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PASSIVE IMMUNOTHERAPY - A VIABLE TREATMENT FOR ALZHEIMER'S DISEASE

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SUMMARY

Passive immunotherapy is one of the most exciting and extensively researched areas in the field of Alzheimer's disease (AD) today, harbouring the potential to become the first disease-modifying treatment for the disease. The interest in immunotherapy as a treatment stemmed from the significant dangers of toxic side-effects and major obstacles in selectivity for currently pursued therapies against amyloid beta (Aβ) proteins and neurofibrillary tangles. Passive immunotherapy especially, has received much limelight, seen as having the potential to be the safer alternative to active immunisation which encountered a significant setback with the notorious AN-1972 trial in which 6% of the vaccinated patients developed meningoencephalitis. At present, passive immunisation research in animal models have exclusively focused on targeting Aβ proteins, a widely accepted pathology of AD.Following on from this, the preliminary results of phase II trials of three distinct passive immunisation strategies were demonstrated at the 2008 International Conference on Alzheimer's Disease (ICAD). The three therapeutic strategies each targeted the N-terminal of Aβ, the central epitope or utilised a polyclonal approach. The results demonstrated potential as well as caution. Efficacy was undoubtedly present but not to the extent that was hoped and side-effects, most notably vasogenic oedema occurred in the N-terminal targeting antibody, bapineuzimab. Lessons have been learnt by identifying the possible cause of the problems and have been taken on board to nurture the proven efficacious results. Key points to be addressed currently are dosage of the agent to ensure that high enough concentrations enter the central nervous system to be available to cause effect and early enough time of administration to cause effect. The results of the efficacy and safety phase III trials and the development of newer passive immunotherapeutic agents addressing the problems are eagerly awaited in the hope of finally yielding a disease modifying therapy of AD.

Key words: *passive immunotherapy - Alzheimer's disease*

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INTRODUCTION

Alzheimer's disease (AD) was first described by Alois Alzheimer and Gaetano Perusini in 1906. It is the most common form of dementia before vascular dementia and as of 2010 has 35.6 million sufferers worldwide. This number is estimated to rise to over 65 million in 2030 (http://www.alz.co.uk/research/statistics) placing an incredibly heavy medical and economic burden on societies around the globe and could lead to a 21st century social crisis.

Alzheimer's disease is a fatal neurodegenerative condition characterised by progressive cognitive impairment, memory, decision-making, orientation, often accompanied in later stages by language deterioration. It is likely to be caused by a combination of factors with age being the greatest risk factor. Genetic and environmental factors are suspected to play a part too, with many potential contributing factors such as mental activity, smoking, cholesterol level and blood pressure to name a few. There is currently no definitive diagnostic tool for Alzheimer's disease. The most common methodology used by clinicians is to discount other causes by running a blood test, physical examination and an MMSE (mini-mental state examination). Treatment for Alzheimer's disease is currently limited to symptomatic treatments and disease-modifying therapy is urgently needed.

THE PATHOPHYSIOLOGY OF ALZHEIMER'S DISEASE

The definition of AD is based on the remarkably accurate case report by Alois Alzheimer in 1907 based on plaques and tangles in the brain of a woman in her fifties who had passed away due to the disease. Despite much controversy over the last 25 years, the consensus on the pathogenesis of AD is now generally accepted. The two major hallmarks of AD are the extracellular amyloid beta (Aβ) plaques and the intracellular neurofibrillary tangles (NFTs). The initiating factor which seems necessary but not sufficient is the production of aggregates containing the Aβ protein, which is formed by the cleavage of a larger peptide, the amyloid precursor protein (APP). In AD, APP and in turn Aβ is overexpressed resulting in the insoluble aggregates.

Furthermore, in familial forms of AD and in cases of Down's syndrome where precocious AD results, a common production of Aβ protein with a longer C-terminal occurs. This form has been found to be more predisposed to produce oligomers and fibrils, which may be present as long as a decade before cognitive symptoms present (Masters 1985). This provides scope for early intervention for prevention of the full onset of AD with the correct technology.

The second step in the pathogenesis of AD is the formation of intracellular NFTs containing the

hyperphosphorylated microtubule binding protein tau. Neurodegenerative diseases can appear just with tau pathology in the absence of Aβ plaque formation. However, these display different clinical presentation and brain regional variation in tau pathology to AD and the two proteins are likely to have a synergistic effect in AD, perhaps Aβ causing a cascade of pathologies including tauopathy. Tau, along with other members of the microtubule associated protein (MAP) family, is involved in modulating microtubule assembly and neuronal stability. The following is thought to be the process by which tau pathology causes AD. Hyperphosphorylation of tau results in reduced binding to microtubules which leads to loss of axonal integrity. It also means that there is a greater pool of tau to form intraneuronal aggregates, causing NFTs, which may also leak out extracellularly causing pathology. Based on these models of AD, various strategies to modify the course of the disease are being researched, mostly attempting to reduce the concentration of Aβ plaques and NFTs. The most active areas of research in this field currently will be briefly discussed in the next section.

CURRENT TREATMENTS AVAILABLE AND RECENT DIRECTION OF RESEARCH

A curative treatment for Alzheimer's disease is still elusive. Even 16 years after its first approval by the US Food and Drug Administration (FDA), donepezil, an acetylcholine esterase inhibitor (AChEI) remains the mostcommonly prescribed drug for Alzheimer's disease. Currently available therapy is limited to drugs that address the symptomatic side of the disease. There are two main types: acetylcholine esterase inhibitorsand N-methyl-D-aspartate (NMDA) receptor antagonists. The basis for administration of AChEIs arises from the aged hypothesis that there is a decline in cholinergic neurone activity in AD. Thus AChEIs are administered to increase the concentration of ACh available to neurones. Glutamate is crucial excitatory neurotransmitter. However in pathological states as in AD, calcium excitotoxity causes the death of neurones and thus NMDA receptor antagonists act to inhibit overstimulation of neurones by glutamate. Only five of these drugs are approved by the FDA. Four of these are AChEIs and are administered for mild to moderate cases of Alzheimer's disease; tacrine, donepezil (Aricept), rivastigmine (Exelon) and galantamine (Reminyl), although tacrine is rarely used due to its hepatotoxicity. The single NMDA receptor antagonist is memantine (Ebixa), exhibiting non-competitive binding and is used for moderate to severe casesin which excitotoxcity becomes a prevalent problem, again reducing the worst of the symptoms rather than modifying the nature of the disease. In an attempt to maximise the efficacy of current limited line of therapy, combination therapy of donepezil and memantine has been pursued and encouragingly, have been shown to reduce clinical worsening with statistically significant results (Atri 2013).

It is evident that preventative and disease-modifying drugs are urgently required. Much research is going on at present into disease-modifying drugs targeting the Aβ plaques and intracellular neurofibrillary tangles. These include γ-secretase inhibitors, α-secretase potentiators, tau kinase inhibitors and tau phosphatase potentiators to name a few. The review of the state of current research will clarify the need for novel methods.

Anti-amyloid aggregation agents

Drugs researched in the anti-amyloid aggregation group of agents have generally given disappointing results. Synthetic glycosaminoglycan 3-amino-1-propaneosulfonic acid (3APS, tramiprosate) is the only Aβ aggregation inhibitor that has reached phase III trial (Gauthier 2009). Even this drug, which inhibits the binding of glycosaminoglycans and Aβ, failed to pass the North American phase III trial and was discontinued in the European phase III trial. Furthermore, 3APS has been shown to promote abnormal aggregation of the tau protein (Santa-Maria 2007). These results show the importance of assessing the effect of the agent across both Aβ and tau proteins as both are key suspected candidates in causing neuronal pathology in AD.

Drugs interfering with metals

A drug known as PBT2 is a promising candidate in this class of agents. Zinc and Copper are both involved in the oligomerisation of Aβ42 and PBT2, a second generation 8-OH quinoline metal-protein-attenuating compound, interferes with the toxic aggregation of Aβ. A very promising study by Faux in 2010 comparing the responses of patients after 12 weeks to treatment of 50mg PBT2, 250mg PBT2 and placebo showed a significant improvement in the patients of the 250mg PBT2 group (Faux 2010). The encouraging results provide a platform for larger-scale research of this drug.

Selective Aβ42 lowering agents

Aβ is formed by the cleavage of the transmembrane protein APP. Aβ is cleaved by two competing enzymes α-secretase and β-secretase. In AD, β-secretase cleavage is the dominant pathway and the subsequent cleavage by γ-secretase produces Aβ40 and Aβ42. Thus inhibition of β-secretase or γ-secretase or the potentiation of α -secretase will work to lower the concentration of Aβ42 (Cummings 2008).

β-secretase enzyme inhibitors

BACE1, a β-secretase enzyme, is a promising target although it has two crucial problems. The first is that it has major physiological roles meaning that its inhibition may have toxic effects. Secondly, the active site of BACE1 is large and many of the bulky compounds that

are likely to block the site will not cross the blood-brain barrier. Moreover many of the peptidomimetic candidates suffer from the common problem of polypeptide drugs, namely poor oral bioavailabilty and susceptibility to P-glycoprotein transport. The direction of research has thus necessarily turned to the design of nonpeptidic compounds, utilising a ligand-based computational approach to identify the molecular properties required to inhibit BACE1 (John 2011).

γ-secretase enzyme inhibitors

γ-secretase is an important nucleoprotein complex composed of at least four different proteins of which preseniline PS-1 and PS-2 appear to be responsible for action on APP. This class of drugs faces great difficulty due to the familiar problem of having many substrates, most notably the transmembrane protein Notch receptor 1, which is crucial for growth and development. The most intensely studied γ-secretase inhibitor, semagacestat (LY-450139), highlighted this problem clearly. In two large phase III clinical trials semagacestat was shown to have detrimental effects on cognition and functionality compared to placebo in patients with mild to moderate forms of AD, thus forcing a premature termination of the trials. The toxicity was attributed to the inhibition of Notch1 and also the build up of the toxic precursor of Aβ, the C-terminal fragment of APP (CTFβ) (Imbimbo 2011). The latter problem is a rather significant obstacle for all drugs in this class since the very nature of inhibiting γ-secretase will cause an accumulation of CTFβ. The additional problem of having to develop a Notch1 sparing agent means that the path ahead will be testing indeed.

α-secretase enzyme potentiation

A group with more promising results and perhaps a way to side-step the problems mentioned above is the class of α-secretase potentiators. A recent randomised, double-blind, placebo-controlled phase II clinical trial with 159 randomised patients with mild to moderate AD showed the potential of etazolate (EHT 0202) to be a safe and well tolerated drug. It is thought to work via potentiating the α-secretase pathway (non-amyloid) and thus reducing the toxic effects of Aβ and salvaging neurons from Aβ induced death. The encouraging initial findings have supported further research into the clinical efficacy of the drug and its tolerance in longer treatment periods (Vellas 2011).

Anti-neurofibrillary tangle agents

Abnormal hyperphosphorylation of tau not only results in reduced binding to microtubules causing axonal instability but also aggregation leading to the formation of intraneuronal NFTs. Drugs targeting the imbalance of kinase and phosphatase activities as well as the aggregation itself are being researched currently with mixed results.

Kinase inhibiting agents

There are many phosphorylation sites of tau and many kinases act on the protein. Of these, glycogen synthase kinase 3 (GSK3β) has emerged as the most likely candidate as a target because it has been co-localised with dystrophic neurones and NFTs (Pei 1997. Yamaguchi 1996). Several agents to inhibit GSK3β are being pursued such as pyrazolopyrazines, pyrazolopyridines and sodium valproate but the most studied so far is lithium. Lithium has its appeals due to its 50 year history of administration for a number of psychiatric illnesses including manic-depressive illness. However, the future of lithium as a reliable therapy for AD is unsure. The mechanism of inhibition of GSK3β is not very well understood and while cell culture and in vivo studies have demonstrated selective inhibition of the enzyme and reduction in tau phosphorylation levels (Munoz-Montano 1997, Stambolic 1996), clinical trials have shown much more disappointing results. A pilot study looking into the feasibility and tolerability of lithium as a treatment for mild to moderate AD was undertaken (Macdonald 2008). After treatment of up to a year, there were no changes in patients' MMSE and many participants reported contra-indications causing the authors to conclude that perhaps lithium as a treatment for AD is limited.

Cdk5 was identified to be tau protein kinase II in 1993 by Kobayashi et al. and it has been suggested to play a key role in tau phosphorylation and subsequent NFT formation in AD (Flaherty 2000). This is a relatively new area of research and clinical trials with selective cdk5 inhibitors are yet to be undertaken.

Phosphatase potentiating agents

Tau is dephosphorylated by protein phosphatase 2A (PP2A) with PP1, PP2B and PP5 having more minor roles. PP2A and PP2B have been selected as possible targets for treating AD as their levels have been detected to be lower in AD relative to controls (Gong 1995, Sontag 2004). Furthermore PP2A has been suggested to regulate GSK3β and cdk5 activity. During starvation, hyperphosphorylation of tau in the hippocampus of mice increased with correlating decrease in both tau protein kinase I (TPKI), GSK3β, cdk5 and PP2A. The results of this study carried out by Planel et al. in 2001 demonstrated that perhaps the activity of the TPKI, GSK3β or cdk5 are not the necessitating factors of hyperphosphorylation of tau but rather that the inhibition of PP2A is the overriding cause. Novel agents selectively potentiating PP2A activity must be developed with attentive research into possible side-effects arising from the complex interplay of enzymes demonstrated from the example above. A possible new direction of insight in this area of research comes from the discovery that memantine, an NMDA receptor antagonist already approved for symptomatic treatment of AD, could reverse the okadaic acid (PP2A and PP1 inhibitor) induced hyperphosphorylation of tau. The research must be evaluated further in a clinical setting

moving on from a hippocampal slice culture model (Li 2004).

Therapies targeting the aggregation of tau

The class of therapies pursuing tau aggregation as possible target of AD disease-modifying treatment is a fairly new realm of research. Tau is usually a very soluble protein which does not spontaneously form paired helical filaments (PHFs) and straight filaments in vitro. The fibrogenicity of tau depends on several factors including post-translational enzymatic action and phosphorylation events at particular epitopes. Current potential prevention methods being studied aim to intervene at various stages of the aggregation cascade, with however, possible drawbacks calling for a novel approach.

Methylene Blue

In 2008, Wischik and colleagues published pioneering research reporting on the possible therapeutic value of methylene blue (MB) for the treatment of AD, building on from the foundation set in 1996 again by Wischik that phenothiazines (MB) can inhibit tau-tau aggregation through their repeat domain while leaving tau-microtubule interactions intact (Wischik 1996). This was a rather unique attempt as MB had previously been used for purposes of dyeing, cheap and effective treatment of malaria and as an antiseptic (Wainwright 2002, Oz 2009). However, MB has undesirable side-effects such as poor bioavailability, toxicity at high dose and the fact that urine is coloured blue, which itself is a major inconvenience for clinical trials as it "unblinds" the patient. Development to put MB with such side-effects remedied on to the market is underway.

A crucial point to note for all potential inhibitors of tau aggregation is that more toxic oligomers may form as a result of the inhibition. Furthermore, many of the strategies against Aβ protein targets enzymes with major roles in the cell, meaning not only that development of the drug is difficult but that the potential side-effects would be devastating. Such reasons would render many of the proposed therapies limited and even harmful. Thus there is an urgent need for novel disease-modifying therapies and an exciting innovative area of research is the use of immunotherapy to target Aβ plaques and the hyperphosphorylated tau aggregates which form NFTs. Research is highlighting the unique benefits potentially offered by this method such as reduced toxicity and increased efficacy of treatment in patients.

PASSIVE IMMUNOTHERAPY FOR TREATMENT OF AD

Passive immunisation is one of the most active areas of research currently in the search for disease-modifying therapy of AD. Vaccination therapy was hailed with great optimism as a potentially effective treatment for AD ever since the pioneering invention by Dale Schenk and colleagues in 1999. However, with the highlighting of potential dangers of active vaccination, epitomised by the notorious case of AN-1792 vaccine causing 6% of the participants to develop subacute meningioencephalitis (Orgogozo 2003), researchers have been enlightened to look elsewhere for safer alternatives. Passive immunotherapy has come forward as the therapy with the potential to be the safer alternative with promising efficacious results. The results of three (Bapineuzumab, Solanezumab, Gammaguard) phase II clinical trials were announced at the 2008 International Conference on Alzheimer's Disease and as well as the importance of safety in these researches, it highlighted the promise of passive immunotherapy. The three therapies are uniquely designed to maximise efficacy and safety by utilising monoclonal N-terminal, central epitope and polyclonal strategies.Subsequently all three have moved on into phase III trials and the results are eagerly awaited, offering the hope of at last yielding a disease-modifying therapy of AD.

Active immunotherapy in human AD

The first report on immunotherapy as a strategy to combat AD by Schenk in 1999 was carried out in mice and utilised active immunisation. With the optimism and excitement of the new potential, this method was remarkably quickly translated to human clinical trials. From the outset, Aβ protein was the main target and focus of the treatment due to the "amyloid cascade hypothesis" and the readily available mice model of Aβ aggregation, although with recent disappointing results concerning Aβ, the possibility of other proteins, namely tau, having a closer correlation with the disease profile is being pursued. In 2000, phase I safety clinical trials for active vaccination of aggregated $A\beta_{1-42}$ (AN1792) took place in 80 subjects with mild to moderate forms of AD. Four vaccinations were given over a six month period and although four patients died during this time, none were suspected to be from the treatment. Some side-effects aside, overall the treatment was generally well-accepted and phase II trials began.

The phase II clinical trial however had to be terminated prematurely in 2002 because four patients began to show signs of meningoencephalitis and in total, 6% (18/300) of the participants developed the condition (Orgogozo 2003). The exact mechanism of cause is still debated but the most suspected is an inflammatory reaction mediated by T-cells since the Aβ42 molecule has been shown to contain a T-cell activating domain. This suggestion is supported by a separate case of AN1972 application in a phase I study (Nicoll 2003) whereby a post-mortem examination showed extensive T-lymphocyte meningoencephalitis as well as macrophage infiltration of white matter causing clearance of Aβ plaques. Alternatively, inflammation may have been part of the sign of the natural clearing process of Aβ plaques by the treatment. In transgenic

mice vaccinated with Aβ monthly for 3-5 months, concomitant transient microglial activation has been observed. Thus it is possible that periods of inflammation are also concurrent with Aβ immunisation (Arendash 2001).

Much has been learned from this study and despite the premature termination of dosage, unblinded post-analyses have been essential for insight into the unexpected possible adverse effects of this kind of therapy. Dangers highlighted from the study have stimulated the endeavour to discover safer, efficacious alternatives and passive immunisation utilising humanised monoclonal antibodies has emerged as one of the strongest contenders.

Passive immunisation in animal models

The potential of passive immunotherapy was reported in the advent of immunotherapy through two reports by Bard 2000 and DeMattos 2001(Bard 2000, DeMattos 2001). The two reports differ with respect to the proposed mechanism of antibody action with Bard's group suggesting direct access and binding to Aβ protein in the brain whereas Dematttos suggests that m266 (monoclonal antibody to the central domain of Aβ) carries out its effect by altering plasma clearance of Aβ. The exact mechanism of action is still debated. The upshot at this stage was that along with many other studies passive immunotherapy was shown to improve behaviour performance and seemed to reduce Aβ plaque pathology in transgenic animals. The studies included a range of antigenic binding sites and variance in the form of Aβ but the therapeutic effect was universal. However, the interesting and contentious finding centres around the very pathophysiology of AD. The widely accepted view of Aβ plaque directly causing the behavioural symptoms of the disease was challenged. The beneficial behavioural effect was observed rather quickly (within one day of administration) than would be expected if the therapeutic effect came from overt Aβ plaque removal (Lichtlen 2008). Clearly the mechanism of action of vaccination as well as the pathophysiology of AD still has much to be identified but recent reports have suggested the existence and toxic role of soluble, prefibrillar oligomers of Aβ. A study by Cleary et al. (2005) proposed that the soluble dimers and trimers of prefibrillar Aβ were both necessary and sufficient to cause impairment of hippocampal long term potentiation of short term memory in a rapid, potent and transient manner. If the soluble forms of oligomerised Aβ precede the explicit synapse degeneration and neuronal death (Lee 2006; Walsh 2004), it becomes a crucial target for disease-modifying agents and reinforces the importance of developing an early detection and prevention methodology.

Transgenic mouse model experiments have not only provided evidence of efficacy but also suggested possible mechanisms of action which are not always the most expected. Several studies propose very little actual penetration of the CNS (only 0.02% to 1.5% of the total antibody is distributed to the CNS) and furthermore, only 3% to 4% of total Aβ produced daily is cleared by passive immunotherapy (Bacher 2008). The observed calculations advocate a more complex mechanism of efficacy which goes beyond the simple binding to and clearance of Aβ proteins by antibodies.

Mechanisms of action of passive immunisation

Following the initial transgenic animal studies, several possible mechanisms of the action of passive immunisation were proposed. The most plausible of these are being tested via clinical trials with some having promising progress to phase III trials.

The peripheral sink hypothesis

The observation of limited antibody actually entering the CNS has led to the suggestion that the antibody mediates its therapeutic action via peripheral over central mechanisms. The peripheral sink hypothesis postulates that the peripherally circulating antibodies draw out the centrally located Aβ protein, eventually leading to its degradation and clearance. This thereby indirectly modifies disease progress in the CNS (DeMattos 2001). DeMattos' follow up study reported in the journal Science in 2002 lends support to this theory as well as proposing an interesting potential method of early AD diagnosis using monoclonal antibodies (DeMattos 2002).

The effect of m266 on plasma Aβ levels was assessed (DeMattos 2002).

Mice were killed and neuropathological assessments-were carried out by investigators blind to the plasma Aβ levels. One hemisphere was investigated by quantitative Aβ -immunofluorescent and thioflavine-S (amyloid) staining to ascertain the Aβ and amyloid load of the hippocampus and cingulate cortex. The other hemisphere was assessed by ELISA. The study revealed two interesting points (DeMattos 2002): 1. The level of plasma Aβ rise markedly after administration of m266 supporting the "peripheral sink" hypothesis since the monoclonal antibody seems to somehow draw the Aβ from the CNS. 2. There is a high correlation between the plasma Aβ40 and Aβ 2 level and load of Aβ in the assessed brain regions. Taken together, not only does the monoclonal immunoglobulin therapy have potential therapeutic value but also the encouraging premise of the presence of the antibody quantifying Aβ load or progression in the brain, offering potential for early detection of those at risk or disease progression in those diagnosed (DeMattos 2002).

The case is by no means closed and the"peripheral sink"hypothesis is being tested in perhaps the ultimate way. The central epitope of Aβ to which m266 binds is coveredduring oligomerisation or aggregation of Aβ. As m266 enters phase III clinical trials its efficacy will provide support or opposition to the hypothesis since

m266 is completely devoid of binding to the oligomers or aggregates of Aβ.

Antibodies targeting the N-terminal of Aβ protein

Many voices in the field of immunotherapy development argue that the most beneficial approach of antibody mediated dissolution and clearance of Aβ plaque is by direct antibody binding to the N-terminal epitope of Aβ and therefore most avidly recognising the pathological feature of the disease. Studies in transgenic animals have displayed efficacy with this approach and thus warranted the prototypical monoclonal antibody in this group, Bapineuzumab, to be tested through rigorous human clinical trials, the details of which will be further discussed later.

Polyclonal methodology

Yet another different approach within passive immunisation is using a multivalent or polyclonal antibody therapy. This is a rather holistic tactic and the antibodies will target the dissolution of Aβ plaques, inactivation of soluble oligomers of Aβ and the drawing out of Aβ via the "peripheral sink" mechanism.The antibodies are naturally occurring human immuno-globulins (IVIg) against Aβ. IVIgG treatment has been shown to increase CSF and serum levels of anti- Aβ antibody and decrease CSF Aβ level, possibly by the "peripheral sink" effect (Dodel 2004). The harnessing and research of these antibodies is spear-headed by the collaboration of Alzheimer Cooperative Study Group and Baxter inc. and subsequently, naturally occurring Aβ antibodies have been deemed worth further investigation, entering human clinical trials.

Further investigations of passive immunotherapy in Human AD

Many agents of passive immunisation, having shown promise in animal models and preliminary human trials, are currently undergoing testing in phase III clinical testing. There are currently twelve passive immuno-therapeutic agents undergoing clinical trials, all utilising various mechanisms of action. The clinical trials of key three studies, prototypical of each of the mechanisms introduced above will be discussed.

Clinical trials of Solanezumab (LY2062430) and the testing of the "peripheral sink" hypothesis

Solanezumab is the humanised monoclonal analogue of murine antibody m266. In a single dose clinical trial involving 16 patients with mild to moderate AD, solanezumab was seen to be well-tolerated with only self-contained infusion reaction symptoms. MRI scans and CSF analyses revealed no meningoencephalitis, vasogenic oedema or microhaemorrhages (Siemers 2010). A significant dose-dependent increase in soluble Aβ in plasma and CSF was detected and this was attributed to the binding of Aβ by solanezumab. With no significant safety concerns, phase II trials went ahead.

The primary purpose of phase II trials was to again test the safety and tolerability of the agent but this time in a multiple dose regimen. The pharmacokinetics of solanezumab was also observed to gauge dosage suitability for future clinical trials. The cognitive efficacy of the agent was studied as well using ADAS-cog.

Solanezumab was again seen to be well- tolerated with no evidence of meningoencephalitis or vasogenic oedema. Treatment-emergent adverse effects were not significantly different from those of placebo treated individuals. As expected, there was no statistically noteworthy difference in cognition between placebo and solanezumab treated patients since the period of study was over 12 weeks during which no significant decline was observed for placebo patients. An interesting point here highlights the caution with which transgenic animal model studies must always be approached. Mice treated with a single dose of m266 were shown to have improved performance in a memory test (Dodart 2002) whereas no such cases are reported in human trials. This shows that direct translation and prediction from animal models to humans cannot be assumed.It was deemed appropriate to use unit dosing of solanezumab rather than by body weight because although the clearance of antibody was weight dependent, the scale was not clinically significant.

It was shown shows quite conclusively that an increase in plasma Aβ levels followed solanezumab infusion, lending support to the "peripheral sink" hypothesis. Based on the successful tolerability and safety results of the phase II trial, as well as the determination of a suitable dosing program and clinical efficacy, solanezumab is currently undergoing phase III trials. The trial tests clinical efficacy on cognition with 400mg of solanezumab administered once every 4 weeks by i.v. infusion for 100 weeks and is estimated to be completed in 2014 (clinicaltrials.gov).

Clinical trials of Bapineuzumab

Bapineuzumab presents an interesting case study because its progress and eventual termination highlights the sheer difficulty of developing a new agent for patient use.Bapineuzumab entered phase II trials with much promise. 234 patients were randomly assigned to receive bapineuzumab or placebo in an 8:7 ratio. The agent was administered 6 times in total every 13 weeks as an i.v. infusion for a period of 18 months. The trial gave intriguing results distinguishing between APOE ε4 carriers and non-carriers. The major safety concern was the 9.7% incidence of vasogenic oedema in patients treated with bapineuzumab (Salloway 2009). The cases of vasogenic oedema seemed to positively correlate

with the dose of bapineuzumab and APOE ε4 gene dose. The aetiology of vasogenic oedema is still not fully understood but it is suspected to be caused by increased amyloid burden in the vasculature in APOE ε4 carriers than non-carriers (Chalmers 2003). This resulted in two implications for phase III trials; 1. Administration of lower doses of bapineuzumab 2. Recruitment of APOE ε4 non-carriers.Although the phase II trial failed to give evidence of definitive clinical efficacy, due to overall favourable safety and measurable test scores, accordingly tailored phase III trials were carried out. However the phase III trial, fully named "A long term safety and tolerability study of bapineuzumab in Alzheimer's disease patients" was terminated in August 2012 because two large phase III studies showed no clinical protection from cognition and functional decline. Despite the disappointing outcome, many believe that there is still a role for immunotherapeutic intervention but that it may have to be given in the prodromal phase. The fact that too little a dose had to be given has been attributed to the lack of efficacy as well.

Clinical trials of IVIg

The results of phase I trial of IVIg werepromising with six out of eight participants having increased or stable MMSE after treatment.After 6 months a group mean increase of 2.5 in MMSE was observed. This is substantially better than the mean decline of 1.5 expected in AD patients typically over a 6 month period. Furthermore, the reversal of the cognitive benefits and increase in CSF Aβ40 and Aβ42 after 3 months cessation of IVIg treatment, coupled to the regain of cognitive stability and reduction in CSF Aβ40 and Aβ42 levels with re-induction of IVIg holds promise for passive immunisation for AD. With no serious adverse effects such as meningoencephalitis or intracerebral haemorrages, phase II trials went ahead (Relkin 2009).

The results of the nine month interim for the phase II trial was carried out by Relkin and received much attention at the 2008 international conference of Alzheimer's disease (ICAD). Four patients each received four different doses of the IVIg treatment and 8 received placebo for 24 weeks with evaluation at 12 and 24 weeks (clinicaltrials.gov). Although the scale of the study was small, some of the results displayed much potential. The nine month interim data showed statistical improvement in primary outcome measures such as ADAS-Cog and ADAS-Clinical global impression of change. However, the results of secondary outcome measures such as ADAS-Activities of daily living scales, CSF analyses, PET scans and global cognitive measures were not published. Furthermore, the poster of the findings created confusion requiring Relkin to explain that the main conclusion to draw from the phase II trials is that the symptomatic improvements seen in phase I remain stable for nine months in this second study. Due to the small scale of the studyand the fairly crude criteria of cognitive studies, a larger and more detailed study must be carried out. A double-blind, placebo-controlled, two dose arm, parallel study looking into the safety and effectiveness of human intravenous immunoglobulin for treatment of mild to moderate AD phase III trial is currently underway, estimated to be completed in 2013 (clinicaltrials.gov).

TOXICITY

Although the serious adverse-effect of meningoencephalitis as in the AN1792 trial is not present with passive immunisation, the vasogenic oedema seen with bapineuzumab is a concern. Although bapineuzumab trials have been terminated, the aetiology of vasogenic oedema is still not precisely characterised and in theory could also arise with other antibody therapy.

There are many other safety concerns in any antibody infusion to consider when assessing the risk-benefit evaluation. Intracerebral haemorrhage, posterior reversible encephalopathy, ischaemic stroke, myocardial infarction, pulmonary embolism and acute renal failure are some important examples of potential adverse events (Belmouaz 2008, Gupta 2001). The risk of myocardial infarction, stroke and haemorrhage are often increased in AD patients already as underlying cardiovascular diseases become more prevalent with age (Hefer 2004). This is a crucial point that should influence the administration of passive immunotherapy if and when it becomes available for the general population. Even before, the exclusion or enrolment of such predisposed patients in clinical trials should be carefully conducted and reported as reducing the risk of adverse events is just as critical as improving efficacy of treatment.

DISCUSSION

The emergence of passive immunotherapy through its trials and successes has been discussed with the evaluation of the latest developments in the field. The overall feedback of recent trials emphasises a mixed view of the potential of passive immunotherapy for the treatment of AD. However, the failure of a particular trial does not suggest or imply that agents pursuing a passive immunisation strategy should be abandoned. Valuable lessons are learned from each trial (such as the potential to utilise passive immunisation as a biomarker of the gravity of AD) and by taking these on board, greater understanding of the mechanism of action, dosage and time of administration can be obtained to finally achieve the first "disease-modifying" agent for AD. The two key lessons to be taken on board for passive immunotherapy are: time of administration and dosage.

The state of the disease at the time of therapy administration is absolutely crucial in determining the efficacy of the agent. This may explain some of the disappointing results seen in the trials discussed above. The biochemical reasoning certainly makes sense since for example solanezumab (LY2062430) is likely to have little utility in modifying disease state once oligomeric Aβ and Aβ plaques begin to form as these are not its substrates. Similar reasoning may extend to the N-terminal targeting agent bapineuzumab and polyclonal IVIg therapies that they are most efficacious at the early stages of the disease.

Furthermore, the correct dosing of the agents is a vital challenge to address. The dosage of agent administration had often been limited due to the side-effect of vasogenic oedema such as the case with bapineuzumab. Undoubtedly, vasogenic oedema needs to be minimised but the underlying issue may have been dosing, not lack of efficacy. This gives scope for refinement and development of the agent in question to be able to increase the dosage for a greater clinical effect but still minimise side-effects.

CONCLUSION

Passive immunotherapy certainly needs to overcome some fundamental challenges for it to become a viable treatment for AD. The animal models and human clinical trials have given useful insights into the problems that need to be addressed such as optimal time of administration, dosage and epitope targeting. Passive immunisation is one of the most active areas of research in AD currently and with the development of agents that address these caveats, passive immunotherapy is certainly a viable option which could become the first disease-modifying therapy for AD.

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