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ANIMAL MODEL STUDIES OF ALZHEIMER'S DISEASE

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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disorder that nowadays affects more than 40 million people worldwide and it is predicted to exponentially increase in the coming decades. Because no curative treatment exists, research on the pathophysiology of the disease, as well as the testing of new drugs, are mandatory. For these purposes, Animal disease models are considered important in the development of drugs for Alzheimer's disease. Experimental models of Alzheimer's disease (AD) are critical to gaining a better understanding of pathogenesis and to assess the potential of novel therapeutic approaches. The most commonly used experimental animal models are transgenic mice that overexpress human genes associated with familial AD (FAD) that result in the formation of amyloid plaques. However, AD is defined by the presence and interplay of both amyloid plaques and neurofibrillary tangle pathology. This review takes a tour through several aspects of mouse models of AD, such as the generation of transgenic models, the relevance of the promoter driving the expression of the transgenes, and the concrete transgenes used to simulate AD pathophysiology. Then transgenic mouse lines harboring mutated human genes at several loci such as APP, PSEN1, APOE 4 and ob (leptin) are reviewed.

KEYWORD: Alzheimer's disease, Animal models, ApoE, APP.

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease affecting an estimated 5.4 million people globally, mainly the elderly. There is currently no cure for AD, but some symptomatic treatments are available. The disease is characterized by its hallmark histopathological findings of extracellular b-amyloid (Ab) plaques and intracellular neurofibrillary tangles of tau and by neuronal and synaptic loss in brain regions involved in learning and memory processes.[1] The interest in finding a cure or prevention for AD is understandably great. Proper animal models of human AD are considered desirable if not essential in this process and much research effort has been put into that effect. As no perfect model exists, the question becomes whether 'the best models available' are good enough. What exactly can be inferred from the results and what not? Or, differently put, how do they contribute to our understanding and decisionmaking. The objective, therefore, of this briefreview is to discuss the potential role of the current animal disease models for AD in drug development. Experimental models are essential to further understand AD pathogenesis and to perform preclinical testing of novel therapeutics. To date, the vast majority of experimental models are animal models, almost exclusively consisting of transgenic mice that express human genes that result in the formation of amyloid plaques (by expression of human *APP* alone or in combination with human *PSEN1*) and neurofibrillary tangles (by expression of human *MAPT*).[2] Other models have included invertebrate animals such as *Drosophila melanogaster* and *Caenorhabditis elegans*, as well as vertebrates such as zebrafish; however, given these models' greater distance from human physiology they are less extensively used.[3] Since the development of the first transgenic mouse model with substantial amyloid plaque burden in 1995.[4] there has been a proliferation of new transgenic models, each with a different phenotype of AD-associated pathology.[2,3]

The development of transgenic models offered much promise about the understanding of AD pathogenesis, allowing questions to be answered that were previously impossible to examine in humans. Accordingly, the number of studies using AD transgenic models rapidly increased. However, questions have been increasingly raised about the validity of relying on the available transgenic models, particularly in light of the very high failure rate of clinical trials of AD therapeutics (of ~99.6%), many of which were successful in preclinical testing using these animal models.[5] These results highlight the often overlooked fact that these animal models do not have AD, they only recapitulate specific pathological features, most commonly in a non-physiological manner designed to allow for efficient

experiments. The majority of animal models (both transgenic and physiological models) develop only the amyloid accumulation that defines AD. This often (but not always) results in specific memory-associated cognitive impairments. Importantly however, these models often lack the widespread presence of other pathological features that define AD including neuronal loss and most importantly, neurofibrillary tangle development. This lack of additional AD associated pathology could at least partly account for the lack of translation between preclinical and clinical trials,[6] although there have also been a few clinical trial failures for approaches not initially tested in transgenic models.[7] As such, it is important to have a good understanding of the exact neuropathology present in each model, particularly regarding how well this correlates with human AD, so that results can be interpreted more accurately and the likelihood of translation to human studies can increase. Results generated from experimental models can be exceptionally informative about specific aspects of AD if researchers are aware of the limitations associated with each model. Therefore, in this review we will discuss our understanding of the pathogenesis of AD and the features and limitations of the major experimental models of AD that reflect this pathology, including transgenic mice, transgenic rats, physiological models of sporadic AD, invertebrate animals and *in vitro* human cell culture models.

Animal models of Alzheimer's disease

The aetiology of AD is unknown, but there is still a general consensus in favour of the 'amyloid hypothesis', even if it has been questioned. A wide range of animal models have been developed to mimic the human context of the disease for the purpose of developing therapeutics or disease modifying agents. In fact, in most of the animal models the first goal is to simulate the neuropathological findings of AD followed by the correlation of cognitive function without knowing whether the neuropathological agents have similar biological consequences in humans and in animal models. It is beyond the scope of this review to discuss all the different models. Here we will briefly summarize some potential animal models.

Transgenic mice expressing tau

Wild-type mouse tau does not develop neurofibrillary tangles. This is likely due to the sequence differences between mouse and human tau (share only 88% sequence homology) and the fact that adult mice only express 4R isoforms, not a mixture of 3R and 4R isoforms that are present in humans. Importantly, expression of all 6 isoforms of human tau only results in tangle formation in mice lacking endogenous tau, showing that endogenous mouse tau inhibits the aggregation of human tau.[2] In contrast, NFTs readily form in transgenic mice that express human tau containing mutations associated with FTLD; the most commonly used models being those that express 4R tau with P301L or P301S mutations.[8] These mice develop NFTs, neurodegeneration, atrophy and motor deficits. The necessity of these mutations for NFT development is an obvious limitation of these transgenic mouse models, as these mutations are not associated with AD in humans and the development of mutated tau may influence its toxicity or interaction with Aβ in a way that is not representative of what occurs in AD. Furthermore, over-expression of mutated tau results in significant motor deficits that are not seen in AD and interfere with cognitive testing.[9,10]

Transgenic mouse models

The vast majority of animal models used in AD research are transgenic mice. Wild-type mouse APP (695 isoform) has 97% sequence homology with human APP. Importantly, sequence differences between mice and humans include 3 amino acids within the Aβ sequence (R5G, Y10F and H13R).[13] These differences impair Aβ aggregation and prevent the formation of amyloid plaques in wild-type mice. Therefore, expression of human APP is necessary for the formation of amyloid plaques in mice. Initial transgenic models expressed wild-type human APP in mice, however while these transgenic mice had increased Aβ production, they failed to consistently show extensive AD associated neuropathology.[2] In contrast, expression of human APP containing mutations associated with FAD resulted in consistent plaque pathology and varying amounts of consequent downstream AD-associated pathological features. Multiple transgenic strains have been generated and the exact phenotype for each transgenic strain strongly depends on the FAD mutation, the promoter used and the background mouse strain. Since the vast majority of AD transgenic models have pathology that is dependent on the expression of FAD mutations and most AD clinical trials are conducted in sAD patients, in whom AD pathogenesis has significant distinctions from FAD, this represents one stumbling block for the translatability of success in these models. The neuropathology and associated cognitive impairments for the transgenic mouse strains most commonly used in AD research. It should be noted that the degree to which each model is characterized in terms of the sensitivity of the cognitive testing performed, amount of tau related pathology and the extent of synaptic pathology (demonstrated by ultrastructural studies and/or electrophysiology) greatly varies, making absolute comparisons between models difficult.[11,12]

Transgenic mice with both plaques and tangles

A limited number of studies have reported the development of animal models that display both plaques and tangles.[14-18] These models rely on concurrent expression of mutated forms of APP, MAPT and occasionally also PSEN1 or PSEN2 to drive plaque and tangle formation in the same model. However, the consistent and abundant expression of both plaques and tangles has proven troublesome, and development of both plaques and tangles is typically not observed until old age in these models. Of all of the models reported,

only the 3xTg mouse model has been widely used in AD studies and is considered the most complete transgenic mouse model of AD pathology available.[18] 3xTg mice first develop intraneuronal Aβ at 3–4 months, followed by plaque development at approximately 6 months in the cortex and hippocampus. NFTs form at approximately 12 months, initially in CA1 and then in the cortex; however, they are much less extensive compared to AD tissue. Mice also have minor, localized neurodegeneration, evidence of synaptic impairment and cognitive deficits from 6 months. However, 3xTg mice are still limited by the production of mutated Aβ and tau that is not representative of that in sAD and is highly over-expressed in a non-physiological manner. Furthermore, widespread presence of plaques and tangles are typically not observed until old age in these mice and even then the pathology is less then typically seen in AD.

Transgenic mice expressing human APP and PSEN1 with FAD mutations

The initial transgenic mouse models developed expressed APP with an individual FAD mutation. The first example of such models was the PDAPP mouse, which expressed human APP with the Indiana mutation (APP^{V717F}) driven by the PDGF-β promoter, which caused dramatic over-expression (>10-fold) of APP.[19] This resulted in pathology associated with human AD including plaque formation in the cortex and hippocampus, CAA, gliosis, synaptic impairment and cognitive impairment). The generation of the Tg2576 mouse model closely followed. Tg2576 mice expressed human APP with the double Swedish mutation ($APP^{K670N/M671L}$) driven by the PrP promoter, which also resulted in significant over-expression of APP (>5-fold).[20] Tg2576 mice developed plaques in the frontal, temporal and entorhinal cortices, hippocampus and cerebellum. APP23 mice also express $APP^{K670N/M671L}$; these mice contrast with Tg2576 mice through expression of the APP751 isoform driven by the Thy1 promoter (in comparison to the APP695 isoform driven by the PrP promoter expressed in Tg2576 mice).[21] APP23 mice have more pronounced CAA, immediately form compact plaques in comparison to the predominantly diffuse plaques found in Tg2576 mice, and have localized neurodegeneration that is not seen in the Tg2576 mice.[22] These differences are despite similar expression levels of the APP transgene, showing that the promoter and APP isoform can greatly influence the type and time-course of AD associated neuropathology in transgenic models.

Unique transgenic mouse models useful for AD research

A number of transgenic mouse models have been developed that are particularly good at replicating a specific pathological feature of AD. For example, the Tg-SwDI transgenic mouse model is a particularly good model of CAA.[23] This model expresses Swedish ($APP^{K670N/M671L}$), Dutch (APP^{E693Q}) and Iowa (APP^{D694N}) APP FAD mutations. The Dutch and Iowa mutations are associated with hereditary cerebral hemorrhage with amyloidosis (HCHWA), where there is extensive CAA with more limited plaque pathology.[24] Tg-SwDI mice develop robust accumulation of fibrillar vascular Aβ and less prominent diffuse parenchymal plaques, starting at 3 months of age.[29] CAA is mainly present in capillaries, in contrast to the prominent arteriolar CAA in AD. Tg-SwDI mice also have localized neurodegeneration of cholinergic neurons and cognitive impairment. Testing the ability of therapeutic approaches to reduce vascular amyloid deposits without complication is of particular importance. In the on-going passive immunization AD clinical trials a major complication has been vasogenic edema (or encephalitis) with/or without hemorrhage (termed amyloid-related imaging abnormalities with edema (ARIA-E) or with hemorrhage (ARIA-H).[11,25] ARIAs are also a major issue in the recently reported aducanumab trial (affecting 55% of patients in the high-dose and APOEε4 carriers arm, associated with a 35% patient drop-out rate due to the development of this side effect).[26] Hence developing a therapy that is effective against CAA without inducing vasogenic edema/encephalitis is of critical importance.[11,27] Hence the preclinical testing of therapeutic approaches in models with extensive CAA (which virtually all individuals with AD and about a third of aged cognitively normal individuals have).[28] and showing that it does not induce microhemorrhages is of importance.

Transgenic rat models

A smaller number of transgenic rat models of AD have also been developed. Transgenic rats have a number of potential advantages over transgenic mice; they are more similar to humans in their physiological, morphological and genetic characteristics, their larger brain makes CSF collection, electrophysiology and imaging easier and they have a richer behavioral phenotype, making more complex behavioral testing possible.[29] Three transgenic rat models have been well characterized in the literature.[30-32] Transgenic rats have a similar phenotype and limitations as transgenic mice; expression of multiple FAD mutations accelerates the development of pathology. The distribution, extent and localization of APP expression is dependent on the promoter used. All models have robust amyloid plaque expression (albeit at lower levels than in transgenic mice) and interestingly, TgF344-AD rats have NFTs.[30] despite expression of only endogenous rat tau, not human tau. This is likely due to the greater similarities between rat tau and human tau, in that there are also 6 isoforms of endogenous rat tau. All rat models have some degree of cognitive impairment; however, the degree of impairment has only been extensively characterized in the McGill-R-Thy1-APP rats.[31] In sum, transgenic rats are potentially useful in AD research and offer specific advantages over transgenic mice; however the comparatively minimal use of these models means that greater characterization needs to be done to properly determine their suitability as models of AD.

Physiological models

Two of the major limitations of transgenic rodent models is that they model FAD and not sAD and that the pathology development in these models is typically non-physiological. Finding a naturally occurring model of AD is appealing because they would more accurately represent changes that occur in sAD. Multiple species naturally develop neuropathological features similar to those seen in AD brain, and their potential as naturally occurring models of sAD has been examined.[32]

Non-human primates

The species with the most well characterized AD neuropathological features are non-human primates. The advantages of using non-human primates to model AD include their biological proximity to humans, behavioral complexity, large brains that are favorable for imaging studies or CSF collection and a natural accumulation of Aβ that has 100% sequence homology with human Aβ.[33-35] There have been relatively few AD studies that have characterized AD pathology in great apes (chimpanzees, gorillas and orangutans) because of their long lifespan and ethical concerns of using great apes for research studies. Great apes accumulate Aβ in the brain, resulting in the development of amyloid plaques and CAA in aged animals.[36-41] Plaques are predominantly diffuse and less abundant than that found in human AD. Typically, great apes have more prevalent CAA, which is more likely to contain fibrillar Aβ than plaques. Despite very high sequence homology between great ape and human tau (100% and 99.5% sequence homology between human tau and chimpanzee or gorilla tau respectively), tauopathy is rare. Focal neurons and glia containing phosphorylated tau have been observed in gorillas, but NFTs and tau positive dystrophic neurites are not present.[39]

Knock-in mouse models

The most recently developed transgenic mouse models that replicate AD associated pathology are the knock-in mice. These mice are considered to be a much more physiological model of AD as they are designed to avoid the confounding effects of APP over-expression present in all other transgenic mouse models by humanizing mouse Aβ and knocking in specific APP FAD mutations. As a result, knock-in mice have the same expression of APP and AICD as wild-type mice and APP expression occurs in a physiological manner in the correct brain regions and cell types. Similar to other transgenic mouse models, the timing of pathology depends on the mutations expressed. For example, knock-in of the Swedish, London and Dutch mutations only results in the development of plaques if bred onto a $PS1^{M146V}$ knock-in background.[42] In contrast, knock-in of Swedish and Iberian mutations results in plaque development beginning at 6 months, and gliosis, synaptic alterations and memory impairment from 18 months.[43] Additional knock-in of the Arctic mutation into these mice results in more rapid pathology development including plaque development beginning at 2 months that is more widespread throughout the brain and memory impairment from 6 months.[43] While these transgenic mice represent a significant step forward in the generation of more physiological transgenic models, it still must be acknowledged that they are models of FAD and not sAD and that pathology only develops after knock-in of a combination of specific multiple FAD mutations.

Cell culture models

The use of experimental models derived from human tissue bypasses concerns associated with confounding effects due to species differences. However, one of the major limitations associated with generating representative adult human cell-based experimental models is the lack of available, quality post-mortem tissue. The development of induced pluripotent stem cells (iPSCs) addresses this limitation.[44] iPSCs have now been generated from multiple human donor cell types including fibroblasts, blood cells and urine derived epithelial cells. Multiple groups have characterized iPSC lines from donor cells from FAD and sAD patients, which show increased production of Aβ, particularly Aβ42, and tau hyperphosphorylation in comparison to iPSCs derived from age-matched non-demented controls.[45-48] Some iPSC lines also have evidence of additional AD-associated pathology such as increased activation of GSK3β, increased number of large endosomes.[45] and accumulation of intraneuronal Aβ oligomers.[46]

Other physiological models

Other species naturally develop AD associated pathology with age, the most well characterized examples being dogs and the guinea pig relative Octodon degu. Aged dogs have the same Aβ sequence as humans and they develop plaques and CAA starting at 8–9 years of age.[49-51] Plaques first develop in the prefrontal cortex and later in the temporal and occipital cortices, following a similar, but not identical, pattern to humans. However, these plaques differ from those in human AD as they are primarily diffuse, and therefore may represent an earlier stage of plaque development. A limited number of compact plaques are evident in a small number of aged dogs. AβN3pE is present in a subpopulation of plaques. Other neuropathological features present in aged dogs include cortical atrophy, declined ratio of CSF Aβ42:40, increased Aβ oligomers, and presence of oxidative damage and mitochondrial dysfunction.[52] NFTs are typically not observed; however, pretangles and possible NFTs have been observed in a very limited number of aged, demented dogs.[50,51] In addition, synaptosomes from demented dogs contain increased total and phosphorylated tau than non-demented dogs, suggesting that cognitive impairment in aged dogs may result from synaptic impairment.[51]

Factors to consider when choosing the model

There are many available models of AD pathology, each with their own benefits and limitations. It is

exceptionally important to acknowledge that none of the available models replicate all features of human AD, and therefore cannot be considered to be representative models of AD as a complete disease. What we believe to be the most important factors to consider when using experimental models in AD are discussed below. Very few models have both plaques and tangles, particularly ones that develop physiologically. The presence of both plaques and tangles is required for diagnosis of AD and how the complex interaction between plaques and tangles affects the development of AD is still being determined. It is evident that crosstalk between Aβ and tau can significantly influence toxicity; increased Aβ production results in NFT formation in FAD and Down Syndrome, while there is also evidence to show that tau increases Aβ-associated toxicity (particularly synaptotoxcity), suggesting that the presence of both pathological features are important to replicate the toxicity that occurs in human AD.[53] Therefore, it is particularly important to determine the effect of a new therapeutic on both plaques and tangles, ideally in a model that contains both so that the pathological effect of the crosstalk between the two can be addressed. It is difficult to interpret downstream pathological changes in animal models that have non-physiological expression of Aβ and tau. It must be considered that downstream pathology may be artifacts that result from overexpression of APP, PS1 or tau, or from other APP cleavage products besides Aβ (eg N-APP, APP C-terminal fragments, AICD). These additional APP cleavage products are also capable of causing toxicity independent of Aβ.[54,55] Furthermore, APP overexpression was recently suggested to be the underlying cause of two prominent AD phenotypes, rather than a downstream response to Aβ as was initially suggested based on studies using transgenic mice, calling into question whether this may also be the case for other interpreted examples of downstream AD pathology observed in transgenic mouse models.[56] The issue of non-physiological over-expression of APP or tau can be addressed by using knock-in mouse models, which have physiological expression of humanized endogenous mouse proteins. The additional toxic effects of APP cleavage products besides Aβ is more technically challenging to address, however the use of viral vectors to induce expression of specific isoforms of Aβ in rodent brains have shown promise and could complement the use of transgenic animal models.[57] It must also be considered that endogenous rodent proteins and/or protein pathways may react differently in response to non-physiological expression of specific human proteins and as such, downstream effects cannot be assumed to also occur in humans. The most obvious example comes from results from animal models solely expressing human PS1 with FAD mutations. Despite some mutations in PS1 causing the earliest onset of FAD in humans, sole expression of human PS1 with FAD mutations doesn't result development of plaques in transgenic mice showing that the response of endogenous mouse proteins to human PS1 is different from that in humans. Furthermore, it is likely that the lack of NFT development in mouse models that overexpress Aβ is due to the endogenous differences between mouse and human tau. An elegant study supporting this hypothesis showed that crossing the APP E693Δ-Tg model with wild-type human tau mice resulted in robust formation of NFTs, which never developed in mice with endogenous mouse tau.[58] These are just two examples of instances where the downstream effects of the human protein expressed in transgenic mice differs from what would occur in humans because of endogenous protein differences, supporting the concept that downstream pathological effects (or lack thereof) should be interpreted carefully. Transgenic animal models represent partial models of FAD and not sAD. Much more research in humans is necessary to determine the similarities and differences between FAD and sAD. Currently it is known that the distribution of Aβ and tau accumulation is different in FAD and sAD, with more present in subcortical regions in FAD. There is also more grey matter atrophy in subcortical regions in FAD, and atypical cognitive symptoms are more likely to be present in FAD.[59]

Genetic studies have identified multiple loci that convey increased risk for sAD. It will be important for future studies to determine how these genetic risk factors contribute to AD associated pathology, and whether this is replicated in animal models of the disease. Studies examining the role of ApoE4, which is the strongest identified genetic risk factor linked to sAD, have suggested that this may be more complex than first anticipated in animal models due to species differences. Transgenic mouse studies confirmed that ApoE was necessary for the formation of fibrillar amyloid plaques and CAA, however they also identified important differences between mouse and human ApoE. Expression of mouse ApoE resulted in greater plaque formation than expression of human ApoE, and mouse ApoE preferentially promoted the formation of parenchymal plaques, while human ApoE promoted the formation of CAA.[59,60] This is further complicated by the fact that expression of different isoforms of human ApoE in transgenic mice results in different levels of plaque and CAA burden with apoE4 expression enhancing amyloid deposition compared to apoE3 or apoE2.[3,60]

The most prevalent symptom of AD in humans is cognitive impairment. While the majority of animal models show some degree of cognitive impairment, the type and the timing of this impairment must be carefully considered, particularly in preclinical studies. As mentioned above, cognitive impairment occurs at a different stage of pathology development in transgenic mouse and rat models in comparison to humans; occurring at or before the onset of plaque development in rodents and many decades after plaque development in humans. In contrast, initial studies show that more physiological knock-in mouse models develop cognitive

impairment many months after plaque development,[59] which is more similar to humans. This raises the question of whether the process that mediates cognitive impairment in transgenic animal models is the same as the one that mediates cognitive impairment in humans.

CONCLUSION

Careful examination of neuropathology and cognitive impairment in multiple species, including those closest to humans, shows that AD is a uniquely human disease. Many of these Animal models have the obvious advantage of providing the option to do preclinical testing *in vivo*, allowing the testing of general toxicity of new therapeutics and providing a system in which cognitive testing can be done. New knock-in mouse models are potentially more representative and physiological models of AD; however, they still need to be further validated in future studies. Non-human primates offer the unique advantages of greater genetic similarity to humans and a more physiological relevant development of pathology that better resembles that in found in sAD compared to transgenic models, but studies are limited by availability, costs, time until onset of phenotype and the inconsistent presence of pathology in all animals. New human cell culture models have the advantage of allowing high-throughput screening of novel therapeutics directly using human cells; however these models obviously cannot replace *in vivo* models for preclinical testing. Therefore, going forward it will be necessary to perform preclinical testing in multiple animal models that each exemplifies a unique aspect of AD pathology, until a more complete and physiological animal model of sAD is available to ensure greater translation of preclinical results to human clinical trials.

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