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Cognitive evaluation of disease-modifying efficacy of Galantamine and Memantine in the APP23 model

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Abstract With increasing knowledge of molecular, biochemical and cellular events causing synaptic dysfunction and neurodegeneration in Alzheimer-diseased brain, preventive treatment strategies are emerging. Neuroprotective capacities have been attributed to galantamine and memantine. The age-dependent cognitive decline in the APP23 model was employed to evaluate disease-modifying efficacy of chronic treatment with both compounds. At age 6 weeks, heterozygous APP23 mice were subcutaneously implanted with osmotic pumps delivering saline, galantamine (1.3 or 2.6 mg/kg/day) or memantine (7.2 or 14.4 mg/kg/day). After 2 months of treatment, a 3-week wash-out period was allowed to prevent bias from sustained symptomatic effects. Subsequently, cognitive evaluation in the Morris water maze commenced. Galantamine low dose significantly improved spatial accuracy during probe trial. Memantine improved acquisition performance (path length) and spatial accuracy during probe trial in a dose-dependent manner. This is the first study reporting disease-modifying efficacy of galantamine and memantine in transgenic mice modeling Alzheimer's disease.

1. Introduction

Alzheimer's disease (AD) has an insidious onset and a slow, progressive course. At present, only partially known genetic and environmental risk factors initiate a cascade of neuropathological events that engages the accumulation of extracellular amyloid plaques and intracellular neurofibrillary tangles. Consequently, a gradual loss of synapses, and later, neural somata emerges. Neuronal degeneration and death in some areas of the neocortex and hippocampus produce a decline in cognitive capacities, ultimately, depleting compensatory circuitry, and hence, behavioural and functional symptoms appear (Selkoe, 2001).

The compounds currently used for treatment of AD (donepezil, rivastigmine, galantamine and memantine)

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provide symptomatic relief. Whether some of those agents are efficacious as disease-modifying treatments, remains a matter of debate. Ideally, intervention in the disease process will endorse restoration of neural integrity and function. As neurodegeneration advances in AD, and secondary or tertiary changes ensue, such reversal becomes increasingly implausible. Other approaches provide secondary prevention by targeting the prodromal stage of AD in an attempt to ward off further progression of mild symptoms. Beyond question, the best approach is primary prevention; neuroprotective agents that attenuate the process of AD pathogenesis in its latent phase would delay or avoid the manifestation of symptoms altogether. For a therapeutic intervention to slow down or even halt disease progression, i.e. be disease-modifying, it must interfere with a central pathway in the pathophysiological disease process. Both galantamine and memantine are internationally approved for symptomatic treatment of AD, but additionally, disease-modifying capacities have been assigned to both compounds.

The discovery of loss of cholinergic neurons in basal forebrain and subsequent reduction of cholinergic transmission in cerebral cortex of AD brain (Davies and Maloney, 1976; Coyle et al., 1983), led to further enquiry into the contribution of cholinergic receptors. A consistent, substantial loss of nicotinic acetylcholine receptors (nAChRs) was perceived in post-mortem brain tissue of AD patients (Whitehouse et al., 1986; Nordberg and Winblad, 1986), and especially the number of $\alpha 4$ subunit-containing nAChRs was selectively abridged (Warpman and Nordberg, 1995; Martin-Ruiz et al., 1999). Neuroimaging techniques confirmed these findings and revealed that these receptor changes present an early phenomenon in the course of the disease (Nordberg, 1992; Nordberg et al., 1995), which is, furthermore, closely associated with augmented levels of the amyloid β $(A\beta)_{1-42}$ peptide (Perry et al., 2000). Galantamine boosts central cholinergic activity through a dual mode of action; it acts as a brain-selective, reversible, competitive acetylcholinesterase inhibitor (AChE) inhibitor to stimulate cholinergic transmission (Thomsen and Kewitz, 1990). Additionally, galantamine has been identified to be an allosteric potentiating ligand (APL) that amplifies the action of the neurotransmitter at the nAChR (Schrattenholz et al., 1996; Albuquerque et al., 1997). Noteworthy, galantamine distinctively operates upon the $\alpha 4/\beta 2$ nAChR subtype (Samochocki et al., 2003).

Excessive activation of glutamate receptors might be responsible for part of the neuronal damage observed in AD. Although it is likely that a glutamate-related alteration is not the primary etiopathological factor in AD, extracellular glutamate might contribute importantly to aggravate and accelerate neuronal degeneration (for review see; Doble, 1999). Under physiological conditions, the glutamatergic *N*-methyl-D-aspartate (NMDA) receptor channel is blocked by Mg^{2+} ions in the absence of its ligand. In case of neurodegeneration, a maintained low release of glutamate displaces Mg^{2+}, leading to a sustained influx of Ca^{2+}, subsequently triggering a cascade of enzyme activations converging to neuronal death. Memantine is a non-competitive, voltage-dependent NMDA receptor antagonist that blocks the ion channel in the presence of this sustained low-level release of glutamate, thereby inhibiting the toxic Ca^{2+} influx (neuroprotection) and reducing the intracellular Ca^{2+} pool (for review see; Parsons et al., 1999b).

In order to establish that a treatment has an impact on disease progression, a (clinical) trial must clearly distinguish between the symptomatic and disease-modifying effects of the treatment. The most obvious of such designs is a so-called withdrawal design (Leber, 1996; Bodick et al., 1997; Kittner et al., 2000). Subjects are randomly assigned to receive either active compound or placebo (i.e. sham treatment) for a specific duration (Period I). They are subsequently withdrawn from treatment and then followed for an additional period of time (Period II), that is thought to be of sufficient length to eliminate ('wash-out') any symptomatic effect of the treatment. In a clinical setting, this type of study design permits assumption about both symptomatic and disease-modifying effects of a certain treatment. Adding an extension with re-randomization during period II can abolished the disadvantage that there is no blinding with respect to the treatment received during the wash-out period. After period I, subjects receiving active compound are re-randomized to either remain on the active compound or switch to placebo during period II. A similar procedure is followed for the placebo-treated subjects (Whitehouse et al., 1998). The switch from placebo in phase I to active compound in phase II evaluates a delayed onset of treatment, which was however not pursued in our animal study. Re-randomization though, was not necessary in our animal trial since researchers evaluating the animals, were blinded for treatment status.

APP23 mice can be considered a valid model for AD as they mimic several pathological hallmarks (Sturchler-Pierrat et al., 1997; Sturchler-Pierrat and Staufenbiel, 2000), and both cognitive and behavioural alterations typical for AD patients (Kelly et al., 1999; Lalonde et al., 2002; Van Dam et al., 2003; Kelly et al., 2003; Vloeberghs et al., 2004; Van Dam et al., 2005a,c). The APP23 model displays an age-related decline in visual–spatial learning capacities, as tested in a hidden-platform Morris-type water maze (MWM) paradigm. Cholinergic dysfunction, as indicated by decreased acetylcholinesterase and choline acetyltransferase activity levels in basal forebrain nuclei, in addition to alterations in other neurotransmission systems might underlie this cognitive decline (Van Dam et al., 2005b). Therefore, APP23 mice are of major interest to evaluate pharmacological interventions commencing before learning deficits and pathological alterations are apparent (Van Dam et al., 2003). Analogously with clinical trials, we employed a withdrawal design to investigate putative disease-modifying capacities of galantamine and memantine. Outcome parameters of this trial assessing putative disease-modifying efficacy of both compounds were MWM acquisition and probe trial performance.

2. Experimental procedures

2.1. Transgenic mouse model

APP23 mice were generated and bred as previously described (Sturchler-Pierrat et al., 1997). The model overexpresses human amyloid precursor protein (hAPP) containing the Swedish double mutation at position