

## Current State of the Art in Preclinical Research in Alzheimer's Disease – A Focus on Mode of Action in Pharmacological and Non-pharmacological Approaches

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### Abstract

Alzheimer's disease (AD) is an age-related neurodegenerative disorder characterised by progressive memory deficits and other cognitive disturbances. Neuropathologically, AD is characterised by synaptic deficits, progressive loss of neocortical, limbic and basal forebrain cholinergic neurons and the abnormal extracellular accumulation of amyloid-beta (A $\beta$ ) and the intracellular aggregation of the cytoskeletal protein tau. Currently available AD therapies either only temporarily delay disease progression or address the symptoms but are unable to alter the underlying mechanisms of disease. Therefore, ongoing AD research is focused at better understanding pathogenesis and at developing disease-modifying experimental therapeutic approaches. This review will summarise the main areas of preclinical research for AD therapeutics that includes those aimed at modulating the processing of amyloid precursor protein (APP) and the production of A $\beta$ ; ameliorating the pathological accumulation of A $\beta$  or tau; augmenting neuroprotective activities in the AD brain; and augmenting neurorestoration in the AD brain. The review will also discuss a novel multimodal therapeutic approach to AD using Cerebrolysin, a peptidergic mixture with neurotrophic-like effects.

### Keywords

Neurotrophic factors, synapses, ageing, neurodegeneration, Cerebrolysin

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Alzheimer's disease (AD) is the seventh most prevalent cause of death in the US and is the leading cause of dementia, affecting more than 5 million Americans and 26 million people worldwide. Without an effective therapy it is estimated that the number of patients with AD will double by the year 2050.<sup>1</sup> Cognitive impairment in patients with AD is closely associated with loss of synapses and the formation of neurofibrillary tangles (NFT) in the neocortex and limbic system.<sup>2–6</sup> The two major neuropathological findings in patients with AD are extracellular plaques formed mainly of the amyloid-beta (A $\beta$ ) peptide,<sup>7–9</sup> and intracellular NFT, containing hyperphosphorylated tau.<sup>10–12</sup> Several lines of investigation support the view that increasing levels of A $\beta$ <sub>1–42</sub>, the proteolytic product of amyloid precursor protein (APP) metabolism, might be centrally involved in the pathogenesis of AD<sup>7–9,13</sup> and it has been proposed that in AD, progressive accumulation of A $\beta$  might be involved in the mechanisms underlying NFT formation and synaptic loss.<sup>14–17</sup> More specifically in recent years the potential role of neurotoxic A $\beta$  oligomers has emerged as a topic of considerable interest.<sup>18–21</sup>

AD medications currently prescribed are aimed at individuals with mild to moderate AD and include drugs such as donepezil, rivastigmine and

galantamine, all of which are acetylcholinesterase inhibitors and work by preventing the breakdown of acetylcholine and stimulating nicotinic receptors to release acetylcholine in the brain. Memantine, another drug currently approved for use in moderate to severe AD, is an N-methyl-D-aspartate (NMDA) receptor antagonist and acts on the glutamatergic system by blocking the toxic effects associated with excess glutamate, thereby regulating glutamate activation. In addition to its activity at the NMDA receptor, memantine also acts as a non-competitive antagonist at the 5-hydroxytryptamine (5-HT) serotonin and nicotinic receptors. Although each of these drugs has demonstrated treatment effects on the cognitive, functional and behavioural problems commonly associated with AD, these drugs simply slow the progression of AD but do nothing to tackle the underlying pathogenesis. In this context, there has been real interest in elucidating the main pathways involved in AD pathogenesis and developing therapies acting on these key pathways.

This review will focus on preclinical experimental therapies being investigated for AD, with particular focus on the role of multimodal therapies, typified by Cerebrolysin (CBL), a peptide mixture with neurotrophic-like effects.

## Areas of Preclinical Therapeutic Research

The last few decades have seen an exponential increase in our understanding of mechanisms underlying AD pathogenesis, and this has led to increased diversity in the modes of action of experimental AD therapies (see *Figure 1*).

Many of the current experimental approaches towards AD therapy fall into the following broad categories:

- those aimed at modulating the processing of APP and the production of A $\beta$ ;
- those aimed at ameliorating the pathological accumulation of A $\beta$  or tau;
- those aimed at augmenting neuroprotective activities in the AD brain; and
- those aimed at augmenting neurorestoration in the AD brain.

Each of these major approaches will be addressed below; additionally we shall highlight CBL, a unique neurotrophic therapeutical approach for AD that has a multimodal mechanism of action. However, it should be noted that some key approaches to AD therapy, such as modulation of the inflammatory response, are topics in their own right and are too broad for the scope of this review; for a discussion on this topic the reader is referred to a number of recent review articles.<sup>22,23</sup>

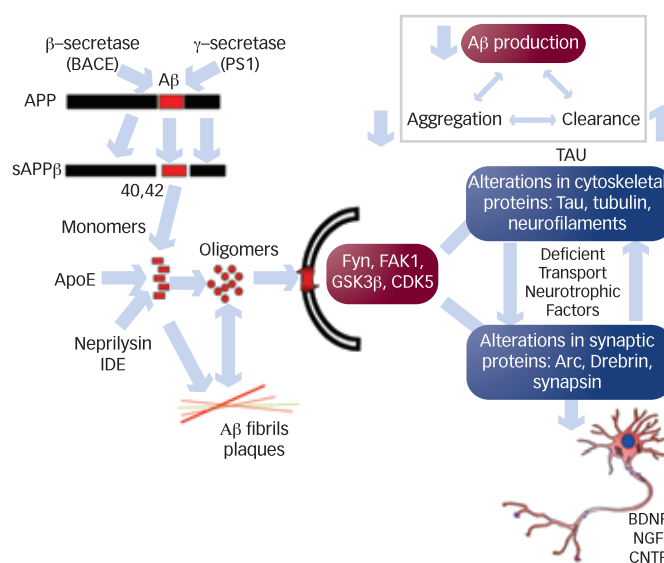
## Therapies Aimed at Modulating Amyloid Precursor Protein Processing

A $\beta$  is formed by the proteolytic cleavage of the APP, a single-pass transmembrane protein with a large extracellular domain. APP processing occurs as a result of the sequential action of a group of enzymes called secretases. Secretase processing can occur via two separate pathways, the first of these is the non-amyloidogenic pathway, in which the sequential action of  $\alpha$ - and then  $\gamma$ -secretase results in the formation a C-terminal fragment, a soluble-APP $\alpha$  and eventually the APP intracellular domain (AICD).<sup>24,25</sup> The second pathway is the amyloidogenic pathway and involves the initial cleavage of APP by  $\beta$ -secretase followed by the action of  $\gamma$ -secretase which now generates multiple forms of A $\beta$  with A $\beta$ <sub>1-40</sub> and A $\beta$ <sub>1-42</sub> being the most common.<sup>7,9,13,25,26</sup> Many lines of investigation have shown that A $\beta$  generated via the amyloidogenic pathway has a strong propensity to form fibrils and to aggregate, with A $\beta$ <sub>1-42</sub> being more aggregenic than A $\beta$ <sub>1-40</sub>.<sup>13,27</sup> The processing of APP to A $\beta$  and the subsequent aggregation of A $\beta$  are thought to be key pathological events in the AD cascade and a great deal of research has focused on factors involved in APP processing and methods to modulate processing along the amyloidogenic pathway.<sup>7,9,26</sup>

Most therapeutic approaches for AD have been focused at reducing A $\beta$  accumulation by decreasing APP metabolism by blocking  $\beta$ - or  $\gamma$ -secretase activity,<sup>28-33</sup> by preventing aggregation of A $\beta$ <sup>34,35</sup> or by promoting clearance.<sup>36,37</sup>

Secretase inhibitors have been designed against both  $\beta$ - or  $\gamma$ -secretases and both have been shown to lower plasma A $\beta$  in rodents,<sup>38</sup> and in cerebrospinal fluid (CSF) and plasma in primate models of the disease.<sup>39</sup> However, the development of these inhibitors has been hampered by the need to have them pass through the blood-brain barrier (BBB), by off-target effects (such as those associated with  $\gamma$ -secretase inhibitors) and their ability to cleave Notch (a transmembrane receptor involved in regulating cell fate

**Figure 1: Points of Entry for Experimental Treatments for Alzheimer Disease**



A $\beta$  = amyloid-beta; ApoE = apolipoprotein E; APP = amyloid precursor protein; BDNF = brain-derived neurotrophic factor; CDK5 = cyclin-dependent kinase 5; CNTF = ciliary neurotrophic factor; FAK1 = focal adhesion kinase 1; GSK3 $\beta$  = glycogen synthase kinase 3 beta; IDE = insulin-degrading enzyme; PS1 = presenilin 1; NGF = nerve growth factor; sAPP $\beta$  = soluble APP beta.

decisions) and finally with adverse side effects and toxicity.<sup>40,41</sup> Two large Phase III clinical trials of semagacestat, a  $\gamma$ -secretase inhibitor, in mild to moderate AD patients were prematurely terminated due to cognitive and functional side effects of the drug.<sup>42</sup> The recent results from clinical trials with secretase inhibitors seem to suggest that despite showing early promise at the preclinical stage, they have failed to live up to expectations in clinical trials. At present, most *in vivo* experimental research has been focused at testing  $\gamma$ -secretase modulators and at developing more specific and BBB penetrating  $\beta$ -secretase inhibitors. Work is also underway to develop compounds that stimulate  $\alpha$ -secretases.<sup>43</sup>

## Therapies Aimed at Ameliorating the Pathological Accumulation of Amyloid-beta or Tau

This includes therapies aimed at reducing the aggregation of A $\beta$  and tau<sup>44-47</sup> and at increasing clearance. Strategies contemplated for the removal of pathologically accumulated proteins include approaches such as chaperone-mediated clearing, stimulation of autophagy or proteosomal activity or increasing the proteolytic cleavage (for example by using Neprilysin to cleave A $\beta$ ) or by modulating chaperone-like activity.<sup>48,49</sup>

Chaperone-mediated clearance co-opts the natural ability of all cells to conduct a quality control process designed to prevent the build-up of abnormally folded or toxically aggregated proteins. Chaperones can inhibit protein misfolding, interfere with abnormal aggregation and may even promote the correct folding of misfolded proteins.<sup>50,51</sup> Chaperones can also target misfolded proteins for degradation via various cellular pathways including the ubiquitin-proteasome pathway or chaperone-mediated autophagy.<sup>52-54</sup> The best characterised chaperones are the heat shock proteins (HSPs)<sup>55-57</sup> and a number of studies suggest that up regulation of HSPs can suppress A $\beta$  aggregation and toxicity.<sup>58,59</sup> Collectively, these studies support a potential therapeutic application of chaperone function modulation for disorders

that are characterised by protein misfolding and aggregation, such as AD. Moreover, HSPs such as Hsp70 and Hsp90 have been shown to play a significant role in tau clearance and processing.<sup>60,61</sup>

An alternative approach to the clearance of proteins has been the use of antibodies targeting pathological forms of A $\beta$  or tau. The observation that elderly AD patients express autoantibodies against A $\beta$ <sup>62</sup> and tau<sup>63</sup> suggests that the immune system is capable of mounting a response against the pathological forms of these proteins. In this context a number of groups conducted studies aimed at inducing or enhancing this immune response. To date, immunotherapeutic approaches to AD have mostly targeted A $\beta$ , as it is a secreted protein that can be found in plasma and CSF and is easily accessible to circulating antibodies.<sup>64</sup>

The first immunotherapeutic approach based on preclinical pioneering work by Shenck et al.<sup>65,66</sup> to reach the clinical trial stage was an active immunisation protocol using the AN1792 antibody (Elan Pharmaceuticals, Inc). A number of positive features of this trial included the ease of administration and the prospect of lifelong immunity. However, this trial was halted in 2002 when a small number of participants reported adverse side effects,<sup>67</sup> these effects have since been linked to the choice of adjuvant and a T cell recognition site. Subsequent clinical trials have included active immunisation with CAD-106 (Novartis), a peptide vaccine that contains a short N-terminal fragment of A $\beta$  which reportedly does not induce the T cell response observed with AN1792.<sup>64</sup> Results from this trial report no significant differences between CSF A $\beta$  levels and magnetic resonance imaging (MRI) whole brain volumes between treated and placebo patients.<sup>68</sup>

Based on preclinical studies in the platelet-derived growth factor beta-APP (PDAPP) model of AD,<sup>69-71</sup> a transgenic (tg) mouse model of AD that overexpresses mutant APP, a number of passive immunisation approaches have also reached clinical trial stage including the Phase II Elan/Wyeth antibody Bapineuzumab trial, which showed side effects such as vascular oedema in the high dose cohort (2.0 mg/kg) resulting in this dose being excluded from the Phase III trial.<sup>72-74</sup> Bapineuzumab has also been reported to reduce cortical PiB retention in AD patients.<sup>75</sup> While recent Bapineuzumab clinical trial results have failed to produce significant cognitive improvements in patients, results from animal models suggest that this approach may be better suited to the early, preclinical stages of the disease and therefore may have a more preventative rather than therapeutic function. Another passive immunisation approach is the humanised monoclonal antibody Solanezumab from Eli Lilly, which was also well-tolerated at lower doses and showed a dose-dependent increase in CSF and plasma levels of A $\beta$ .<sup>76</sup>

In recent years some groups have investigated the possibility of utilising immunotherapy to target abnormally phosphorylated tau. As previously mentioned, NFT form the other neuropathological hallmark of AD, these are intracellular accumulations of tau, a microtubule associated protein, which is hyperphosphorylated at multiple epitopes.<sup>77-79</sup> This hyperphosphorylation has been linked to tau propensity to aggregate and a loss-of-function with regard to the ability of tau to stabilise the microtubule structure and facilitate axonal transport.<sup>80-84</sup>

Two recent studies have shown that immunisation against phosphorylated forms of tau might be effective at reducing NFT

pathology *in vivo* and slowing the progression of behavioural deficits in tg mouse models of AD.<sup>85-87</sup>

Unlike A $\beta$ , which is a predominantly extracellular protein, tau and the hyperphosphorylated forms of tau are intracellular and have traditionally thought to be inaccessible to antibodies. However, recent work has shown that aggregates of  $\alpha$ -synuclein, an intracellular synaptic protein that accumulates in the brains of patients with Parkinson's disease (PD) and AD were reduced following active<sup>88</sup> and passive<sup>89</sup> immunisation against  $\alpha$ -synuclein in a tg mouse model, indicating that intracellular proteins could also be potential targets of immunisation. This might be related to recent findings in *in vivo* models showing that aggregated  $\alpha$ -synuclein<sup>90</sup> and tau<sup>91,92</sup> can be released from neurons and propagate to other cells in a prion-like fashion.

In 2007, Asuni et al. demonstrated that active immunisation with the tau peptide 379–408, phosphorylated at serine residues 396 and 404 (Tau379–408: Ser396/404) was effective at reducing the levels of tau aggregates in the brain of P301L tg mice, a model of tauopathy.<sup>85</sup> These particular epitopes were chosen as they were known to be pathological forms of tau found in AD brains and had been reported to increase the fibrillogenic nature of tau increasing its propensity to assemble into paired helical filaments (PHFs). Asuni et al. showed that active immunisation with the Tau379–408: Ser396/404 peptide was capable of inducing antibodies against the phosphorylated forms of tau and in the immunised mice the reduction in tau aggregation was accompanied by an amelioration of the sensorimotor deficits associated with tau pathology.<sup>85,87</sup>

## Neuroprotective Approaches to Alzheimer's Disease Involving Key Pathways and Signalling Molecules

Oxidative stress has been proposed to be a key pathway underlying pathogenesis in AD.<sup>93,94</sup> A $\beta$  has been reported to bind to mitochondrial membranes, interact with heme and interfere with the normal electron flow through the respiratory chain, resulting in a faulty mitochondrial energy metabolism and in an increased production of reactive oxygen species (ROS).<sup>95</sup> Antioxidant therapies such as vitamin E and resveratrol have been investigated in animal models for their therapeutic potential in AD, with varying degrees of success.<sup>95</sup> While memantine, a currently available AD therapy, targets the NMDA receptor, further studies on the glutamate system in AD have focused on the role of other glutamate receptors including alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) and metabotropic glutamate receptors.<sup>96</sup>

The loss of AMPA receptor-mediated transmission in AD has been reported to be modulated by A $\beta$ <sub>1-42</sub>.<sup>97-99</sup> A recent study found that an agent that blocks AMPA receptor desensitisation, cyclothiazide, prevented oligomer-induced reduction of excitatory postsynaptic currents (EPSCs), consistent with A $\beta$  acting by inhibiting glutamate uptake.<sup>100</sup> Furthermore, micromolar concentrations of synthetic A $\beta$ <sub>1-42</sub> oligomers, especially in the presence of cyclothiazide, can rapidly trigger AMPA receptor-dependent inward currents and delayed neurodegeneration in cultured cortical neurons.<sup>101</sup> Taken together, these findings indicate that agents designed to directly boost AMPA receptor function in AD may have a relatively narrow therapeutic window.

Stimulation of Group 1 metabotropic glutamate receptors (mGluR 1 and 5) leads to activation of a wide variety of signalling pathways and other

downstream protein kinases, such as extracellular signal-regulated protein kinases (ERK) 1/2 and Akt, which in turn have been implicated in cellular growth, differentiation and survival.<sup>102</sup> mGluR1/5 signal transduction is complex and involves multiple partners, and alterations in mGluR signalling have been implicated in neurodegenerative disorders such as AD, PD<sup>103</sup> and Huntington's disease.<sup>104</sup> mGluR5 inhibitors, currently employed for the treatment of Fragile X syndrome, have been shown to reduce A $\beta$  production in rodent models.<sup>102</sup>

A number of groups have investigated signalling pathways involved in AD, and the Wnt pathway has been a particular area of interest. Tight regulation of Wnt signalling is a prerequisite for normal neural development as well as for the maintenance of neuronal homeostasis and synaptic plasticity in adults.<sup>105,106</sup> Previous studies have linked Wnt signalling to neurodegenerative disorders such as AD.<sup>107,108</sup> In fact, strong evidence suggests that a loss of Wnt function is implicated in the pathophysiology of neuronal degeneration of AD. Compounds such as rosiglitazone, a peroxisome proliferator-activated receptor- $\gamma$  agonist, and lithium have been shown to attenuate the neurotoxic effects of A $\beta$ , both of these drugs activate Wnt signalling.<sup>109</sup> In addition to its effect on glycogen synthase kinase 3 beta (GSK3 $\beta$ ), lithium is known to modulate cyclin-dependent kinase 5 (CDK5), another kinase that has been linked to the abnormal hyperphosphorylation of tau in AD.<sup>110,111</sup>

Oxidative stress, glutamate signalling and the Wnt signalling pathway are only a few of the pathways thought to play a role in AD.<sup>112</sup> Given the number of alternate pathways implicated in AD, such as p38, Akt, c-Jun N-terminal kinase (Jnk), among others, it seems likely that the most efficacious AD therapies would act upon a number of these pathways in tandem.

### Neurorestorative Approaches Aimed at Neurogenesis and Synaptic Plasticity

Since the first report of the production of new neurons in the adult hippocampus,<sup>113</sup> research has shown that various neurotransmitter systems, growth factors, neurotrophins, cytokines and hormones are major regulators of neurogenesis.<sup>114</sup> Studies in tg animal models of different neurological disorders have shown significant alterations in neurogenesis in the hippocampus under pathological conditions.<sup>115</sup>

Interestingly, a number of molecules central to AD have been found to play a regulatory role in adult neurogenesis.<sup>116–118</sup> Mutations of presenilin 1 (PS1) have been shown to negatively affect the production of new neurons.<sup>116,119,120</sup> Similarly, the proliferation and survival of neuronal precursor cells was shown to be reduced in tg mice expressing a chimeric mouse-human APP-695swe (APPswe) polypeptide, a mutated form of APP that causes early onset familial AD (FAD).<sup>121</sup>

In the mature brain, neurogenesis is believed to play an important role in maintaining synaptic plasticity and memory formation in the hippocampus.<sup>87</sup> In AD, the most significant correlate to the severity of cognitive impairment is the synaptic loss in the frontal cortex and the limbic system.<sup>122–125</sup> Synaptotoxic effects have been observed with soluble A $\beta$  oligomers prepared from multiple sources such as synthetic A $\beta$  peptides, APP-transfected cell culture supernatants, APP tg mouse brain and even human AD brain tissue.<sup>126</sup> At nanomolar to low micromolar concentrations, soluble A $\beta$  oligomers impair excitatory synaptic transmission, inhibit long-term potentiation (LTP), induce loss of dendritic spines and impair rodent spatial memory.<sup>127–133</sup> In contrast to suppression of LTP, long-term depression (LTD) is unaffected or

even enhanced by A $\beta$ .<sup>134</sup> Thus, in terms of synaptic plasticity, exposure to A $\beta$  seems to favour the weakening and oppose the strengthening of synapses. Consistent with its functional effects on LTP and LTD, prolonged exposure to A $\beta$  leads to morphological loss of synapses.<sup>126,135,136</sup> A $\beta$ <sub>1–42</sub>, which is more prone to aggregation and more toxic than A $\beta$ <sub>1–40</sub>, is also more effective at impairing LTP and reducing spine density. Furthermore, dysregulated cholinergic signalling is an early hallmark of AD and it is interesting to note that A $\beta$  oligomers have been shown to induce cholinergic neurodegeneration in the nucleus basalis<sup>137</sup> and, at nanomolar concentrations, to inhibit the activity of choline acetyltransferase (ChAT).<sup>138</sup>

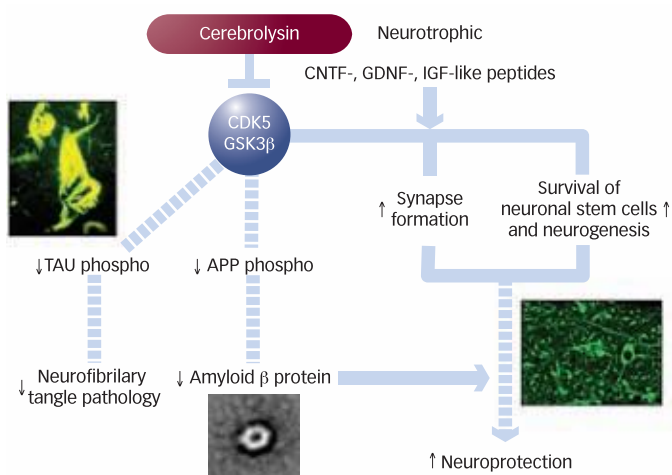
Given the deficits in neurogenesis, synaptic plasticity and deficits in glutamatergic and cholinergic systems in AD, it would be reasonable to hypothesise that compounds capable of intervening in these processes would show a serious promise in the development of new treatments. One such type of compound may be related to modulation the activity of neurotrophic factors (NTFs). NTFs are secreted proteins that promote the differentiation, growth and maintenance of developing neurons and the survival of adult neurons<sup>139–141</sup> and include members such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), signalling molecules in various cellular pathways, which regulate development, proper functioning, survival and regeneration of nervous tissue under physiological conditions and most importantly after injury. NTFs including NGF and BDNF have been widely reported to be altered in a number of neurodegenerative disorders including AD,<sup>142–144</sup> and many studies have looked at NTF replacement strategies in an effort to stave off neuronal dysfunction and death in these disorders with a number reporting beneficial effects.<sup>145</sup> The results of the first clinical trial of NGF gene therapy in AD were published in 2005<sup>146</sup> and reported no long-term adverse effects of implanting autologous fibroblasts genetically modified to express human NGF into the forebrain.

BDNF gene delivery into tg mice expressing mutated (Swedish K670M/N671L, London V717I) human (h) APP751 under control of the mouse thymus cell antigen-1 (mThy-1) promoter (mThy1-hAPP751 tg mice; Line 41)<sup>147</sup> reverses synapse loss, partially normalises aberrant gene expression, improves cell signalling and restores learning and memory.<sup>148</sup> These outcomes occur independently of effects on amyloid plaque load. In aged rats, BDNF infusion reverses cognitive decline, improves age-related perturbations in gene expression and restores cell signalling.<sup>148</sup> The therapeutic effects of BDNF have also been assessed in non-human primate models, where BDNF gene delivery to the entorhinal cortex, significantly ameliorated lesion-induced entorhinal cortical neuronal death, improved hippocampus-dependent memory and increased neuronal size.<sup>148</sup>

Given the large molecular weight of NTFs, such as NGF and BDNF in comparison with chemical compounds, the key challenge in the field of growth factor therapy is drug delivery to the CNS and to this end a number of NTF-based therapies are currently in the clinic, including those focusing on methods of delivering NTFs, particularly NGF (AAV-NGF, [CERE-110, NCT00876863]), encapsulated cell biodelivery of NGF [NCT01163825].

AD, like many neurodegenerative disorders, occurs as a result of the progressive loss of structural or functional integrity of neurons. In this context, many groups have tried to replace damages/lost neurons in the hope of restoring neuronal function and have explored stem cell therapy as an alternative neurorestorative approach in models of

**Figure 2: Multimodal Mechanisms of Action of Cerebrolysin in Preclinical Models of Alzheimer Disease**



APP = amyloid precursor protein; CDK5 = cyclin-dependent kinase 5; CNTF = ciliary neurotrophic factor; GDNF = glial derived neurotrophic factor; GSK3β = glycogen synthase kinase 3 beta; IGF = insulin-like growth factor.

neurodegenerative conditions such as PD.<sup>149</sup> Rodent AD models receiving neural precursor cell (NPC) grafts demonstrate increased hippocampal synaptic density and increased cognitive function associated with BDNF production.<sup>150,151</sup> Combining engineered growth factor overexpression with the benefits of NPC integration into neural networks may provide an enhanced approach to treating AD. The results of these will be interesting in light of recent stem cell studies in PD that have shown host to graft transmission of  $\alpha$ -synuclein.<sup>152,153</sup>

Given the multiple mechanisms associated with AD pathology, and the apparent failure of single-target approaches to therapeutics, it is likely that the most efficacious AD therapy will target multiple components of the AD pathophysiological cascade from the processing of APP, the abnormal accumulation of A $\beta$ , hyperphosphorylation of tau combined with a neuroprotective and neurorestorative neurotrophic factor-like effect.<sup>154</sup> It is most likely that truly disease-modifying treatment for AD will be a multi-target compound with actions at intersecting nodes on pathogenic pathways associated with AD.

## A Multimodal Approach to Alzheimer's Disease – Preclinical Studies in Transgenic Mice

One example of a multimodal approach to the treatment AD is the J147 compound, a curcumin derivative which has a broadly neuroprotective effect including the ability to prevent memory deficits in a tg mouse model of AD associated with an increase in levels of BDNF and BDNF responsive proteins, enhancement of LTP, synaptic preservation, reduction oxidative stress and inflammation and lower levels of soluble A $\beta$ <sub>1-42</sub> and A $\beta$ <sub>1-40</sub>.<sup>155</sup>

Another interesting set of compounds that display a multimodal activity are antidepressants such as fluoxetine. This family of compounds increase levels of BDNF, glial derived neurotrophic factor (GDNF) and NGF, stimulate neurogenesis, are neuroprotective and stabilise mood.<sup>156-158</sup> A further example of a model multimodal drug is CBL, a neuropeptide preparation produced by a standardised enzymatic breakdown of purified brain proteins (see Figure 2). CBL contains low-molecular-weight peptides (<10 kDa) and free amino acids and has been reported to have beneficial effects in tg models of

AD<sup>159-162</sup> and in human clinical trials.<sup>163-165</sup> CBL is known to be composed of small peptides with neurotrophic activity similar to ciliary neurotrophic factor (CNTF), GDNF and insulin-like growth factors-1 and -2 (IGF-1, IGF-2) and it is the activity of these small peptides that are thought to be involved in the neuroprotective effects of CBL.<sup>166</sup> In addition to effects on disease-related proteins, CBL has been reported to have neuroprotective actions, typified by studies that have shown that CBL protects cholinergic neurons after fimbria-fornix lesion<sup>167-169</sup> as well as protecting the CNS in models of stroke.<sup>170</sup> CBL has recently been shown to modulate the pro-NGF/NGF balance in the mThy1-hAPP751 tg mice resulting in a concomitant amelioration of cholinergic deficits in these mice.<sup>171</sup>

In addition to its neurotrophic-like effects, CBL has been reported to reduce amyloid deposition; this has been linked to CBL-regulated modulation of APP maturation and its intracellular distribution.<sup>161</sup> Studies in mThy1-hAPP751 tg mice have shown that CBL is able to modulate the activity of kinases involved in APP phosphorylation by reducing the levels of the active form of CDK5 and its activators p35 and p25 and increasing the level of inactive GSK-3 $\beta$ .<sup>161</sup>

Furthermore, since GSK-3 $\beta$  and CDK5 are known to phosphorylate tau at epitopes critical for the formation of NFTs, the effect of CBL treatment was investigated in mThy1-hAPP751 tg mice that received bilateral intrahippocampal adeno-associated virus (AAV2)-mutTAU injection and results from these studies confirmed the previously reported modulating effect of CBL on the activity of CDK5 and GSK-3 $\beta$  but also demonstrated a decreased abnormal phosphorylation of tau, resulting in improved neurodegenerative pathology in the hippocampus in the treated mice.<sup>172</sup>

Collectively these studies demonstrate that CBL is able to modulate the aggregenic properties of the key neuropathological hallmark proteins in AD, by either the modulation of APP maturation or modulation of tau hyperphosphorylation, in both cases CBL appears to be having this effect by virtue of its activity on key signalling molecules such as GSK3 $\beta$  and CDK5.

Neuropathological examination of mThy1-hAPP751 tg mice treated with CBL have shown that CBL promotes synaptic regeneration. After treatment with CBL for four weeks, brain sections immunolabelled with antibodies against synaptophysin, a synaptic marker protein showed preservation of synaptic terminals in the frontal cortex and hippocampus of treated mice,<sup>159</sup> an effect which correlated with improved behavioural performance in the Morris water maze – a sensitive procedure to test spatial orientation and learning in rodents.<sup>159</sup>

The neuroregenerative effects of CBL are supported by its ability to promote neurogenesis. The effects of CBL on neurogenesis were studied in mThy1-hAPP751 tg mice injected once-daily with bromodeoxyuridine (BrdU, a marker for the dividing cells) followed by daily intraperitoneal injections of CBL.<sup>160</sup> Compared with wild-type controls, saline-treated tg mice showed decreased numbers of neural progenitor cells (BrdU+ and doublecortin [DCX+]) in the subgranular zone of the dentate gyrus. However, mThy1-hAPP751 tg mice treated with CBL demonstrated a significant increase in the number and migration of these newborn nervous cells, and a decrease in terminal transferase Tdt-mediated dUTP-biotin end labelling (TUNEL) apoptotic activity. CBL had no effect on cell

proliferation or the ratio of neural progenitor cells converting to neurons and astroglia in the neurogenic area of the hippocampus.<sup>160</sup> These findings are in line with previous studies in cultured neural progenitor cells and in normal adult rats investigating the effects of CBL on dentate gyrus neurogenesis.<sup>173</sup>

The protective and reparative effects of CBL have been translated into clinical trials demonstrating that CBL is a well-tolerated treatment for neurological disorders like ischaemic stroke, dementia and traumatic brain injury.<sup>173-176</sup> Clinical trials with CBL have shown that it improves cognition in patients with mild to moderate AD<sup>177-179</sup> and the activities of daily living and psychiatric deficits in patients with moderate to moderately severe AD.<sup>164</sup> Several randomised double-blind studies in patients with AD have shown that CBL is consistently superior to placebo at reducing cognitive alterations.<sup>180,181</sup>

Moreover, the behavioural and other functional benefits, as assessed by neuropsychiatric tests and activities of daily living, respectively, were noted to persist for several months after stopping CBL treatment in patients with AD or vascular dementia.<sup>180,181</sup> A similar persistence of beneficial effect on spatial learning and memory following treatment interruption has been reported in the mThy1-hAPP751 tg mice.<sup>162</sup>

Collectively, the results from animal and human studies show that CBL has the potential to effect AD at many points along the pathological cascade and as such potentially provides a much broader protection than therapies aimed at a single pathological mechanism alone. It is likely that this broad-spectrum approach, coupled with a biomarker-driven earlier detection of AD may represent the future of AD therapy. ■

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