for the treatment of AD (24).

In AD brain, tau was by about 3-4 fold more phosphorylated at Ser-199/202 than tau from control brains (26). Hyperphosphorylation of tau leads to massive detachment from microtubules that in turn greatly reduce microtubule stability in nerve cells. Several protein kinases (MAPKs) such as cdk5 and glycogen synthase kinase 3 (GSK-3beta) may be involved in tau phosphorylation. In addition, MAPKs contribute to excitotoxicity, synaptic plasticity and neuroinflammation. The stress-activated enzyme p38 capable of inducing apoptosis is increased in AD brain compared to age-matched normal brain; moreover, phospho-p38-immunoreactivity correlates with high incidence of senile Abeta plaques and neurofibrillary tangles (20, 27). Obviously, the GTPase-dependent p38 MAPK signalling pathway contributes in different ways to the pathological feature of AD (28).

The most important tau phosphatase is protein phosphatase-2A (PP2A). AD-induced decrease of PP2A also contributes to tau hyperphosphorylation due to impaired tau dephosphorylation (29).

Also calcium homeostasis is affected in AD pathology as Abeta induces L-type voltage-sensitive calcium channels (LVSCC) that in conjunction with the over-excitation of postsynaptic NMDA receptors causes an excessive entry of calcium into the post-synaptic neuron eventually leading to neuronal death (30).

Hypercholesterolemia is another risk factor for developing AD. As apolipoprotein E (APO-E) transports cholesterol and lipoproteins in the circulation it has a large influence on blood lipid levels. APO-E deficiency leads to higher levels of blood cholesterol, as shown in APO-E knockout mice fed with low fat diet that had increased blood cholesterol levels compared with the wild-type control group. The most common allele APO-E epsilon3 is expressed in more than 70 percent of the population, whereas only about 15 percent express APO-E epsilon4. APO-E epsilon4 increases the individual risk for developing AD by 3-4 fold and 40 percent of AD patients exhibit the APO-E-epsilon4 allele. According to Small *et al.* (2000) (31) carriers of APO-E epsilon4 who developed AD have lowered parietal, temporal and posterior cingulate glucose levels, leading to significant cognitive impairment after two years of longitudinal follow up. Apparently APO-E epsilon4 is also involved in Abeta-clearance and tau phosphorylation. C-terminal-truncated aggregated APO-E epsilon4 fragments caused NFT-like inclusions in cultured neuronal cells as well as in APO-E epsilon4 transgenic mice (32).

During the last two decades research and therapeutic strategies were mainly targeting the Abeta hypothesis thereby ignoring the fact that no correlation has been found between Abeta plaques and dementia. On the contrary, drugs influencing Abeta clearance or production that have been successfully tested on animals failed to show a significant benefit in humans and occasionally even worsened the condition of treated AD patients compared to placebo (19). For that reason recent therapeutic research has shifted from the Abeta focused strategies to tau pathology due to an obvious correlation between NFTs and dementia (33).

It is well established that brain glucose metabolism is impaired in Alzheimer's disease; vice versa AD is known to deteriorate already existing pathologic insulin changes (34). Impaired brain glucose metabolism leads to abnormal hyperphosphorylation of tau and neurofibrillary degeneration via downregulation of tau O-GlcNAcylation in Alzheimer's disease (35). Consequently, restoration of brain tau O-GlcNAcylation could be an option for the treatment of AD. In AD brains, e.g. levels of insulin receptor substrate mRNA, tau mRNA, and phosphotidylinositol 3-kinase were reduced, whereas glycogen synthase kinase-3beta activity and APP mRNA expression were enhanced. As in addition the expression of genes encoding insulin and insulin-like growth factors were reduced in a manner that resembled changes observed in diabetes mellitus, AD was referred to as "diabetes type 3" (36).

In insulin-receptor substrate 2-knockout mice tau phosphorylation at Ser-202 was increased (37). Functional insulin receptor signalling induces GSK-3beta inactivation, whereas dysfunction of insulin receptor signaling provoking insulin resistance caused dephosphorylation and activation of GSK-3beta. Thus in insulin-resistant individuals, such as type 2 diabetes patients, there is an increase in GSK-3beta activity due to glucose clearance (38). Also primary cultured hippocampal neurons with a mutation in presenilin 1 gene showed an increased GSK-3beta activity, whereas down-regulation of GSK-3beta via the Akt signaling pathway caused less Abeta and NFT formation (39). In summary, these findings support a correlation among AD-associated tau phosphorylation, impaired glucose metabolism, and reduced insulin sensitivity.

Apart from Abeta plaques and NFTs other pathologies have been observed in AD brains. In AD patients brain mass was decreased by 5-fold compared to normal loss of 0.5. percent of the brain mass/year. Impaired cell differentiation and regeneration due to developed AD pathologies mainly in the hippocampus result in rapid progression of the neurodegeneration in AD patients (19).

In conclusion, AD is a multifactorial disease. As the underlying pathomechanisms take place years before clinical onset an adequate therapy should comprise inhibition of neuronal degeneration and stimulation of neuronal regeneration as well as rebalancing the cholinergic input.

4. DRUGS APPROVED FOR THE TREATMENT OF ALZHEIMER'S DISEASE

Four approved medications comprising three AChE inhibitors and memantine are currently available to

Table 1. Basic properties of AChE inhibitors and memantine

Medication	Oral dose (mg/d)	Mode of action	Pharmacokinetics	
Rivastigmine[1] (Exelon®)	3 – 6 patch: 4.6 or 9.5 mg/24 h)	Brain-selective, carbamate-type, "pseudo" irreversible inhibitor of AChE and BuChE	T_{max} Half-life	0.5 – 2 h 1.5 h (10 h pharmacodynamic)
			Protein binding Bioavailability Metabolism Excretion	40% 36% non-hepatic renal
Donepezil (Aricept®)	5 – 23 (once daily)	Specific, uncompetitive reversible inhibitor of AChE, mainly CNS active	T_{max} Half-life Protein binding Metabolism Excretion	3 – 4 h 70 h 95% CYP2D6/CYP3A4[2] renal
Galantamine (Razadyne®)	8 – 24	Potent, competitive and reversible inhibitor of AChE	T_{max} C_{max} Protein binding Bioavailability Metabolism Excretion	1 h 4 – 5 h 18% 90% CYP2D6/CYP3A4[2] renal
Memantine (Namenda®)	5 – 20	Uncompetitive NMDA receptor antagonist	T_{max} Protein binding Metabolism Excretion	3 – 8 h 45% partial hepatic, not CYP[2] dependent renal

[1]: Transdermal patches of rivastigmine are available; they may exhibit a favourable tolerability profile compared to oral dosages,
[2]: CYP: cytochrome P450 monooxygenase

treat AD. All AChE inhibitors (donepezil, Aricept®; rivastigmine, Exelon®; galantamine, Razadyne®) are indicated for the treatment of *mild to moderate* AD; in the US and Japan, donepezil can be used for *severe* AD as well (40, 41,42). Tacrine (Cognex®) was the first drug for the treatment of the memory problems of AD approved by the FDA in 1993, but was of limited clinical significance due to the potential of severe side effects, in particular hepatoxicity. In the US, tacrine has been withdrawn from use in 2013, because of concerns on safety and availability of other AChE with a better adverse event profile. The un-competitive NMDA receptor antagonist memantine (Namenda®) is indicated to treat *moderate to severe* AD (43).

4.1. Acetylcholine esterase inhibitors

Among the different therapeutics that may be used to modify cholinergic neurotransmission, only AChE inhibitors have been modestly effective and have been approved as medicinal drugs indicated for AD (41). They prolong the actions of the neurotransmitter acetylcholine at the synaptic cleft stimulating presynaptic muscarine and nicotine receptors, the number of which is decreased in patients with AD (44).

The three commonly used cholinesterase inhibitors, rivastigmine, donepezil and galantamine, have virtually the same general mechanism of action, but differ substantially in terms of other pharmacological properties (Table 1), which may affect their safety and tolerability profiles. For a given AChE inhibitor the incidence of adverse effects is directly related to the dose administered. Nausea, vomiting, and diarrhoea are the most common adverse events are; cardiovascular and neurologic adverse effects are comparable (45). Rivastigmine is in addition available as transdermal patch (Exelon®) that appears better tolerated than oral rivastigmine (46).

4.1.1. Rivastigmine

Rivastigmine tartrate is a brain-selective, slowly reversible inhibitor of AChE and butyrylcholine esterase (BuChE). As it has been demonstrated that BuChE levels increase with progressive loss of neurons in AD and may take over the function to metabolise acetylcholine at the synapse, there may be at least a theoretical advantage of a dual inhibitor of AChE and BuChE over a selective AChE inhibitor in the treatment of AD (46). A result supporting this idea was obtained in patients with minimal to mild AD treated for 20 weeks with various AChE inhibitors. The rivastigmine group did not show the widespread cortical atrophic changes in parietotemporal regions reported in untreated AD patients, that were also detectable in the subgroups treated with selective AChE inhibitors (47).

Rivastigmine is a carbamate-type, selective inhibitor of AChE and BuChE. It is cleaved by AChE by transferring the carbamoyl moiety onto the active site of the enzyme that thereby becomes inactivated. *In vitro* studies using rat brain tissue demonstrated that carbamylated AChE required more than 24 h to become re-activated (48-49). Hence, due to the prolonged inhibition of AChE rivastigmine may be classified as pseudo-irreversible agent (48). Rivastigmine lacks binding affinity for dopaminergic, opioid, muscarinic, nicotinic, and α- and β-adrenergic receptors.

Several isoforms of AChE have been identified, with identical amino acid sequences, but with different posttranslational modifications, locations, and functions (49). The predominant form of AChE in the cortical and hippocampal regions of the normal brain is the membrane-bound globular tetrameric form, G4, with a small amount of the monomeric form, G1. This may have clinical significance, as a selective loss of membrane-associated G4 isoform was observed in patients with AD, whereas the level of G1 in these patients was largely unchanged (49). Rivastigmine, by inhibiting the G1 isoform of AChE, could increase ACh levels in the remaining cholinergic terminal in AD. Preferential inhibition of the G1 isoform may also account for the selectivity of rivastigmine for central over

peripheral AChE, since the brain contains appreciable amounts of G1, whereas G4 is the predominant isoform in the presynaptic membrane of the neuromuscular junction in skeletal muscle. This selectivity for central AChE may minimize peripheral adverse events related to heart and muscle (46).

Various clinical phase II and phase III studies with oral rivastigmine demonstrate beneficial effects for patients with mild to moderate AD (50). At daily doses of 6 to 12 mg compared to placebo, improvements were seen in the rate of decline of cognitive function, activities of daily living, and severity of dementia. A post hoc analysis of several studies suggests that rivastigmine may slow the cognitive decline for up to five years (51). Adverse events were consistent with the cholinergic actions of the drug such as nausea, vomiting, diarrhea, and anorexia.

Transdermal patches appear associated with fewer side effects than the corresponding oral dosages at comparable efficacy (46, 52). For example, the IDEAL study, a 24-week, multicentre, double-blind, double-dummy, placebo- and active-controlled trial in 1,195 patients, and its 28-week, open-label phase extension demonstrated comparable efficacy of rivastigmine transdermal patches (9.5. mg/24 h) with rivastigmine capsules (12 mg/d) and an improved frequency of adverse events.

4.1.2. Donepezil

Donepezil, is a piperidine based, potent, specific, non-competitive and reversible inhibitor of AChE (53-54). It is structurally dissimilar from the other cholinesterase inhibitors, rivastigmine and galantamine and has a half-life of approximately 70 h. Accordingly, it is given once daily in contrast to the other oral AChE inhibitors. Some data suggest a neuroprotective effect of donepezil by reducing glutamate excitotoxicity and Abeta toxicity as well as slowed progression of hippocampal atrophy (55-56).

Donepezil was the first rational treatment available in the UK for AD and as such received considerable attention. Patients with mild, moderate or severe AD treated for up 52 weeks with donepezil experienced benefits in cognitive function, activities of daily living and behaviour (57). If well tolerated, donepezil should be continued in the severe stages of AD as long as the patient appears to benefit from a slower clinical decline. In addition to the 10-mg dose, a 23-mg dose is available that is an efficacious therapy for moderate to severe AD, with or without concomitant memantine, extending the treatment opportunities available to manage moderate to severe AD dementia (58). A long-term study with donepezil suggests greater cognitive benefits if donepezil therapy is started early in the course of AD (59).

4.1.3. Galantamine

Galantamine, a phenanthrene-derived alkaloid of the morphine group, is a potent, competitive and reversible inhibitor of AChE. It was for the first time isolated in 1956 by the Bulgarian scientists Paskov and Iwanova-Bubewa from the bulbs of snowdrops, Galanthum nivalis (60). *In vitro* inhibition measurements of AChE and BuChE in rat striatum and mouse forebrain homogenates demonstrated a distinct selectivity of galantamine for inhibiting AChE over BuChE (61). In addition, galantamine allosterically modulates nicotine receptor function in a positive manner (62). This effect could be beneficial for the treatment of AD considering a correlation of severity of cognitive impairment in AD with the loss of nicotinic receptors (63). Furthermore it is expected that prolonged direct activation may cause desensitization rather than increased activation of the remaining nicotine receptors.

Galantamine appears to exhibit also neuroprotective effects *in vitro* and *in vivo* (64). Moreover, it has been demonstrated to allosterically modulate rat microglial nicotine receptors (expressing the alpha7 subunit), thereby enhancing microglial Abeta phagocytosis (65). It is postulated that this mechanism may be involved in galantamine-enhanced Abeta clearance observed in the brains of Abeta-injected rats and APdE9 mice.

The clinical efficacy of galantamine on cognitive function is comparable to the other AChE inhibitors, rivastigmine and donepezil (41). Significant gains for people taking galantamine were also found for functional and global outcomes (40). The beneficial effect of galantamine sustained for up to 36 months in mild to moderate AD patients with 50 percent improvements over expected scores in untreated Alzheimer subjects (66).

4.2. Memantine

Memantine is currently the only approved drug for AD that targets the glutamatergic system by preventing excessive glutamatergic activity (13). Although memantine is indicated for the treatment of *moderate to severe* disease stages, it is often pre-scribed off-label for *mild* AD (67).

However, a meta-analysis of clinical trials on memantine in a total of 431 patients with mild AD came to the conclusion that memantine was ineffective (68). Also the benefits for patients with moderate AD were inconsistent. Support comes from multiple large-scale, controlled clinical studies, demonstrating efficacy against overall and individual items of cognitive function (69-70). A Cochrane review concluded that memantine at a dosage of 20 mg per day over six months had a slight positive effect on cognition (71). In addition, memantine improved behavioural scores, and in particular, subscales associated with agitation and aggressive behaviour (72).

Memantine is generally well tolerated and is often used in combination with AChE inhibitors. It is, however, controversially, discussed whether it produces clinically meaningful improvements (13, 42, 67). In patients with mild disease, combination therapy has shown no significant benefits over monotherapy with AChE inhibitors.

4.3. Conclusion

A clinical benefit based on symptomatic treatment with AChE inhibitors and/or memantine is widely assumed to last only for 6 to 12 months. Although this therapeutic window period appears rather limited at first glance it

should be considered that interventions that could delay both disease onset and progression by only 1 year, would lead to nearly 9.2. million fewer expected cases of the disease in 2050 (1). Moreover, several studies show AChE inhibitors to slow cognitive decline for a period of 3 - 5 years and being more effective if AChE therapy is started early in the course of AD (73). Such an early onset of therapy appears reasonable in view of the neuroprotective effects suggested for some AChE inhibitors.

5. PROMISING NEW THERAPEUTIC APPROACHES

This section briefly summarizes promising approaches in the treatment of AD focused on neuroprotection targeted at either tau hyperphosphorylation or NMDA receptor-associated formation of reactive oxygen species.

Drugs targeting Abeta virtually failed to show a cognitive benefit in humans possibly because intervention in the Abeta cascade could only be successful at an early stage of the disease or even before developing any symptoms or specific AD hallmarks in the brain. For example, in clinical studies with several direct-acting (e.g. CTS-21166, LY2811376) and indirect-acting (e.g. ACI-91 corresponding to pirenzepine) beta-secretase inhibitors and several gamma-secretase inhibitors (e.g. avagacestat, JNJ-40418677 and semagacestat), there was a lack of efficacy or their use was associated with safety concerns (74). Early studies with solanezumab (LY2062430), a monoclonal antibody that binds to a soluble Abeta fragment suggested a decrease in the amount of Abeta in neuritic plaques by enhancing Abeta clearance from the body. However, two phase-3, double-blind trials (EXPEDITION 1 and EXPEDITION 2), including 1,012 and 1,040 patients, respectively, with mild-to-moderate AD failed to show a significant improvement in the primary outcomes, cognitive impairment and functional ability. The patients received placebo or solanezumab administered intravenously at monthly doses of 400 mg for 18 months. Also statistical re-analysis of EXPEDITION 2 focusing on patients with mild AD did not provide evidence for a clinical benefit of solanezumab (75). Currentlly, the efficacy of Abeta immunotherapies is investigated in early onset familial AD (18).

5.1. Drugs directed at tau pathology

Hyperphosphorylated tau representing the major protein of neurofibrillary tangles in AD is most probably the result of an imbalance of tau kinase and phosphatase activities in the affected neurons. Accordingly, inhibition of tau hyperphosphorylation appears a promising therapeutic approach in AD. As PP2A is a major regulator of tau phosphorylation that showed reduced enzymatic activity in AD (76), maintaining or restoring PP2A activity may prevent or counteract further progress of the disease, e.g. by reducing cleavage and translocation of I2(PP2A), an endogenous inhibitor of PP2A. This could be achieved by direct inhibition of I2(PP2A) or by inhibition of asparaginyl endopeptidase that causes cleavage of the inhibitor (19, 77).

Also overexpression of GSK-3beta resulted in increased formation of NFTs. Consequently, chronic inhibition of GSK-3beta by lithium chloride significantly decreased the levels of hyperphosphorylated tau (78). Considering the potential toxicity of lithium Nunes *et al.* (2013) (79) studied the effect of lithium at a microdose of 300 µg. The drug was administered to AD patients once daily for 15 months. The lithium-treated patients maintained the performance in the mini-mental state examination test in contrast to the control group with significant differences 3 months after the onset of treatment and progressively increasing thereafter. Accordingly, this clinical study shows clinical benefits of microdose-lithium therapy by preventing ongoing cognitive loss associated with AD (79).

Another study investigated the disease-modifying properties of long-term lithium treatment for amnestic and mild cognitive performance in 45 AD patients. The treatment group (n=24) titrated to target lithium serum levels of 0.2.5–0.5. mM exhibited significantly less levels of phosphorylated tau in the cerebrospinal fluid and improved performance on the cognitive subscale of the AD Assesment Scale and attention tasks. In healthy volunteers this concentration range of lithium caused a 50 percent reduction in GSK-3beta activity in leukocytes. Overall tolerability of lithium was good during the study with an adherence rate of 91 percent (80).

Jayaplan and Natarajan (2013) (81) investigated the tertiary structure of tau and the role of CDK5 and GSK-3beta in abnormal tau hyperphosphorylation. CDK5 appeared more relevant in tau hyperphosphorylation than GSK-3beta. Hence therapeutics that target CDK5 could be particularly promising in avoiding NFT formation. A strong correlation between elevated levels of phosphorylated tau and CDK5 was also observed in the APP/presenilin 1 mice (82).

In a clinical phase II study with methylene blue, this inhibitor of tau aggregation only a low dose (60 mg) showed improvement of memory function, whereas the higher dose (100 mg) failed to show any benefits (19).

Active and passive immunotherapy for tau significantly reduced density of NFTs as well as improved cognitive function in transgenic mouse models. Moreover, a tau-immunization based vaccine is currently tested in a phase I clinical trials investigating inhibition of the spread of the tau pathologies to the extracellular space. This clinical approach seems promising considering that extracellular tau is correlated with cognitive decline (19).

5.2. Combined treatment of memantine plus vitamin D

A dysfunction of glutamatergic neurotransmission is assumed being involved in AD. NMDA receptor over-excitation due to enhanced y combines O^{2-} under formation of peroxynitrite (OONO$^-$). The free radicals as well as peroxynitrite damages neuronal DNA, membrane lipids and cell proteins in a dose-dependent manner finally leading to delayed neuronal

apoptosis as a consequence of multiple enzyme inactivation.

The clinical benefit of memantine is related to inhibition of massive calcium influx through NMDA receptors. Accordingly, glutamatergic neurons are protected from over-excitation, and consequently immediate neuronal necrosis can be avoided. However, as memantine exhibits low NMDA receptor affinity in conjunction with rapid association/dissociation kinetics the NMDA receptors remains functional in surviving cells and enables glutamate-induced calcium entry. Together with the Abeta-mediated up-regulation of LVSCC this constant calcium influx into the post-synaptic neurons may trigger oxidative stress and hence may induce apoptosis. Accordingly, combining memantine with a drug targeting the delayed neuronal apoptosis seems promising for prevention of NMDA receptor-associated formation of reactive oxygen species leading to apoptosis.

Vitamin D is a key regulator of intracellular and extracellular calcium homeostasis in the brain for example by decreasing the expression and density of the Aβ-targeted LVSCC-A1C channels (83-84). A significant increase in hippocampus LVSCC channels was described to correlate with increased cell death. In addition, vitamin D day regulates the extracellular calcium-binding proteins calbindin-D28K, parvalbumin and calretinin that are suggested to play an important role in processes involved in neuroprotection and prevention of apoptosis (30, 86-86). Exposure of rat mesencephalic neurons to glutamate (1 mM for 10 min) caused significantly less damage if 100 nM vitamin D was substituted 24 h before exposure. Vitamin D may target due to its anti-oxidative properties controlling the intracellular free radicals produced by reactive species of oxygen and NO. Moreover, vitamin D is assumed to influence the activity of gamma-glutamyl transpeptidase, a key regulator of the antioxidant glutathione metabolism (30, 87).

As vitamin D is known to have neuroprotective effects that include regulation of neuronal calcium homeostasis as well as antioxidant, neurotrophic and anti-inflammatory properties, the combination of memantine plus vitamin D may provide enhanced protection against several degenerative processes linked to AD (30, 88).

5.3. Conclusion

AD is a multifactorial disease and the underlying pathomechanisms take place years before clinical onset. Accordingly, an adequate therapy should comprise inhibition of neuronal degeneration, stimulation of neuronal regeneration as well as symptomatic treatment by rebalancing the cholinergic input. Moreover, a reliable diagnostic is needed that detects an initial disease stage and thus allows an early initiation of therapy. In default of such an ideal therapeutic approach measures are recommended that may help to maintain neuronal plasticity by providing a functional biochemical microenvironment.

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