Physiological and Pathological Ageing

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Physiological and pathological ageing (as exemplified by Alzheimer's disease, AD) are characterized by a progressive decline that also includes cognition. Therapeutic ultrasound presents as a new modality to decrease the pathological load and restore cognition in AD mouse models. Moreover, it is an excellent modality to increase uptake (and hence, efficacy) of therapeutic antibodies in AD mouse models. With clinical applications in mind, the review also discusses the challenges of AD therapies more generally and what needs to be considered when therapeutic ultrasound is employed.

Keywords: Alzheimer's disease ; aducanumab ; amyloid ; cognition ; focused ultrasound ; memory ; neuromodulation ; scanning ultrasound

1. Alzheimer's Disease–A Disorder of Pathological Ageing

Age is the major risk factor for dementias and neurological diseases more generally. The incidence of AD as a major dementia is doubling every five years after the age of 65, and currently, there are around 32 million people with AD worldwide, at a cost of over US$1.1 trillion, with total cases expected to rise to 100 million by 2050 (Alzheimer's Association 2018). Globally, 9 million people died from neurological disorders in 2016 [1]. This corresponds to a loss of 276 million disability-adjusted life-years. Neurological disorders account for close to 17% of all deaths globally, second only to cardiovascular disease. AD and other dementias account for a quarter of these deaths.

Prevalent dementias other than AD are frontotemporal lobar degeneration (FTLD), dementia with Lewy bodies, and vascular dementia. Dementia is also present in movement disorders such as Parkinson's disease (PD). What these diseases have in common is the accumulation of insoluble protein deposits in the brain as a result of either an increased production or impaired clearance. In fact, the assumption shared by many researchers is that these proteins and their aberrant aggregation are causal and drive disease progression. What are these proteins in AD? Alois Alzheimer and Oskar Fischer, more than a century ago, independently described the cardinal histopathological features of the disease that now bears the Alzheimer's name [2]. With light microscopic examination of histologically stained brain sections, they captured the two most prominent hallmarks; extracellular amyloid plaques (which contain oligomeric and fibrillar forms of the aforementioned Aβ peptide) and intracellular neurofibrillary tangles (which contain the microtubule-associated protein Tau in its oligomeric and fibrillar forms). Pathological Aβ and Tau are thought to drive, at least in part, the loss of synapses and neurons in vulnerable brain regions, leading to the symptoms commonly associated with AD.

A causative role for Aβ in disease pathogenesis is further supported by observations in familial AD, which accounts for up to an estimated <3% of all cases. Here, autosomal dominant mutations have been identified in genes encoding the amyloid precursor protein, APP (from which Aβ is derived by proteolytic cleavage), and components of the protein machinery that generates Aβ (presenilin-1 and -2). While no causative mutations have been identified in the Tau-encoding *MAPT* gene, they have been identified in a significant subset of FTLD with Tau (FTLD-Tau). Importantly, in AD, Tau has been more closely linked to dementia than Aβ [3]. Together, this points to Aβ and Tau as pathogenic agents. The situation is less clear for the predominantly sporadic cases of AD and FTLD-Tau, which have a later age of onset. Because the two forms (sporadic and familial) do not differ in their general histopathological features and clinical presentation, it has been suggested that while the initiating, upstream signals might differ, they likely converge in a common downstream pathogenic signalling pathway.

In modelling AD in animals, transgenic mouse models have been particularly useful. The early mouse models targeted the expression of AD-relevant proteins to neurons in general, whereas region-specific and cell-type-specific approaches, as well as inducible systems, are increasingly being employed to understand, for example, aspects of regional vulnerability and the spreading of AD pathology. Such brain area and cellular specificity can be achieved by introducing the gene of interest under the control of specific promoters and regulatory elements [4]. In the animal modelling of AD, a major emphasis was initially placed on reproducing key lesions that are specific to the human disease. Despite the opportunities offered by these transgenic models, several caveats remain. The human transgenes contain either no or not all non-

coding sequences, making it impossible to study human genomic interactions and the role of splice variants. Although these models were crucial in proving the role of distinct genes and their associated mutations in AD, the human pathology is generally only incompletely recapitulated. Compared with the Aβ plaques in human brains, those in many rodent models are either diffuse or, even when they are condensed, exhibit fewer crosslinked fibrils. Neurofibrillary tangles have proved to be even more difficult to model, as even when Tau filament-like structures form, they appear different from those in human brains on the basis of negative-stain electron microscopy. Another caveat, intrinsic to the way in which transgenic animals are usually generated, is that neither the integration site nor the copy number of the inserts can be controlled, leading to a large and uncontrollable range of expression patterns and levels, a situation that is further complicated by the potential for integration artefacts. Another concern regarding overexpression models is the presence of secondary effects. In an attempt to overcome what is seen by some as an overexpression artifact of the classical Tau transgenic models, knock-in mice have been generated, some of which are now more widely used [5][6]. Nevertheless, these models also have their limitations. As is the case with transgenic mice, they recapitulate only certain aspects of the disease. For example, APP knock-in mice (with or without additional mutations) do not present with Tau pathology. To generate Aβ deposits, these models also combine several gene mutations that do not co-occur in AD and that could complicate the analysis of downstream effects.

So far, our discussion has focused on Aβ and Tau; however, the field has produced many hypotheses to explain what initiates and drives the pathogenic process for the predominantly sporadic forms of AD. These range from impaired neurotransmitter systems as presented by the cholinergic hypothesis, a role for mitochondrial dysfunction (shared e.g., with Parkinson's disease), inflammation including changes to the innate immune system, viral infections, and an interaction between the nervous system and the gastrointestinal tract, which consists of multiple connections, including the vagus nerve, the immune system, and bacterial metabolites and products [7]. However, even if one were to focus on Aβ and Tau only, the challenge remains, as these species undergo a process that generates different sizes (proteolytic cleavage in the case of Aβ and alternative splicing plus proteolytic cleavage for Tau), a host of posttranslational modifications (Tau undergoes acetylation, O-linked N-acetyl-glucosamination, ubiquitylation, sumoylation, methylation, glycosylation, isomerization, and truncation, whereas Aβ undergoes phosphorylation, glycosylation, and isomerization), and a process of oligomerisation and fibrillization that leads to extracellular amyloid plaques and intraneuronal Tau-containing neurofibrillary tangles [8].

Finally, it needs to be acknowledged that age is the most important risk factor for AD. Consumer organisations would define AD in an operational manner: Regarding short-term memory and learning new information, possible changes due to normal ageing may be sometimes forgetting people's names or appointments but remembering them later, whereas in people with dementia, they may forget the names of close friends or family, or they forget recent events such as visitors they had that day. The question thus arises in which aspects healthy, physiological ageing can be discriminated from AD. A recent review discussed nine hallmarks of ageing: stem cell exhaustion, altered intercellular communication, genomic instability, telomerase attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, cellular senescence, and mitochondrial dysfunction, and all these aspects are more severely affected in AD [9]. Structurally, healthy ageing is more strongly associated with a decline in frontal regions, whereas middle-aged individuals more likely to develop AD have shown greater grey matter reductions in dorsolateral and medial prefrontal, parietal, and lateral temporal regions. They have also shown a loss of white matter integrity in regions including the cingulum, corpus callosum, superior longitudinal fasciculus, and left uncinate fasciculus. Additionally, midlife volumetric reductions in the fronto-striatal executive network seem to be a normal part of ageing, whereas reductions in the medial temporo-parietal episodic memory network seem to indicate pathological ageing [10]. Finally, entorhinal cortex and hippocampal atrophy rates appear to diverge in healthy and pathological brain ageing, but it is not yet known whether this divergence is relevant to midlife [11]. Despite these differences, discriminating healthy brain ageing and changes of AD remains a grey zone [12]. Given the overlap between physiological and pathological ageing, one could argue that by slowing the ageing process or aspects thereof, neurodegenerative processes such as those in an AD brain could also be slowed down.

2. Alzheimer's Disease Therapies–Treatment Options and Challenges

The current treatment of AD is largely only providing symptomatic relief. With the observation of a degeneration of (although not exclusively) cholinergic neurons, the foundation was laid for the development of acetylcholine esterase inhibitors (donepezil, rivastigmine, and galantamine) that stabilize the neurotransmitter acetylcholine in the synaptic cleft. These drugs are prescribed to patients with mild to moderate AD; however, they do not target the underlying degenerative process. The drugs were approved between 1996 and 2001 under the proprietary names Aricept, Exelon, and Razadyne. The only other drug that has been approved is memantine (in 2003 under the proprietary name Namenda), which is prescribed to cases of moderate to severe AD. This drug is an antagonist of the NMDA receptor, blocking excitotoxicity.

In the past decades, more than 200 clinical trials have been performed, resulting in zero approvals as AD drugs. In a recent assessment of the clinical trial landscape, with a censor date of 27 February 2020, 121 agents were in clinical trials for the treatment of AD. Twenty-nine of these were in 36 phase III trials, 65 agents in 73 phase II trials, and 27 agents in 27 phase I trials. Twelve agents in trials targeted cognitive enhancement, and 12 were intended to treat neuropsychiatric and behavioural symptoms. There were 97 agents in disease modification trials. Interestingly, compared to the 2019 pipeline, there was an increase in the number of disease-modifying agents targeting pathways other than Aβ or Tau [13].

Of the strategies targeting Aβ and Tau, comprehensive data are available for immunotherapies, a strategy that was first adopted for Aβ and then Tau. Following disappointments in the wake of failures of a whole battery of therapeutic antibodies in meeting their primary end points, the outlook is now more positive following the recent U.S. Food and Drug Administration (FDA) approval of aducanumab, an anti-Aβ antibody that targets Aβ aggregates including insoluble fibrils and soluble oligomers by binding to the amino terminus of Aβ at residues 3–7 in a shallow pocket in the antibody [14]. This human IgG1 antibody had been isolated from the B cells of cognitively healthy elderly humans and has low affinity for monomeric Aβ [15]. Of four clinical-stage anti-Aβ antibodies studied (aducanumab, gantenerumab, bapineuzumab, and solanezumab), only aducanumab had an impact on the aggregation kinetics and production of oligomeric aggregates [16].

In a Biogen-sponsored phase Ib clinical trial (PRIME) of aducanumab in prodromal and mild AD patients, a striking reduction in amyloid plaques, as measured by positron emission tomography, was reported following one year of monthly intravenous antibody infusions at doses ranging from 3–10 mg/kg. One of the two phase III trials of aducanumab, EMERGE, unlike ENGAGE, showed reductions in cognitive decline, possibly reflecting the effects of higher accumulated doses of the antibody [17]. Biogen was granted conditional FDA approval for aducanumab on 7 June 2021, pending a post-market commitment of a phase IIIB re-dosing trial that has recently been launched, becoming the first anti-amyloid agent and first antibody treatment for AD. However, it remains to be determined whether aducanumab is a disease-modifying therapy that achieves significant clinical benefits, i.e., improves cognition, in AD patients [18]. The interpretation of the cognitive data from these trials is complex because, during the trial, dosing was altered and stopped, and the magnitude of the cognitive effect was relatively small. Sevigny and coworkers demonstrated 50% plaque reduction in APP mutant Tg2576 mice after treatment with a mouse IgG2a aducanumab analogue; however, the effects of the immunotherapy on behavioural read-outs in mice have not been reported [15]. Antibodies in ongoing advanced clinical trials include Eli Lilly's donanemab, which targets a specific form of Aβ (N3pG-Aβ); Biogen's BAN2401 (which bears similarities with aducanumab); Eli Lilly's solanezumab, which binds to monomeric Aβ; and Roche's gantenerumab, which recognizes a conformational epitope. In this context, it is important to note that deposition of Aβ in the brain is not sufficient to develop dementia, and similarly, removing the peptide in patients with AD by itself is not sufficient to restore their cognitive functions [19].

Conceptually, targeting Tau in AD may present a more compelling approach than targeting Aβ, insofar as Tau burden correlates better with cognitive decline, while simultaneously posing the nontrivial challenge of targeting a protein that is primarily intraneuronal [3][20]. With limited exceptions, therapeutic monoclonal antibodies are of an IgG isotype, predominantly binding to pathogens in extracellular compartments. Anti-Tau IgGs on the other hand must penetrate both the blood–brain barrier (BBB) and the neuronal membrane to induce neutralization and/or clearance of intracellular pathogenic species of Tau. The Tau antibody approach could circumvent this issue if prion-like spreading does indeed play a central role in disease progression, as neutralizing Tau seeds in the extracellular compartment could in principle slow down spreading without the need for neuronal uptake [21]. On this ground, most of the earliest Tau passive immunotherapies in clinical trials were aimed at targeting extracellular Tau, and lead candidate screening is still often performed by assessing the antibody's capacity to neutralize extracellular Tau seeds [22][23]. Yet, pathological Tau predominantly resides within neurons, and to what degree spreading effectively contributes to Tau pathology remains unclear. Hence, it is likely that targeting both intra- and extracellular pools of Tau will maximize efficacy. To this end, it follows that therapeutic ultrasound could be beneficial as a combination treatment by increasing the fraction of circulating antibody that reaches the brain parenchyma and targeted neurons.

Tau immunotherapy is still in its early days of development relative to the anti-Aβ field, with a total of ten antibodies currently in early clinical trials of AD and other primary tauopathies, six of which have advanced to phase II trials. Perhaps unsurprisingly, two of the latter antibodies targeting extracellular Tau, Biogen's gosuranemab and AbbVie's tilavonemab, have both been discontinued as candidates for progressive supranuclear palsy (PSP) after their respective phase II trials failed to meet efficacy primary endpoints [24][25]. These outcomes are relevant to the argument at hand in that targeting extracellular Tau may not be sufficient to modify disease progression. This approach may be particularly ill-suited in PSP given that evidence for prion-like spreading is much more limited than in AD and that Tau levels in cerebrospinal fluid (CSF) are not as significantly increased [26][27]. Both antibodies continued into phase II trials in AD with the hope that targeting extracellular Tau will yield more promising outcomes in the AD pathological context. Additional disappointments

were recently released at the AD/PD 2021 conference with preliminary results reported of the phase II TAURIEL trial (NCT03289143) showing that Roche's semorinemab decreased CSF Tau, but not other markers of inflammation and degeneration (e.g., sTREM2, IL-6, or NfL) in patients suffering from mild AD.

More recent trends in Tau antibody development have shifted from predominantly targeting Tau's N-terminus to targeting the protein's mid-region, as well as phosphorylated epitopes characteristic of toxic species. Although several recent studies have demonstrated the clear superiority of mid-region antibodies in blocking proteopathic spread in vitro, it remains to be validated whether these preclinical studies accurately predict clinical performance [23][28]. Of the newer generation of Tau antibodies, only Janssen's JNJ-6373657 and UCB's UCB0107 have recently advanced to phase II trials, and Eisai's E2184 was recently selected as the biological anti-Tau arm in the DIAN-TU trial. Phase II AD trials for Eli Lilly's zagotenemab and tilavonemab are due for completion in 2021, and efficacy outcomes could be decisive in shaping the future of Tau immunotherapies.

Why is it so difficult to find a treatment for AD? Drug development in general is a challenging field: drugs must enter the bloodstream, reach their target organ, and then exert a therapeutic effect. Different forms of drug administration pose different levels of challenges. These issues are present in most forms of drug delivery, and the industry has developed ways to overcome them. However, for neurological disorders, there is an additional challenge that the pharmaceutical industry has to overcome: the BBB. Nearly all large drug molecules and 98% of small molecules cannot pass the BBB. In comparison to the one-layer epithelium in other organs, the BBB is formed by a tight neurovascular unit (NVU) of endothelial cells (ECs), pericytes, and astrocytes that interact to create a tight barrier. Furthermore, even if a drug were to enter past the tight junctions of ECs, the ECs and astrocytes are equipped with a large variety of efflux pumps, which recognize a wide range of molecules and export them back into the bloodstream. The extreme limitations on molecular structure and cut-off size imposed by the BBB create a trade-off that has been difficult to balance thus far. To design an effective drug, pharmaceutical companies first need to create a molecule that they hypothesize will have the desired effect on neurons when it enters the brain. Additionally, there are requirements for the drug to make it into the bloodstream, survive, and then access the brain. These properties are often contradictory to those needed to cross the BBB.

Additional challenges for AD and other dementias are the incomplete understanding of the etiology and progression of the disease (as discussed further up); a failure to diagnose at presymptomatic stages; a lack of robust and sensitive biomarkers (which presents a problem for validating clinical trials and showing efficacy); in some cases, a short therapeutic window; and the inaccessibility of damaged or degenerating brain tissue.

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