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Themed Issue: Translational Neuropharmacology – Using Appropriate Animal Models to Guide Clinical Drug Development

REVIEW

Animal models in the drug discovery pipeline for Alzheimer's disease

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With increasing feasibility of predicting conversion of mild cognitive impairment to dementia based on biomarker profiling, the urgent need for efficacious disease-modifying compounds has become even more critical. Despite intensive research, underlying pathophysiological mechanisms remain insufficiently documented for purposeful target discovery. Translational research based on valid animal models may aid in alleviating some of the unmet needs in the current Alzheimer's disease pharmaceutical market, which includes disease-modification, increased efficacy and safety, reduction of the number of treatment unresponsive patients and patient compliance. The development and phenotyping of animal models is indeed essential in Alzheimer's disease-related research as valid models enable the appraisal of early pathological processes – which are often not accessible in patients, and subsequent target discovery and evaluation. This review paper summarizes and critically evaluates currently available animal models, and discusses their value to the Alzheimer drug discovery pipeline. Models dealt with include spontaneous models in various species, including senescence-accelerated mice, chemical and lesion-induced rodent models, and genetically modified models developed in *Drosophila melanogaster*, *Caenorhabditis elegans*, *Danio rerio* and rodents. Although highly valid animal models exist, none of the currently available models recapitulates all aspects of human Alzheimer's disease, and one should always be aware of the potential dangers of uncritical extrapolating from model organisms to a human condition that takes decades to develop and mainly involves higher cognitive functions.

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Abbreviations
Aβ, amyloid-β; AD, Alzheimer's disease; APP, amyloid precursor protein; BACE1, β-site APP-cleaving enzyme 1; BPSD, behavioural and psychological signs and symptoms of dementia; EGFP, enhanced green fluorescent protein; GSK-3β, glycogen synthase kinase 3β; NFT, neurofibrillary tangle; PSEN, presenilin; SAM, senescence-accelerated mouse; SAMP, SAM-prone; TILLING, targeted induced local lesions in genomes; ZFN, zinc finger nuclease

Introduction

As the prototype of cortical dementias, Alzheimer's disease (AD) presents with prominent cognitive deficits. Initially, patients display limited forgetfulness with disruption of memory imprinting, which evolves to short-term memory disruption and, eventually, to long-term memory deficits. At more advanced stages, patients show executive dysfunctioning leading to advanced helplessness. Besides cognitive deterioration, patients display behavioural and psychological signs and symptoms of dementia (BPSD). BPSD is an umbrella term that embraces a heterogeneous group of noncognitive symptoms and behaviours, including paranoid and delusional ideation, hallucinations, activity disturbances,

aggressiveness, diurnal rhythm disturbances, affective disturbances, anxieties and phobias (Reisberg *et al.*, 1987). The concept of BPSD is a descriptive one and does not reflect a diagnostic entity but rather highlights an important clinical dimension of dementia that has until recently been ignored from both research and therapeutic points of view. In contrast with cognitive symptomatology, BPSD do not show a progressive course. The impact of BPSD is emphasized by the fact that they increase patient suffering, impose tremendous strain on caregivers and significantly increase the financial burden on the family and society.

The histopathological hallmarks of AD brain are extracellular amyloid-β (Aβ) plaques and intracellular neurofibrillary tangles (NFT), accompanied by decreased synaptic density, which eventually leads to widespread neurodegeneration, loss of synapses and failure of neurotransmitter pathways, particularly those of the basal forebrain cholinergic system.

The incidence and prevalence of dementia in general, and AD in particular, have been studied extensively. AD is the most common form of dementia, and the most elaborate European epidemiological study – The Rotterdam Study – demonstrated 72% of all dementia cases to be of AD origin (Ott *et al.*, 1995). The number of affected individuals is likely to grow in the decades to come due to demographic changes and our still-rising life expectancy. The worldwide societal cost of dementia, based on a dementia population of 34.4 million demented persons, was estimated to $422 billion in 2009 (Wimo *et al.*, 2010). It is forecasted that the number of demented elderly will rise to 114 million by the year 2050 (Wimo *et al.*, 2003). Therefore, as prevalence rates are predicted to experience a steep rise over the next 50 years, intense and meticulous AD-related research is imperative.

Clinical research focuses at diagnosis of AD and related conditions in an early stage based on specific biomarkers. With increasing predictive efficacy for conversion of mild cognitive impairment to dementia (De Meyer *et al.*, 2010), disease-modifying treatment strategies become indispensable. Despite intensive research, underlying pathophysiological mechanisms remain insufficiently documented for purposeful target discovery. The development and phenotyping of animal models is essential in AD-related research as valid models enable the appraisal of early pathological processes – which are often not accessible in patients. The identification of biological targets with an explicit role in early stages of the disease process allows rational development and preclinical evaluation of therapeutic strategies to alleviate or prevent this neurodegenerative condition.

Animal models aiming at studying human diseases, emerged in the 1800s and experienced a major boost during the last decades. Of primary concern to neuroscientists is the selection of the most relevant animal model to achieve their research goals. Researchers are challenged to develop models that recapitulate the disorder in question, which is often not as straightforward as it may seem. Quite often they are confronted with the choice between models that reproduce cardinal pathological features of the disorders caused by mechanisms that may not necessarily occur in the patients versus models that are based on known aetiological mechanisms that may not reproduce all clinical features.

Alzheimer's disease is by its prevalence and nature an important burden on the life of patients and caretakers, as well as poses major consequences for the health and aged care systems. This review will therefore, focus on animal models of AD. Readers interested in animal models of other dementia types, including, for example, normal pressure hydrocephalus, Parkinson's disease, frontotemporal dementia, and forms of vascular and toxic dementia, are referred to a recent Springer Science + Business Media Neuromethods book entitled: 'Animal models of Dementia' (De Deyn and Van Dam, 2010a).

Various types of animal models can contribute to our growing understanding of the molecular pathways involved in disease development and progression in AD. In general, animal models of human disease can be classified into spontaneous, induced, negative and orphan models, of which the latter two types do not apply to the field of Alzheimer modelling. Spontaneous models are presumed to develop their condition without experimental manipulation, but selective breeding is often compulsory to establish and maintain the desired line. Especially for psychiatric and neurological conditions, including AD, few spontaneous models exist and experimentally induced pathology is often necessary.

Spontaneous models

Few species, including dogs (Cummings *et al.*, 1993; 1996; Rofina *et al.*, 2006), cats (Head *et al.*, 2005; Gunn-Moore *et al.*, 2006), (polar) bears (Cork *et al.*, 1988; Uchida *et al.*, 1995; Tekirian *et al.*, 1996), goats and sheep (Braak *et al.*, 1994), wolverine (Roertgen *et al.*, 1996), as well as several nonhuman primate species (Bons *et al.*, 1994; Gearing *et al.*, 1994; 1997; Lane, 2000; Geula *et al.*, 2002; Kimura *et al.*, 2003; Sani *et al.*, 2003; Lemere *et al.*, 2004; 2008), spontaneously develop plaque pathology and some species even exhibit tauopathies. In addition, these histopathological changes can be accompanied by cognitive decline (Cummings *et al.*, 1996; Voytko and Tinkler, 2004; Gunn-Moore *et al.*, 2006; Rofina *et al.*, 2006). Unfortunately, the use of these species for experimental research is limited by availability, economical (based on long lifespan) and/or ethical reasons. Nevertheless, the dog has been pointed out as an especially appropriate model for the study of human brain ageing and neurodegenerative diseases in general, and AD in particular (Sarasa and Pesini, 2009), based on its phylogenetic proximity to humans, the in-depth knowledge of canine (behavioural) neurology, and the histopathological and molecular similarities between clinical AD and the canine variant. In particular, the amino acid sequence of canine $A\beta_{1-42}$ is identical to the human form, whereas the murine form differs three amino acids from the human form. The severity of cognitive decline represents a spectrum that captures normal ageing, mild cognitive impairment and early/mild AD in humans. Given these similarities, dogs have been frequently used in preclinical AD studies. Dogs are ideally suited for longitudinal studies, and have therefore been mainly used to study the beneficial effects of an antioxidant diet, behavioural enrichment and Aβ immunotherapy (for review, Cotman and Head, 2008).

Ageing rodents do not spontaneously develop AD-like histopathological hallmarks, and are therefore of no use to the development of drugs targeting these pathological hallmarks. Their contribution to the AD-related drug discovery

pipeline is based on the occurrence of senescence-related cognitive decline and behavioural alterations linked to AD-relevant neurochemical and morphological alterations (Erickson and Barnes, 2003), including age-associated cholinergic hypofunction (Sherman and Friedman, 1990). In addition, they aid in uncovering the boundary between normal and pathological ageing, allowing in-depth investigation of basic neural mechanisms underlying brain ageing.

Natural age-associated deterioration has culminated in the senescence-accelerated mouse (SAM), a model which was established through phenotypic selection from a genetic pool of AKR/J mice in the early 1980s. The SAM model includes nine major SAM-prone (SAMP) substrains and three major SAM-resistant substrains. SAM strains have been extensively used as models for various age-related disorders; SAMP mice undergo accelerated ageing while SAM-resistant mice undergo normal ageing processes. The SAMP8 substrain in particular has drawn attention in dementia-related research because it shows age-associated learning and memory deficits in association with Aβ deposition (Yagi *et al.*, 1988; Takeda, 1999). Interestingly, genes and proteins that undergo significant alterations in SAMP8 brains are related to the following functional categories: neuroprotection, signal transduction, immune response, energy metabolism, mitochondrion, protein folding and degradation, reactive oxygen species production, cytoskeleton and transport, lipid abnormalities and cholinergic dysfunction (Butterfield and Poon, 2005; for review, see Sowell and Butterfield, 2010). The SAMP8 strain has proven to be a relevant model for AD and several treatment strategies have been studied in these mice, including antioxidants (Farr *et al.*, 2003; Poon *et al.*, 2005; Nishimura *et al.*, 2006; Shih *et al.*, 2010), antisense oligonucleotides, directed at the Aβ region of the amyloid precursor protein (APP) gene (Kumar *et al.*, 2000; Banks *et al.*, 2001; Poon *et al.*, 2004; Ali *et al.*, 2009), consistent with the notion that SAMP8 cognitive changes are associated with Aβ-associated oxidative stress. Besides pharmacological interventions, dietary restriction as a way to increase lifespan and improve health, and its effect on various functional categories that are affected in SAMP8 with ageing, as described above, was recently evaluated (Tajes *et al.*, 2010).

Pharmacological, chemical and lesion-induced rodent models

The disruption of multiple neurotransmitter systems in AD plays an important role in the pathophysiology of cognitive and behavioural disturbances associated with the illness. The majority of animal models within this category are based on the cholinergic hypothesis of AD. Degeneration of cholinergic neurons in the nucleus basalis of Meynert, situated in the basal forebrain and primarily projecting to the neocortex, occurs early in the course of the disease (Davies and Maloney, 1976; Whitehouse *et al.*, 1982). A correlation between cholinergic deficits and both cognitive symptomatology and the extent of neuropathological alterations in AD was reported (Martin *et al.*, 1987; Bierer *et al.*, 1995; Dournaud *et al.*, 1995). The basal forebrain is the anatomical region ranging from the septum to the midbrain, which passes under the anterior commissure and groups together both the telencephalic and diencephalic structures. The bilateral cholinergic centres display a high density of cholinergic isodendritic neurons. Classically, three cholinergic groups or 'nuclei' were defined: the medial septal nucleus, the nuclei of the diagonal band of Broca, and the nucleus basalis of Meynert. Histochemical research specified different sets of cortical projecting neurons with neurotransmitters other than acetylcholine intermeshed among the cholinergic neurons, as well as a better understanding of the cortical targets, and the relationship between cholinergic groups and neighbouring structures. The ascending cholinergic system, comprising sectors Ch1–Ch4 is a topographically organized cholinergic regulatory projection system, which innervates the entire neocortex, in addition to several limbic and olfactory structures. Sectors Ch5 and Ch6 provide cholinergic innervation to the thalamus, and are essential components of the ascending reticular activating system, thereby indirectly regulating cortical activity via a noncholinergic system between thalamus and cortex (Mesulam *et al.*, 1983; Selden *et al.*, 1998).

The most commonly used pharmacological model related to AD is scopolamine-induced amnesia (Sunderland *et al.*, 1986; Ebert and Kirch, 1998), which has increased our knowledge of the role of the cholinergic system in cognition and allows preclinical evaluation of symptomatic efficacy of cholinomimetics, including mainly compounds with presumed acetylcholinesterase inhibiting activity (e.g. Trabace *et al.*, 2000; Ahmed and Gilani, 2009; Wong *et al.*, 2010) and muscarinic receptor 1 agonists (Malviya *et al.*, 2008).

Scopolamine, a tropane alkaloid drug with muscarinic antagonist effects, has a primary influence on processes related to information acquisition (Rush, 1988). The use of the scopolamine-induced amnesia model is however limited by the fact that cholinergic hypofunction is not associated with the development of pathological AD hallmarks, and the lack of disease progression at the level of cholinergic and cognitive dysfunction. Blockade of nicotinic receptors by mecamylamine also induces learning impairment (Moran, 1993; Estapé and Steckler, 2002). In AD patients, not only the muscarinic but also the nicotinic receptors are markedly decreased (Whitehouse and Au, 1986; Nordberg *et al.*, 1989). Therefore, blockade of both receptors may offer a better amnesia model (Levin *et al.*, 1990; Riekkinen *et al.*, 1990).

Besides scopolamine-induced amnesia, the cholinergic hypothesis of AD has led to the development of a number of lesion models for studying the pathogeny of cortical cholinergic involution. Focal neurotoxic, electrolytic or mechanical lesions of the cholinergic centres of the basal forebrain, as well as more general lesions of all the cholinergic neurons of the basal forebrain, are most frequently used to obtain such models. Focal lesions are especially directed at the nucleus basalis magnocellularis (Lescaudron and Stein, 1999; Vale-Martínez *et al.*, 2002), the rodent analogue of the human nucleus basalis of Meynert, the septal area (Mulder *et al.*, 2005), or consist of fimbria/fornix transection leading to septo-hippocampal cholinergic denervation (He *et al.*, 1992; Alonso *et al.*, 1996). Lesioning can be achieved by surgical or electrolytical procedures, and intraparenchymal or intracerebroventricular microinjections of neurotoxic substances, such as quinolic, kainic, N-methyl-D-aspartic, ibotenic and quisqualic acids, the cholinotoxin AF64, and the immuno-

toxin 192 IgG-saporin (for review, see Toledana and Álvarez, 2010). These models increase our understanding of the role of cholinergic innervations in the aetiology and treatment of cognitive disorders. In-depth anatomical knowledge of the target area, including its neuronal and glial types, regional neuronal circuits and their connections with other brain areas, and mode of action of the toxin employed are essential because lesion characteristics depend on the type of agent employed and its capacity to cause selective harm to different subtypes of neurons, nerve fibres passing through the affected area, glial cells and blood vessels. However, the suitability of these models is also much debated about because conflicting results may be obtained and is it is essential to take into account a wide range of factors influencing the outcome of the study; such as, the species or strain used, its physiopathological characteristics (e.g. age at induction) and maintenance (e.g. housing conditions), the model protocol, including the location and extent of the lesion and whether a unilateral or bilateral lesion is opted for, the lesion-inducing agent, the type and concentration of toxin used, and even the morphological, histochemical, biochemical and cognitive methods used to phenotype the model (for review, see Toledana and Álvarez, 2010).

Alzheimer's disease-related memory deficits can also be (partially) reproduced by specifically lesioning brain structures or pathways essential for different aspects of learning and memory, such as the hippocampus, striatal and cortical regions (Gray and McNaughton, 1983; Glenn *et al.*, 2003; Sloan *et al.*, 2006; Castañé *et al.*, 2010). These models are mainly used to increase our knowledge of the neural mechanisms underlying memory dysfunction. As for basal forebrain lesion models, the major disadvantages are the lack of disease progression, AD-typical pathology and the fact that only selected lesion are studied compared with a more global disease process in AD.

Some chemically induced models focus on only one specific pathophysiological pathway thought to underlie AD. Such partial models have been developed to mimic, for example, brain inflammation or glucose/energy metabolism impairment and study the effects on neurodegeneration. Brain inflammation can be experimentally induced by the infusion of endotoxins, like lipopolysaccharide (Hauss-Wegrzyniak *et al.*, 1998), or proinflammatory cytokines (Wenk *et al.*, 2003). Brain metabolism can be disrupted through interference with mitochondrial metabolic pathways (Szabados *et al.*, 2004), or neuronal insulin signal transduction (Ishrat *et al.*, 2009).

Amyloid-β infusion rodent models

The amyloid cascade hypothesis of AD states that, regardless of whether the disease is familial or sporadic, cerebral accumulation and aggregation of Aβ peptides to form amyloid plaques is the primary culprit driving AD pathogenesis (Selkoe, 2000; Hardy and Selkoe, 2002), and additional disease processes (NFT formation and inflammation) result from the imbalance between Aβ production and clearance. More recently an updated version of this theory has assigned a pivotal role in AD pathogenesis to soluble Aβ oligomers, which can rapidly block long-term potentiation, and therefore cause memory failure (Gong *et al.*, 2003; Lacor *et al.*, 2004; Walsh and Selkoe, 2007).

Aspects of AD can be mimicked by intracerebral or intracerebroventricular infusion of Aβ peptides in the rodent brain (for review, see Lawlor and Young, 2010). Aβ species can be administered acutely, using a single stereotactic injection (Harkany *et al.*, 1998; 2000), or repetitively, using injections through an implanted cannula (Yamada *et al.*, 2005). To better mimic the progressive nature of AD, chronic and continuous administration is accomplished by connecting an implanted cannula to an osmotic mini-pump (Nakamura *et al.*, 2001; Olariu *et al.*, 2002) or a micro-infusion pump (Nag *et al.*, 1999), or with microdialysis (Harkany *et al.*, 2000).

Direct intracerebral injection of Aβ peptides causes learning and memory deficits, as well as AD-like behavioural alterations (Harkany *et al.*, 1998; Yamada *et al.*, 2005; Sipos *et al.*, 2007), with the severity of the deficits dependent upon the species of Aβ infused and the time interval between Aβ administration and behavioural testing. In addition to measurable deleterious effects on cognition and behaviour, exogenous administration of Aβ species can lead to neuropathological changes reminiscent of human AD, although the full complexity of the human pathology is not reproduced and these pathologies are not widespread as in the human condition. Accumulation of Aβ deposits in brain parenchyma (Frautschy *et al.*, 1996; Sipos *et al.*, 2007) can be associated with, for example, inflammation and microglial activation, oxidative stress, and local cell loss (Weldon *et al.*, 1998). More specifically, disruption of cholinergic function was reported (Harkany *et al.*, 1998; Yamada *et al.*, 2005). Within the published literature there is a wide variation in the reported behavioural and neuropathological effects of Aβ infusion. These inconsistencies may be due in part to variations in methodologies; the species of peptide infused (e.g. $A\beta_{1-40}$, $A\beta_{1-42}$ or $A\beta_{25-35}$), the aggregation state and concentration of the peptide preparation, the duration of the infusion, the site of infusion, the time interval between Aβ administration and behavioural testing, and even the solvent used to dilute peptides (for review, see Lawlor and Young, 2010). These methodological differences need to be considered when designing an *in vivo* Aβ infusion model and interpreting data obtained from such AD models.

Models based on intracerebral Aβ infusion support the Aβ cascade hypothesis, provide insight in mechanisms and secondary effects of Aβ toxicity and allow preclinical evaluation of drugs targeting Aβ, as well as test the protective effects of pharmacological modulation of microglial signalling, because infusion of peptide induces inflammation and microglial activation. Rodent Aβ infusion models also offer some advantages over the use of APP transgenic models. Overexpression of APP results not only in increased production of $A\beta_{1-40}$ and/or $A\beta_{1-42}$, but in elevated levels of other APP fragments, which can have neuroprotective, neurotoxic or signalling functions, and influence learning and memory. The infusion model allows researchers to administer defined amounts of a specific Aβ species of known sequence and length or to introduce controlled co-factors related to plaque development. Moreover, rather than waiting several months for pathology to develop with ageing in transgenic animals, Aβ infusion models can deliver experimental results (including plaque pathology) within a timeframe of a few weeks (Frautschy *et al.*, 1996).

On the downside, in addition to providing only a partial model of AD, and largely bypassing the effect of ageing on AD progression, a major caveat of this approach is the fact that administered Aβ concentrations are much higher than Aβ levels found in the brain or cerebrospinal fluid of AD patients (Vickers *et al.*, 2000). In contrast to transgenesis, the invasive nature of Aβ infusion inevitably brings about brain injury, which – in addition to the potential neurotoxic effects of vehicles used – may contribute to the induction of inflammation observed in these models. These potentially confounding effects can of course be controlled for by including proper sham and/or scrambled peptide groups.

Transgenic models for AD

The past two decades have witnessed an extraordinary expansion in our knowledge of the molecular basis of neurological diseases, including AD. Much of this progress is based on mapping gene loci in families with genetically determined neurological diseases. Prior to the current revolution in applied molecular genetics, the only practical method to study the regulation and function of mammalian genes was to utilize spontaneous mutants. Since the 1970s, it has been possible to introduce DNA fragments into prokaryotic and eukaryotic cells *in vitro* and to induce the expression of the foreign DNA in these cells. Although the evaluation of gene expression is relatively straightforward (determination of gene product in culture medium), the activity of a specific gene at the cellular level does not yield satisfactory information about the regulation of the gene among the complex physiological interactions of the whole organism. The development of transgenesis techniques to create genetically manipulated models has provided us with powerful tools to study pathophysiological mechanisms and evaluate new treatment strategies *in vivo*. Over the past decades, several species have been used to create genetically altered phenocopies of human AD; in particular of course mice, and to a much lesser extent, rats, as well as nonmammalian species, like zebrafish (*Danio rerio*), nematodes (*Caenorhabditis elegans*) and the fruit fly (*Drosophila melanogaster*).

AD models in *D. melanogaster*

Drosophila has found major application in the analysis of genetic interaction in neurological disorders, including AD, based on both classical phenotype-based genetic screens and techniques for genetic manipulation, including gene knockdown, deletion and transgenic insertions. A large degree of functional conservation of proteins exists between insect and human. Of particular interest to the field of AD research, is the conservation of the proteolytic activity of γ-secretase between *D. melanogaster* and humans. This fruit fly γ-secretase can correctly cleave human APP. Endogenous orthologues of AD-related genes, namely *Appl* (i.e. APP-like) (Rosen *et al.*, 1989; Luo *et al.*, 1990) and *dPsn* (i.e. Drosophila presenilin (*PSEN*s)) (Li *et al.*, 2007), with roles in axonal transport and Notch signalling, respectively, are present in *D. melanogaster*. In normal flies, however, there is no formation of Aβ peptides because of the lack of β-secretase (BACE1) activity and sequence differences between APPL and APP at the positions that constitute Aβ (Rosen *et al.*, 1989).

A complete APP-processing *Drosophila* model was achieved by creating transgenic flies that carry constructs encoding both human APP and human β-site APP-cleaving enzyme 1 (BACE1, i.e. β-secretase). Human APP is cleaved by the transgenic human BACE1, and subsequently, by endogenous γ-secretase, thereby releasing the Aβ sequence. When specifically expressed in the eye, retinal deposition of Aβ plaques and age-dependent neurodegeneration were noted. Ubiquitous expression also led to shortened life span and defects in wing vein development. These APP-based models are useful to screen for genes, drugs or metabolites that modulate APP processing and have the potential to decrease Aβ-induced degeneration, as illustrated by dose-dependent increased survival rates and life span after supplementation of the food medium with a BACE1 inhibitor, and increased survival rates after treatment with a γ-secretase inhibitor (Greeve *et al.*, 2004).

In more simpler models, the Aβ sequence is fused downstream of a secretion signal peptide, which results in expression of secreted peptides in the fly nervous system or in the developing eye. These models have successfully shown progressive intracellular Aβ accumulation, extracellular Aβ plaque deposition and neurodegeneration accompanied by olfactory memory defects, reduced longevity and defective locomotor behaviour. Phenotypical alterations occur in an age- and dose-dependent manner with correlation between Aβ levels and neurodegeneration, as well as between propensity of Aβ to aggregate and disease severity (Finelli *et al.*, 2004; Iijima *et al.*, 2004; Crowther *et al.*, 2005). These secreted Aβ models are useful to study the toxicity of different Aβ species, and asses modifiers of Aβ metabolism and toxicity. The predictive validity of this fly model as a platform for drug discovery was verified by testing the therapeutic efficacy of MK-801, an inhibitor of the excitatory action of glutamate on the NMDA receptor and functionally related to memantine, the noncompetitive glutamate antagonist approved for symptomatic treatment of AD and effective in slowing AD progression (Crowther *et al.*, 2005). Predictive validity of the secreted Aβ fly models was further corroborated with administration of Congo red, which inhibits Aβ oligomerization and had earlier been shown to reduce neurodegeneration in a fly model of polyglutamine repeat disease and a mouse model of Huntington's disease (Crowther *et al.*, 2005).

Some of the phenotypes observed in fruit fly AD models are analogous to those clinically observed in human patients, such as learning and memory defects, and the presence of Aβ plaques and neuronal loss. In addition, other easy-to-score fly phenotypes are used as surrogate markers for neurodegeneration, such as reduced longevity, locomotor defects and rough-eye phenotypes (for review, see Giannakou and Crowther, 2010). While there appears to be good concordance between the phenotypes observed in these two complementary approaches to model Aβ toxicity in *Drosophila*, some differences in the subcellular localization of the peptide may exist. With normal processing of APP, Aβ is generated in an endosomal compartment and may subsequently be released to the extracellular space. In Aβ-expressing fly models, the peptide

may enter the secretory pathway from the endoplasmic reticulum (Crowther *et al.*, 2005).

Although APP and Aβ-expressing fly models mimic one crucial aspect of AD pathogenesis, the role of tau pathology is completely ignored. *Drosophila* tauopathy models developed up to date are (mutated) human tau-overexpression models. The neurotoxicity of (mutated) tau causes rough-eye and longevity phenotypes, with more pronounced deficits in models expressing mutated tau forms (Wittmann *et al.*, 2001). Intracellular inclusion resembling NFT can be provoked in wild-type tau expressing flies when glycogen synthase kinase 3β (GSK-3β) activity is increased (Jackson *et al.*, 2002), which is in accordance with the fact that hyperphosphorylation of tau induced its aggregation. As the Aβ-related models, *Drosophila* tauopathy models can be used in genetic screens for modifiers of tau pathology with rough-eye as the most frequently assessed phenotype (Shulman and Feany, 2003).

AD models in *C. elegans*

Caenorhabditis elegans, a free-living nematode of approximately 1 mm in length, has several characteristics that make it useful as a model organism. The nematodes are transparent, which allows study of embryonic development and gene expression in living animals under the microscope. They also have a very short life cycle (3 days) and a relatively short lifespan (3 weeks), which allow genetic dissection of the mechanisms that affect ageing and lifespan (Brenner, 1974; Byerly *et al.*, 1976). In addition, the mechanism of gene silencing by RNA interference has been discovered in *C. elegans* and has been developed into a potent reverse genetic tool (Fire *et al.*, 1998)

Several AD-related genes and pathways found in humans have orthologues in *C. elegans*. The nematode genome encodes three orthologues for PSEN1; (i) *sel-12*, which has been found in a screen for suppressors of the egg-laying defective phenotype in *lin-12* gain-of-function worms (Levitan and Greenwald, 1995), and which functions mostly during embryonic development to facilitate Notch/lin-12 signalling; (ii) *hop-1*, homolog of PSEN1 (Li and Greenwald, 1997), which is in fact more homologous to human PSEN2; and (iii) *spe-4*, which has no obvious human counterpart (Li and Greenwald, 1997). Three genes, *aph-1*, *pen-2* and *aph-2*, produce proteins that combined together form a functional γ-secretase complex. In addition, an orthologue of Aβ (*apl-1*), has been described in *C. elegans* (Daigle and Li, 1993). Similar to *Drosophila*, the APL-1 protein does not contain the Aβ sequence, neither does *C. elegans* display BACE1-like activity.

Three Aβ-expressing nematode models have been developed. When expressed in muscle cells, $A\beta_{1-42}$ induced the formation of amyloid-immunoreactive inclusions. A subset of these deposits also binds the Aβ-specific dye thioflavin S, indicating that amyloid fibrils are formed, comparable to human AD. In addition, paralysis of the nematodes occurred, thereby indicating a specific toxicity of Aβ to the muscle cells (Link, 1995). Transgenic nematodes expressing $A\beta_{1-42}$ in neurons, also develop amyloid deposits, but display only a very subtle phenotype (Link, 2006; Wu *et al.*, 2006). Interestingly, oligomeric species of Aβ were detected in these strains that might be similar to the neurotoxic Aβ-derived diffusible ligands (Wu *et al.*, 2006). These models provide important insight into toxicity of specific Aβ species, but do not allow screening of genetic or chemical modifiers of APP processing.

To create nematode tauopathy models, both wild-type and mutated human tau protein were expressed in *C. elegans* neurons (Kraemer *et al.*, 2003), inducing a progressive phenotype of defective motility known as 'uncoordinated phenotype', which was more apparent in the mutants. Interestingly, these transgenic lines also exhibit hyperphosphorylation of tau (Kraemer *et al.*, 2003), which is linked to GSK-3β activation. Future genome-wide screens will show what modifier genes are linked to the disease process, and represent diagnostic or even therapeutic targets.

AD models in *D. rerio*

Danio rerio, or zebrafish, is a small (3–5 cm) fresh water tropical fish, which served a premiere model organism to study vertebrate development. *Danio rerio* is very well suited for large-scale forward genetic screens in which phenotypic defects are identified before the identification of the gene causing these defects, due to its large quantity of eggs, short generation time and the external development of the transparent embryos (Amsterdam and Hopkins, 2006). Importantly, orthologues of the genes involved in familial AD have been identified in zebrafish as well, including PSEN1 (*zf-ps1*; Leimer *et al.*, 1999; Nornes *et al.*, 2003), PSEN2 (*pre2*; Groth *et al.*, 2002; Nornes *et al.*, 2003) and APP (*appa*, *appb*; Musa *et al.*, 2001). Zebrafish reverse genetics is slowly catching up with *Drosophila* and/or mouse, as the techniques to perform gene-specific knock downs, target-selected mutagenesis and transgenesis in zebrafish are quickly developing (for review, see Willemsen *et al.*, 2010). Morpholino antisense oligonucleotide injection is the most widely used technique for transient gene knockdown in zebrafish (Bill *et al.*, 2009), although other strategies to establish stable knockout lines, including chemical mutagenesis using alkylating agents (e.g. *N*-ethyl-*N*-nitrosourea) for targeted induced local lesions in genomes (TILLING) (Moens *et al.*, 2008), and zinc finger nuclease (ZFN)-mediated mutagenesis (Miller *et al.*, 2007) are winning ground. At present, no TILLING or ZFN-stable mutant zebrafish lines with knockout mutations in orthologues of human neurodegenerative disease genes have been published yet. Morpholino-based zebrafish models have indicated that PSEN enhancer (*pen-2*), part of the zebrafish γ-secretase complex, plays an important role in promoting neuronal cell survival and protecting from apoptosis (Campbell *et al.*, 2006). Morpholino-based interference with splicing of PSEN transcripts affects multiple PSEN functions, most often linked to altered Notch signalling. Phenotypical alterations, including hydrocephalus and decreased pigmentation have been noted (Nornes *et al.*, 2008).

Methods for generating a transgenic zebrafish are pseudotyped retrovirus infection, transposons, transfection of sperm nuclei and DNA microinjection, with the latter being the most frequently used method for generating transgenic lines expressing a gene of interest (for review, see Willemsen *et al.*, 2010). A first step to study the effect of

mutant human APP expression on the development of AD was achieved by the generation of transgenic zebrafish expressing enhanced green fluorescent protein (EGFP) under control of zebrafish *app* gene regulatory elements. EGFP expression was found to be present in subregions of brain and spinal cord, as well as in vasculature (Lee and Cole, 2007). The logical next step is to apply this vector to clone a PCR product containing mutant human APP. Transgenic zebrafish tauopathy models are already available. Transient and stable transgenic zebrafish expressing human (mutated) tau showed tau accumulations in neuronal cell bodies and proximal axons resembling NFT (Tomasiewicz *et al.*, 2002; Bai *et al.*, 2007; Paquet *et al.*, 2009). Zebrafish kinases are sufficiently conserved with respect to their human orthologues thereby allowing the screening of therapeutic leads focussing on kinase inhibition.

Zebrafish is an ideal vertebrate for primary toxicity studies in whole animals because of their cost-effectiveness, the ease of drug delivery and their high sensitivity to toxins. Applicability of zebrafish in the drug discovery pipeline for dementia was substantiated by several recent studies. GSK-3β is abnormally up-regulated in several diseases including AD, where it has been regarded as a potential drug target. Inhibition of GSK-3β in zebrafish results in a headless embryo. Using this phenotype, chemical libraries were successfully screened to identify GSK-3β inhibiting compounds as potential therapeutic candidates for GSK-3β-related diseases (Zhong *et al.*, 2009; Zou *et al.*, 2010). Inhibition of γ-secretase presents a direct target for lowering Aβ production in the brain as a therapy for AD. However, γ-secretase is known to process multiple substrates in addition to APP, most notably Notch, which has limited clinical development of inhibitors targeting this enzyme. APP-selective inhibitors would be preferable to non-selective inhibitors from a safety perspective for AD therapy. Recently, a high-throughput screening method based on phenotypic differentiation between pan and APP-specific γ-secretase inhibitors was established in zebrafish (Arslanova *et al.*, 2010).

Transgenic rodent models for AD

Modelling of AD in transgenic mice became reality in the mid-1990s with the development of the PDAPP model (Games *et al.*, 1995), followed in subsequent years by the Tg2576 (Hsiao *et al.*, 1996) and APP23 (Stürchler-Pierrat *et al.*, 1997) mouse models, currently the most widely used amyloidosis models in AD-related research. The PDAPP model expresses human APP carrying the Indiana familial AD mutation (V717F) driven by the platelet-derived growth factor-β promoter, whereas both the Tg2576 and APP23 model express human APP with the Swedish mutation (K670N/M671L) driven by the hamster prion protein and murine Thy-1 promoter respectively. All three models support the amyloid cascade hypothesis; they display progressive Aβ deposition in both diffuse and neuritic plaques, cerebral amyloid angiopathy, astrocytosis, microgliosis, (limited) hippocampal atrophy, synaptic and neurotransmitter alterations, and cognitive and behavioural deficits, relevant to the human AD clinical and neuropathological profile (for review, see Van Dam *et al.*, 2005; Basak and Holtzman, 2010; Deacon, 2010; Van Dam and De Deyn, 2010). APP-based models confirm the central role of APP and Aβ in the Alzheimer disease process, allow target identification, and subsequently, the preclinical evaluation of various symptomatic and disease-modifying drugs, mainly targeting the amyloid cascade. The major caveat of these models, however, is the lack of NFT formation, although hyperphosphorylated tau may be present.

The discovery of early-onset AD mutations in the PSEN genes, gave rise to the development of PSEN1 and PSEN2 transgenic mouse models. Despite an increased $A\beta_{1-42}/A\beta_{1-40}$ ratio in some of these models, no plaque pathology and few cognitive and behavioural abnormalities are present. Like APP-based models, they lack NFT development. They have mainly served the basis for the development of double transgenic APP/PSEN mice, which display an increased $A\beta_{1-42}/A\beta_{1-40}$ ratio and accelerated Aβ pathology compared to the single APP model they are based on, thereby supporting the modifying role of PSEN. In addition, these APP/PSEN mice exhibit neuronal loss, amyloid-associated inflammation, cognitive decline and BPSD-like behavioural alterations (McGowan *et al.*, 2006; Van Dam and De Deyn, 2006). The major drawback of all above-mentioned transgenic mouse models, that is, the lack of NFT formation, was partially overcome by the development of (mutated) human tau mice, and the subsequent crossing of tau and APP models, latter featuring enhanced amyloid deposition accompanied by tau phosphorylation, NFT-like formation and overt neuronal loss, thereby supporting the amyloid cascade hypothesis stating that Aβ pathology mediates tau pathology (Götz *et al.*, 2004; Ribé *et al.*, 2005). Unfortunately, there is no co-localization of plaques and NFT in AD-relevant brain regions, for example, hippocampus and cortex, in APP/tau mice. This shortcoming was counterbalanced with the development of the triple transgenic (3 × Tg) mouse (Oddo *et al.*, 2003a,b). Rather than crossing independent mutant mouse lines, two transgenic constructs (mutant APP and tau) were microinjected into single-cell embryos from homozygous mutant PSEN1 mice, thereby preventing segregation of APP and tau genes in subsequent generations. In accordance with the amyloid cascade theory, these 3 × Tg mice develop Aβ plaques prior to NFT pathology with a temporal and spatial profile equivalent to AD, in addition to inflammation, synaptic dysfunction and cognitive decline (for review, see Sy *et al.*, 2010).

Single tau-knockout and (mutated) tau-transgenic models allow further exploration of tau-related neurodegenerative mechanisms in AD and related dementias. Tau-knockout mice appear physically normal, are able to reproduce, and do not display any change in central or peripheral nervous systems, indicating that tau deficiency is likely compensated by other microtubule-associated proteins. With the discovery of mutations on microtubule-associated protein tau in frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17), numerous transgenic models using these mutations were developed, allowing for the development of tau pathology characterized by tau aggregation and neurofibrillary degeneration. They display various phenotypes, with the most prominent one being motor deficits, but also memory impairment, in addition to neurofibrillary (or neuronal-like) tangles or gliofibrillary tangles (for review, see Sergeant and Buée, 2010).

More recently the use of viral vector gene transfer technology has allowed the development of 'somatic transgenic' models, whereby genes putatively involved in AD pathogenesis can be selectively overexpressed in specific AD-relevant brain regions (Hong *et al.*, 2006; Lawlor *et al.*, 2007). Although this promising strategy has been shown to result in the development of both cognitive deficits and Aβ deposits in treated animals, these genetic models require further characterization to show reproducible development of behavioural deficits and neuropathology prior to their widespread adoption as a reliable and useful model of AD.

Given the boost transgenesis and gene targeting techniques have given to the development of valid phenocopies of the human condition the mouse models included in this review are not exhaustive but rather form a representative sample of the available models. For an updated overview of available genetically modified models, we refer the readers to specialized websites, as, for example, http://www.alzforum.org, which also pays attention to models based on late-onset AD genetic risk factors, such as apolipoprotein E and sortilin-related receptor, as well as transgenic lines based on other aetiological hypotheses, for example, mutated human α-synuclein models, human cyclooxygenase-2 overexpression models and anti-nerve growth factor mice.

The time and expense required to make genetically altered mice is considerable, and the importance of this investment is amplified by the long time course of most studies of dementia. Investigators need to be able to make informed choices about the different strategies for transgenics and gene targeting in order to minimize unwanted variation, and to maximize fidelity to the disease. In recent years, the development of large genomic fragments stably cloned in well-characterized libraries, and the ability to make transgenic mice from these clones in inbred strains have greatly increased the power of the transgenic mouse. In addition, new embryonic C57BL/6 cell lines have become widely adopted for gene targeting, allowing knockins, knockouts and conditional alleles to be established much more expeditiously on the standard C57BL/6 background (Conlon, 2010).

The generation of transgenic rodent research models that develop some of the pathological hallmarks of AD has given a sizable boost to drug discovery efforts, and has also raised many intriguing questions about the underlying disease process. However, one should never neglect the potential dangers of uncritical extrapolating from mouse/rat to humans. The fact that at the moment no animal model recapitulates all aspects of human AD reflects the limitations of using a rodent system to model a human condition that takes decades to develop and mainly involves higher cognitive functions.

The merit of animal models in AD drug development

Treatment goals change with disease severity. In mild to moderate AD, the objective is to improve or maintain baseline performance with disease-modifying drugs targeting central aetiological processes. In more progressed cases displaying cognitive and behavioural deficits which impair the wellbeing of patients and caregivers, treatment is intended to slow the rate of decline. These symptomatic therapeutics, however, do not address the cause of the disease. If predisposition for AD will become predictable – for example, based on biomarker profiling in patients with mild cognitive impairment – the development of preventive therapies will be mandatory.

Treatment strategies in AD can be based on the following approaches: (i) neurotransmitter-focussed approach; (ii) prevention based on epidemiological data; or (iii) neuropathological hallmark-based approach. Either of these approaches can be achieved via compounds with quite diverse modes of action. Table 1 provides a nonexhaustive summary of these different approaches or modes of action based on compounds currently under clinical investigation. For each compound, a representative example of a preclinical study supporting the clinical application of that compound is provided, thereby illustrating the merit of animal models in the drug discovery pipeline for dementia.

Table 1 focuses on the major pathophysiological pathways underlying AD and their link to cognitive decline. Besides focusing on cognitive symptomatology, treatment of AD should also include managing BPSD and related behavioural alterations, especially given their major impact on patients, caretakers and society at large, in addition to the fact that atypical antipsychotics or classic neuroleptics display only modest effect size and are associated with some potentially major side effects. A variety of pharmacological agents has been evaluated for the treatment of BPSD, including cholinomimetics, anxiolytics, anticonvulsants, antidepressants, hormonal preparations and antipsychotic (neuroleptic) drugs. With the exception of atypical antipsychotics, clinical evidence is rather anecdotal or based on open-label clinical trials for most of these substances. In addition, although the categories of BPSD are superficially similar to symptoms in, for example, the psychosis of schizophrenia or depression in major affective disorders, the specific nature of these symptoms in AD and related disorders may be different based on AD-specific neurochemical alterations and the interaction with psychological, cognitive and functional factors (De Deyn and Van Dam, 2010b). Preclinical evaluation of (non)pharmacological treatment strategies will undoubtedly contribute to a better clinical management of BPSD. Prerequisite is of course the availability of valid animal models of BPSD, and a need for shifted attention from cognitive disturbances to BPSD-related alterations in animal models of dementia. Certain AD models have already been shown to exhibit both face (Vloeberghs *et al.*, 2004, 2006; Van Dam *et al.*, 2005; Van Dam and De Deyn, 2006) and predictive validity (Vloeberghs *et al.*, 2008) with regards to BPSD-like behaviours.

However, the insuperable species barrier between AD model and patient should prevent uncritical and premature extrapolation of animal model findings to the human condition. In general, high-quality and conscientious research aiming at the validation of a new model or testing a new compound requires thorough standardization of procedures, good knowledge of strains, compounds and paradigm characteristics, and skilled personnel. Keeping in mind basic metabolic, physiological and anatomical differences between humans and other species, it is clear that a 'pluri-species' approach increases the reliability of extrapolation from

Table 1

The merit of animal models in AD drug development

	Compound	Current or recent clinical phase(s)	Preclinical research supporting clinical trials			
			Reference	Animal model	Treatment scheme	Study outcome
Neurotransmitter-based approach						
Cholinesterase inhibitors						
↑ Efficacy ↓ Side effects	Rivastigmine transdermal patch	Phase III–IV	Tse and Laplanche (1998)	Minipig (*Sus scrofa*)	Single i.v., p.o. or transdermal (patch) administration of 18 or 54 mg radioactively labelled rivastigmine	20–40 × higher bioavailability in transdermal versus oral route
Disease-modification	Donepezil	Phase I–II	Meunier *et al.* (2006)	Mouse i.c.v. aggregated $A\beta_{25-35}$ infusion model causing learning deficits on day 7–8	Dose range 0.12–1 $mg \cdot kg^{-1}$: a) i.p. injection 20 min before behavioural testing (antiamnestic) b) i.p. injection 20 min before $A\beta_{25-35}$ infusion (neuroprotective) c) i.p. injection 24 h after $A\beta_{25-35}$ infusion and daily until behavioural testing (neuroprotective)	a) dose-dependent reversal of alternation deficit in Y maze and passive avoidance deficit b) dose-dependent prevention of alternation deficit in Y maze and passive avoidance deficit, and lipid peroxidation c) dose-dependent prevention of alternation deficit in Y maze and passive avoidance deficit, and lipid peroxidation
GLU receptor antagonism						
AMPAkines	CX717	Phase II	Hampson *et al.* (2009)	Adult male rhesus monkeys (*Macaca mulatta*)	i.v. administration of 0.3 to 1.5 $mg \cdot kg^{-1}$ 10 min prior to isotope injection for PET imaging in sleep-deprived monkeys	Reversal of delayed-matching-to-sample task deficit
Disease-modification	Memantine	Phase III	Martinez-Coria *et al.* (2010)	3 × Tg AD mouse model ($hAPP_{695}$Swedish; human tau P301L; human PS1 M146L)	a) 3 month p.o. treatment starting at age 6 months (30 $mg \cdot kg^{-1} \cdot day^{-1}$), mild pathology group b) 3 month p.o. treatment starting at age 9 months (30 $mg \cdot kg^{-1} \cdot day^{-1}$), moderate pathology group c) 3 month p.o. treatment starting at age 15 months (30 $mg \cdot kg^{-1} \cdot day^{-1}$), severe pathology group	a) rescue of visual-spatial learning deficit; reduction of learning deficit in novel-object recognition task; improvement of deficit in short-term and long-term passive avoidance learning; ↓ soluble $A\beta_{1-40}$ and $A\beta_{1-42}$ b) rescue of visual-spatial learning deficit; improvement of deficit in long-term passive avoidance learning; ↓ tau levels; ↓ phospho-tau levels; ↓ GSK-3β levels; ↑ soluble $A\beta_{1-42}$ c) reduction of visual-spatial learning deficit; ↓ tau levels; ↓ phospho-tau levels; ↓ GSK-3β levels; ↑ soluble $A\beta_{1-42}$; ↓↓ insoluble $A\beta_{1-40}$ and $A\beta_{1-42}$; ↓ plaque load; ↓ Aβ oligomers
Prevention strategies based on epidemiological data						
NSAIDs	Ibuprofen	Phase I	Van Dam *et al.* (2010)	APP23 mouse model ($hAPP_{751}$Swedish)	2 month treatment (50 $mg \cdot kg^{-1} \cdot day^{-1}$) starting at age 6 weeks, followed by 3 weeks wash-out period	Prevention of development of visual-spatial learning deficit
Statins	Simvastatin	Phase II–III–IV	Boimel *et al.* (2009)	Double mutant [K257T/P301S] tau tg mouse	a) 1 month treatment in normocholesterolemic aged mice b) 8 month treatment in young mice	a) ↓ NFT and lectin-positive microglia b) ↓ NFT and improved T-maze performance
Oestrogen/HRT/SERMs	Raloxifene	Phase III	Wu *et al.* (1999)	6-month-old ovariectomized rats exhibiting reduced hippocampal ChAT activity	3 or 10 day s.c. treatment 3 weeks post-surgery (3 $mg \cdot kg^{-1} \cdot day^{-1}$)	Recovery hippocampal ChAT activity
Antioxidants	α-Tocopherol	Phase III	Nishida *et al.* (2009)	$Ttpa^{-/-}$APPsw	a) 18-month-old α-tocopherol transfer protein knockout mice crossed with Tg2576 model b) α-tocopherol supplementation (750 $mg \cdot kg^{-1}$)	a) ↑ Aβ brain levels based on ↓ Aβ clearance b) partial ↓ Aβ accumulation
Neuropathological hallmark-based approach						
Targeting the amyloid cascade						
1. Decrease Aβ formation						
Secretase inhibitors	Semagacestat	Phase III	Ness *et al.* (2004)	PDAPP mouse model ($hAPP_{695}$Indiana)	5 month p.o. treatment starting at age 5 months (3, 10 or 30 $mg \cdot kg^{-1} \cdot day^{-1}$)	Dose-dependent ↓ plaque load; ↓ cortical $A\beta_{1-40}$ and $A\beta_{1-42}$ levels; ↓ hippocampal $A\beta_{1-42}$ levels; ↓ plasma total Aβ; ↑ cortical and hippocampal C99 levels
Antimonomer immunotherapy	CAD-106	Phase II	Staufenbiel *et al.* (2006)	APP23 mouse model ($hAPP_{751}$Swedish)	10 month s.c. treatment with monthly administration of 25 mg starting at age 3–4 months	↓ Plaque load; ↓ cortical $A\beta_{1-42}$ levels

Table 1

Continued

	Compound	Current or recent clinical phase(s)	Preclinical research supporting clinical trials: Reference	Animal model	Treatment scheme	Study outcome
2. Increase $A\beta_{1-40}/A\beta_{1-42}$ ratio						
γ-Secretase modulators	CHF5074	Phase I	Imbimbo *et al.* (2007)	Tg2576 mouse model ($hAPP_{695}$Swedish)	9 month p.o. treatment (375 p.p.m. in diet) starting at age 6 months	↑ Hippocampal neurogenesis potential; ↓ cortical synaptophysin intensity; ↓ cortical plaque burden; reversal contextual memory deficit;
3. Inhibit Aβ aggregation						
Aβ inhibitors	Scyllo-inositol	Phase II	McLaurin *et al.* (2006)	TgCRND8 mouse model ($hAPP_{695}$Swedish-Indiana)	a) p.o. treatment starting at age 6 weeks till age 4 or 6 months (preventive trial; 30 $mg \cdot kg^{-1} \cdot day^{-1}$)	a) prevention of visual-spatial learning deficit; ↓ brain Aβ levels; ↓ plaque load; ↓ aggregation into oligomeric Aβ; ↓ synaptic loss; ↓ inflammation; ↓ mortality
					b) 1 month p.o. treatment starting at age 5 months (symptomatic trial; 30 $mg \cdot kg^{-1} \cdot day^{-1}$)	b) improvement of visual-spatial learning deficit; ↓ aggregation into oligomeric Aβ; ↓ brain Aβ levels; ↓ plaque load; ↓ aggregation into oligomeric Aβ
					c) p.o. treatment starting at age 6 weeks till age 4 month (0.3 to 30 $mg \cdot kg^{-1} \cdot day^{-1}$)	c) dose-dependent improvement of visual-spatial learning deficit; ↓ plaque load; ↓ brain Aβ oligomers
Aβ intercalators	Homotaurine = tramiprosate = 3APS	Phase III	Gervais *et al.* (2007)	TgCRND8 mouse model ($hAPP_{695}$Swedish-Indiana) on two different backgrounds resulting in four to five times higher Aβ levels in mice for experiment b	a) 8 week s.c. treatment starting at age 9 weeks (30 or 100 $mg \cdot kg^{-1} \cdot day^{-1}$)	a) ↓ Aβ plaque load; ↓ $A\beta_{1-40}$ and $A\beta_{1-42}$ plasma levels
					b) 9 week s.c. treatment starting at age 9 weeks (500 $mg \cdot kg^{-1} \cdot day^{-1}$) resulting in same Aβ/homotaurine in both groups	b) ↓ soluble and insoluble $A\beta_{1-40}$ and $A\beta_{1-42}$ brain levels
Metal-protein attenuating compound	PBT-2	Phase II	Adlard *et al.* (2008)	a) APP/PS1 mouse model expressing human APP with Swedish mutation and human PS1-dE9 deletion	a1) 11 or 35 day p.o. treatment at age 8 months (10 or 30 $mg \cdot kg^{-1} \cdot day^{-1}$)	a1) improvement of visual-spatial learning deficit; ↓ brain insoluble Aβ levels; ↓ brain oligomeric Aβ levels; ↓ brain phosphorylated tau levels; ↑ brain synaptophysin levels
					a2) single p.o. dose (30 $mg \cdot kg^{-1}$) prior to microdialysis at age 22 months	a2) ↓ interstitial Aβ levels
					a3) single p.o. dose (30 $mg \cdot kg^{-1}$) prior to microdialysis at age 3–4 months	a3) faster ↓ interstitial Aβ levels
				b) Tg2576 mouse model ($hAPP_{695}$Swedish)	b1) 11 day p.o. treatment at age 13.5 months (30 $mg \cdot kg^{-1} \cdot day^{-1}$)	b1) improvement of visual-spatial learning deficit; ↓ brain insoluble Aβ levels; ↓ brain total tau levels; ↑ brain synaptophysin levels
					b2) single p.o. dose (30 $mg \cdot kg^{-1}$) prior to microdialysis at age 18 months	b2) ↓ interstitial Aβ levels
4. Anti-oligomer agents						
Small molecule inhibitors	Curcumin	Phase II	Ahmed *et al.* (2010)	Rat bilateral intrahippocampal amyloid infusion model ($A\beta_{1-40}$ + ibotenic acid)	a) 5 day i.p. treatment starting on day 2 post-infusion (3, 10 or 30 $mg \cdot kg^{-1}$)	a) (partial) recovery of synaptic plasticity-related gene expression levels
					b) 20 day i.p. treatment starting on day 2 post-infusion (3, 10 or 30 $mg \cdot kg^{-1}$)	b) improvement of visual-spatial learning
Anti-oligomer immunotherapy	IVIg	Phase III	Magga *et al.* (2010)	APP/PS1 mouse model expressing human APP with Swedish mutation and human PS1-dE9 deletion	i.v. administration of 1.0 $g \cdot kg^{-1}$ of 10% IVIg starting at age 4 months with twice weekly injections for 1–3 weeks (short-term) or long-term study with weekly injection for 14 weeks	IVIg is able to penetrate the blood-brain barrier; intensity of IVIG immunostaining increased with duration of treatment; highest reactivity in hippocampus; IVIg selectively binds to Aβ deposits in co-localization with microglia
Targeting tau pathology						
Inhibition GSK-3β linked to tau hyperphosphorylation	Lithium	Phase II	Leroy *et al.* (2010)	Tg30tau mice expressing human 4R1N double-mutant tau (P301S and G272V)	a) 8 month treatment starting at age 3 months with lithium carbonate supplemented in food (2.4 $g \cdot kg^{-1}$ chow)	a) no effect on NFT development
					b) 1 month treatment starting at age 9 months with daily gavage (350 $mg \cdot kg^{-1}$)	b) ↓ tau phosphorylation; ↓ aggregated tau; ↓ NFT; no rescue of motor and working memory deficits

Aβ, amyloid-β; AD, Alzheimer's disease; AMPA, α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate; APP, amyloid precursor protein; ChAT, choline O-acetyltransferase; GSK-3β; glycogen synthase kinase 3β; HRT, hormone-replacement therapy; IVIg, intravenous immunoglobulin; NFT, neurofibrillary tangles; NSAIDs, nonsteroidal anti-inflammatory agents; PBT-2, second-generation 8-hydroxy quinoline analogue; PSEN1, presenilin1; SERMs, Selective Oestrogen Receptor Modulators; tg, transgenic.

animal models to humans (Van Dam and De Deyn, 2006). Too often it is taken for granted that a very good correlation between the effect of drugs in so-called 'validated' animal models and human clinical trials exists. A possible failure of a drug in clinical settings is often interpreted as the failure of the basis hypotheses on which the target for the drug was selected, rather than the failure of the animal models in which the drug was active. Several essential neurochemical differences between, for example, rodents and men might hinder a successful clinical development of a candidate drug; for example, (i) the different pharmacology of the same drug for rodent versus human target subtypes; (ii) the different wiring of specific neurotransmitter circuits in rodent versus human brain; and (iii) the difference in drug metabolism which makes it difficult to simulate the human drug exposure. More emphasis on good-quality translational studies, more pre-competitive information sharing and the implementation of multi-target pharmacology strategies in preclinical settings, may allow more reliable translation of preclinical observations to the clinical setting and significantly increase the success rate of the CNS drug discovery pipeline (for review; see Geerts, 2009; 2010).

Conclusion

The conclusions drawn from animal models largely depend on the validity of the model in representing the human condition. Validation of a newly developed model mostly comprises assessment of face, construct, predictive and aetiological validity. The more levels of validity a model satisfies, the greater its value, utility and relevance to the human condition. The perfect model would account for aetiology, symptomatology, treatment and physiological basis. Animal models in general do not meet all of these criteria, but nevertheless, all models described in this review may serve a pivotal role in the drug discovery and development pipeline of dementia to increase our knowledge of pathophysiological mechanisms underlying dementia and predict clinical activity of newly developed treatment strategies. Certainly given the fact that the evaluation of preventive or disease-modifying efficacy is not easily accomplished in a clinical setting. Animal models have the advantages of a rapid development of symptoms and/or pathology, availability of potentially large groups of subjects, accessibility to early-stage CNS changes and the possibility of time-linked observations.

Unmet needs in the current AD pharmaceutical market are disease-modification, improved efficacy, fewer side effects, reduction of the number of treatment unresponsive patients and patient compliance. Translational research based on target discovery and evaluation in animal models will undoubtedly aid in alleviating at least some of these shortcomings of the presently marketed drugs in the years to come.

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Conflict of interest

The authors declare that no conflict of interest exists with regard to the material discussed in this review paper.

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