Neurobiological aspects of Alzheimer’s disease

Kanwaljit Chopra†, Shubham Misra & Anurag Kuhad
Pharmacology Research Laboratory, University Institute of Pharmaceutical Sciences, UGC Centre of Advanced Study, Panjab University, Chandigarh, India

Introduction: The molecular pathogenesis of Alzheimer’s disease (AD) includes a variety of risk factors, extracellular deposition of β-amyloid, accumulation of intracellular neurofibrillary tangles, oxidative neuronal damage and inflammatory cascades. Although amyloid-β-containing senile plaques and phospho-tau-containing neurofibrillary tangles are hallmark lesions of AD, neither is specific to nor even a marker of the disease. From a biochemical point of view the most consistent finding is a decreased level of choline acetyltransferase. In recent years, cumulative evidence has been gained on the involvement of neuronal lipoprotein activity, and on the role of cholesterol and other lipids in pathogenesis. Although basic research has made remarkable progress in the past two decades, currently available drugs are only able to improve cognitive symptoms temporarily and no treatment can reverse, stop or even slow this inexorable neurodegenerative process.

Areas covered: The various neurobiological events associated with development of AD and the multiple treatment approaches for combating this disorder.

Expert opinion: AD is a complex multifactorial disorder and thus a single target or pathogenic pathway is unlikely to be identified. Developing therapeutic interventions demands a greater understanding of the processes and the differential involvement of the various mediators. Effective therapeutics are urgently needed, and it is hoped that anti-amyloid strategies will offer a significant step towards a causal therapy.

Keywords: Alzheimer’s disease, animal models, pathobiology, pharmacotherapy

1. Introduction

Alzheimer’s disease (AD) is the common age-related, chronic debilitating neurodegenerative condition that is associated with progressive cognitive decline and profound neuronal loss [1]. It is the most common form of senile dementia, affecting 10% of individual older than 65 and nearly 50% of those older than 85 [2]. It is estimated that there are currently about 18 million people worldwide with Alzheimer’s disease. This figure is projected to nearly double by 2025 to 34 million. The prevalence of AD in the USA is estimated to be approximately 4.5 million. The clinical symptoms result from the deterioration of selective cognitive domains, particularly those related to memory. Memory decline initially manifests as a loss of episodic memory, which is considered a subcategory of declarative memory. The dysfunction in episodic memory impedes recollection of recent events including autobiographical activities. Elucidating the underlying molecular determinants that trigger the disruption of recent episodic memory, and eventually the decline in the other cognitive domains, is among the most crucial unanswered questions in the AD field [3].

In 1907, Alois Alzheimer described two pathological alterations in the brain of a female patient suffering from dementia. These two lesions represent the hallmark...
Neurobiological aspects of Alzheimer’s disease

Article highlights.
- Amyloid-beta (Aβ) plays central role in Alzheimer’s disease.
- Neurofibrillary tangles are an important target in therapeutics of Alzheimer’s disease.
- Oxidative and nitrosative stress are the main culprits.
- Neuroinflammation, especially activated microglia, is a consistent feature of lesions associated with Alzheimer’s disease.
- The FDA has approved acetylcholinesterase inhibitors for the symptomatic treatment of Alzheimer’s disease.
- Disease-modifying approaches increase the hope of slowing the rate of memory decline in patients with Alzheimer’s disease.
- AD have been studied using four different animal models; i) pathologies associated with normal aging, ii) production of lesion using neurotoxins, iii) using surgical methods to administer chemicals directly into brain and 94 transgenic models.

This box summarizes key points contained in the article.

Pathognomonic features of the disease, and their observation during postmortem examination is still required for a diagnosis of AD. Alzheimer described a ‘peculiar substance’ occurring as extracellular deposits in specific brain regions, which are now referred to as amyloid plaques. It was not until the mid-1980s that it was discovered that the plaques consist of aggregates of a small peptide called amyloid-β (Aβ). The second lesion described by Alzheimer, neurofibrillary tangles (NFTs), occurs intraneuronally. In the late 1980s, it was discovered that NFTs are composed of aggregates of the tau protein, which becomes abnormally hyperphosphorylated. Although plaques and NFTs are pathognomonic, it would be misleading to create the impression that these are the only significant pathological changes occurring in the AD brain. In fact, numerous other structural and functional alterations ensue, including inflammatory responses and oxidative stress [3]. From a biochemical point of view the most clear-cut and consistent finding is a deficit in the cholinergic system, decreased level of choline acetyltransferase, and other cholinergic markers [2].

Extensive research into AD over the past 30 years has greatly enhanced our understanding of the molecular events underlying the development of different pathologies. It is likely that from this burgeoning knowledge a new wave of disease-modifying treatments will be developed [4].

2. Neurobiology of Alzheimer’s disease

Several independent hypotheses have been proposed to link the pathological lesions and neuronal cytopathology with, among others, hyperphosphorylation of cytoskeletal proteins [5], oxidative stress [6], inflammation [7], Aβ metabolism [8], excitotoxicity [9] etc. However, not one of these theories alone is sufficient to explain the diversity of biochemical and pathological abnormalities of AD which involves a multitude of cellular and biochemical changes. Furthermore, attempts to mimic the disease by a perturbation of one of these elements using cell or animal models, including transgenic animals, do not result in the same spectrum of pathological alterations [10]. The relative modality of single-insult models to accurately reflect disease pathogenesis led us to speculate that AD, like cancer, may be the result of serial insults that alone are insufficient to lead to disease and therefore proposed a ‘two-hit hypothesis’ stating that both oxidative stress and mitogenic dysregulation are necessary and sufficient to cause the disease and suggested that it may be a common mechanism for other neurodegenerative diseases as well [11].

2.1 Cholinergic hypothesis

Experimental and clinical observations of deficiency in cholinergic neurotransmission in AD led to the development of cholinesterase inhibitors as the first approved treatment for dementia symptoms. The cholinergic hypothesis states that decreased cholinergic transmission plays a major role in the expression of cognitive, functional and possibly behavioral symptoms in AD [12,13]. The cholinergic hypothesis rests on pathological, biochemical and pharmacological observations. Cholinergic neurons in the ventral forebrain are depleted; many of those that remain contain neurofibrillary tangles. As a result of these pathological changes, there are decreases in biochemical indices of cholinergic function in neocortex and hippocampus that correlate with dementia severity. The hypothesis is further supported by an extensive literature from pharmacological studies using cholinergic agonists and antagonists, ablative lesions of the cholinergic pathway and transgenic animal models that emphasize the close connection between cognition and cholinergic neurotransmission (Figure 1). It has also been proposed that the cholinergic deficit plays a role not only in the cognitive symptoms but also in patients with mild cognitive impairment [14,15].

2.2 Amyloid-beta-mediated neurodegeneration
‘Amyloid’ is a generic term for an abnormal aggregate of proteins or peptides that specifically adopt a regular β-sheet configuration [4]. It is a highly ordered precipitate of extracellular protein; misnamed ‘starch-like’ by Rudolf Virchow because of its reactivity to the periodic acid-Schiff (PAS) stain [16]. Systemic amyloid deposits can occur in any organ and are often large and amorphous; cerebral amyloid deposits take the form of delimited, miliary spheres called plaques (Figure 2). Plaques contain a trace amount of glycosaminoglycans, which explains the PAS positivity. In AD, brain amyloid is composed almost entirely of a 4 kDa Aβ peptide [17] that exhibits microheterogeneity in amino acid sequence and in a variety of biophysical states. Most Aβ is comprised of a peptide designated Aβ40, Aβ41-40, or, in some cases, Aβ38-40. Aβ42 is considered to be the most critical component in plaque development.
2.3 Amyloid beta accumulation

The generation of Aβ from its precursor, the Aβ peptide precursor (APP), is illustrated in Figure 3. APP is first cleaved at the amino terminus of Aβ by a membrane-bound aspartyl protease (β-secretase). This cleavage generates a large secreted derivative (soluble APPβ) and a membrane-bound β-cleaved carboxyterminal fragment of APP (CTFβ; also known as C99). Cleavage of CTFβ by γ-secretase results in the production of the Aβ40 and Aβ42 species described above. The term ‘soluble Aβ’ generally is applied either to newly generated, cell-secreted Aβ or to that fraction of tissue or synthetic Aβ that is taken into the aqueous phase of a non-detergent-containing extraction buffer. ‘Misfolded’ and ‘aggregated’ Aβ are terms used to describe very early, nonspecific changes in Aβ folding states or solubility states, respectively (e.g., aggregated Aβ solutions usually scatter light to a greater extent than do solutions of soluble Aβ). ‘Oligomeric’ Aβ refers to peptide assemblies with limited stoichiometry (e.g., dimers, trimers, etc.), while protofibrils (PFs) are structures of intermediate order between aggregates and fibrils. The term ‘Aβ-derived diffusible ligands’ (ADDLs) is also applied to pre-protofibrillar intermediates based less on a structural definition than on the neurotoxic activity of these oligomers. Indeed, oligomers, PFs and ADDLs are believed to be the assembly states of Aβ with the most potent toxicity and are believed by many in the field to be the proximate mediators of Aβ-induced neurotoxicity, especially in primary neuronal culture models (18,19). The final assemblies, called fibrils, are the basic building blocks of the amyloid plaque and are so named because of their characteristic ultrastructural appearance.

2.4 Amyloid cascade hypothesis

Mismetabolism of APP, and abnormal production, aggregation and deposition of Aβ are central to the pathogenesis of AD, and that the Aβ pathology somehow leads to the tau/NFT pathology and the subsequent neuronal damage observed (Figure 4). This model of disease progression is usually referred to as the ‘amyloid cascade hypothesis’ and is
Neurobiological aspects of Alzheimer’s disease

Figure 2. Formation of β-amyloid plaques and neurofibrillary tangles are thought to contribute to the degradation of the neurons in the brain and the subsequent symptoms of Alzheimer’s disease.

Figure 3. The γ-secretase protein quartet, and its roles in brain development and Alzheimer’s disease. Presenilin-1, nicastrin, anterior pharynx defective 1 homolog (APH-1) and presenilin enhancer 2 (PEN-2) form a functional γ-secretase complex, located in the plasma membrane and endoplasmic reticulum (ER) of neurons. The complex cleaves Notch (left) to generate a fragment (Notch intracellular domain (NICD)) that moves to the nucleus and regulates the expression of genes involved in brain development and adult neuronal plasticity. The complex also helps in generating the amyloid-beta-peptide (Aβ; centre). This involves an initial cleavage of the amyloid precursor protein (APP) by an enzyme called beta-site APP-cleaving enzyme (BACE) (or β-secretase). The γ-secretase then liberates Aβ, as well as an APP cytoplasmic fragment, which may move to the nucleus and regulate gene expression. Mutations in presenilin-1 that cause early-onset Alzheimer’s disease enhance γ-secretase activity and Aβ production, and also perturb the ER calcium balance. Consequent neuronal degeneration may result from membrane-associated oxidative stress, induced by aggregating forms of Aβ (which create Aβ plaques), and by the perturbed calcium balance.
supported by a large number of studies spanning molecular genetics, cell biology, transgenic animal models and neuropathology [20]. The original hypothesis was proposed following the discovery of a mutation within the APP gene (on chromosome 21) that caused a minority of early-onset familial AD. This gene had become of interest and was subsequently cloned after Ab had been biochemically identified as a key component of neuritic plaques in the mid 1980s. These discoveries were complemented by the observation that individuals with Down’s syndrome, who have an extra copy of chromosome 21 (trisomy) and therefore a third copy of the APP gene, inevitably develop AD (should they live long enough). The advent of AD in these cases is thought to be due to a ‘gene dosage effect’, in that more Ab is produced because of the third copy of the APP gene.

The supposition within the original hypothesis was that fibrillar amyloid within plaques was responsible for initiating the disease process. This was supported by the observation that the toxicity of Ab depends on its state of aggregation: cell culture experiments revealed that while fibrillar amyloid was neurotoxic, synthetic monomeric Ab appeared to be harmless. However, the major objection to the proposal was based on the observation that plaque number correlated rather poorly with the severity of dementia observed before death. Eventually, these observations have lead to an increasing body of work focusing on smaller Ab arrays (i.e., pre-fibrillar moieties). Certainly, better correlations exist between soluble Ab (including soluble Ab oligomers), rather than plaque amyloid, and the presence and degree of cognitive impairment observed. Many researchers now believe that soluble Ab oligomers are the critical pathogenic molecules in AD and that plaques may represent inert reservoirs’ of such molecules. Although revisions to the original amyloid cascade hypothesis’ duly acknowledge this shift in opinion, it remains plausible that fibrillar amyloid may also exert some of the pathogenic effects observed [4].

2.5 Microtubule-associated protein tau in Alzheimer’s disease

Pathologic deposition of the microtubule (MT) associated protein tau, in the form of hyperphosphorylated inclusions or filamentous NFTs, is one of the defining features of adult-onset neurodegenerative diseases such as AD, progressive supranuclear palsy (PSP), and frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) [21]. Hyperphosphorylated insoluble tau in the form of NFTs was considered by many to be a relative bystander, despite the persistent association of tau deposition with dysfunctional neurons within diseased brains and cognitive dysfunction in normal aging [22,23].

Tau phosphorylation plays a normal role in decreasing tau’s affinity for MTs and is an important regulator of MT

---

**Figure 4. Amyloid cascade hypothesis.**

APP: Amyloid precursor protein; PHF: Paired helical filamentous tau.
polymerization during development. Tau has approximately 20 – 30 potential phosphorylation sites, many of which are putative targets of proline-directed serine/threonine kinases. Accumulating evidence suggests that two such kinases, glycogen synthase kinase-3β (GSK-3β) and cyclin-dependent kinase-5 (cdk5) are major tau kinases in vitro and in vivo (Figure 5) [24]. In AD, FTD and PSP, insoluble paired helical filamentous (PHF) tau and its other pathologically aggregated forms invariably contain tau hyperphosphorylated on residues that overlap with GSK-3β and cdk5 targets. However, it has been unclear whether aberrant tau phosphorylation by these or other kinases, such as p42/44 MAPK and p38 MAPK [21] can cause NFT formation in vivo.

2.6 Tau hyperphosphorylation causes or enhances NFT formation

Experimental evidences from the fly [25] and mouse [26] suggest that insoluble aggregates such as NFTs and amyloid plaques are signposts of damage already done; tau-related neurodegeneration can occur without, or precede, frank NFT formation. However, processes that accelerate NFT formation inevitably worsen neurodegeneration, supporting the concept that NFTs mark a critical, albeit probably later, process in disease progression. Tau hyperphosphorylation by GSK-3β in the fly [25] and cdk5 in mouse [27] can cause or accelerate NFT formation in vivo, with an attendant worsening of neurodegeneration. However, these effects were observed on a background of either tau overexpression or expression of a mutant tau transgene, raising the question of whether significant tau hyperphosphorylation alone under normal conditions may lead to neurofibrillary tau pathology. If so, perhaps tau kinases become an even more attractive target for therapeutic development.

Cruz et al. [26] suggest that NFT formation is most probably due to the significantly greater cdk5 activation present in this model relative to previous models. The differences in promoters used in each of these models may also be contributory. In any regard, Cruz et al. provide the strongest evidence to date that aberrant tau kinase activity of any kind can lead to neurodegeneration and tau neurofibrillary pathology in vivo in the absence of tau dysregulation or mutations. These findings add to a growing body of evidence that NFTs or NFT-like pathology can be caused and/or accelerated by tau kinase overactivity in vivo [25,27,28] and firmly establishes tau phosphorylation as a potential therapeutic target in AD and FTD [22].

Noble et al. [27] demonstrated that p25/cdk5 overactivation, in conjunction with expression of human tau containing the most common FTD-causing mutation in mouse, is associated with concomitant GSK-3α/β activation. Thus, although p25/cdk5 activation provides the initial insult in the Noble et al. model, GSK-3β is likely to be playing a role. Why GSK-3β activation is not observed in the model developed by Cruz et al. is not known—it may not be relevant in the context of such high cdk5 activity. It has been proposed that GSK-3β may act to produce NFTs in the context of mutant or overexpressed tau, rather than normal tau. However, the persistent localization of GSK-3β with pretangle and tangle-bearing neurons in human AD suggests otherwise.

Town et al. [29] demonstrated that that soluble Aβ (sAβ) is a potent activator of the p25/Cdk5 pathway, resulting in promotion of AD-like tau phosphorylation in vivo. They transfected N2a cells with a p35 vector (N2a/p35 cells) and, after differentiation, challenged these cells with Aβ (1 – 42) peptide in soluble form (sAβ(1 – 42)). Results showed that sAβ (1 – 42) at relatively low levels (1 – 5 µM) dose-dependently increased tau phosphorylation at AD-specific phosphoepitopes in differentiated N2a/p35 cells compared with controls, an effect that is blocked by antisenese oligonucleotides against p35. SAβ (1 – 42)-induced tau phosphorylation is concomitant with an increase in both p25 to p35 ratio and Cdk5 activity (but not protein levels) [29].

Although the circumstantial evidence is strong, it is not yet certain whether the neurodegeneration in the Cruz et al. model of p25 overexpression is a direct consequence of tau hyperphosphorylation by cdk5 or occurs via another signaling cascade. Cdk5 has many substrates, including proteins associated with neurodegeneration, such as APP (which is preferentially hyperphosphorylated by the p25/cdk5 complex) [26,30]. Cdk5 is also involved in several aspects of neuronal differentiation as a link between extracellular signals and the cytoskeleton and thus could lead to cell dysfunction and death via a number of different pathways. Additionally, Cruz et al. demonstrate that tau is hyperphosphorylated on sites that are not known cdk5 targets and that two other known tau kinases may be activated, suggesting that at least some of the effects of p25 overactivation are indirect. Whether this indirect effect occurs through activation of other kinases, by downregulation of phosphatases, or somehow by altering the affinity of prolyl-kinase isomerases for tau (favoring a conformation not permissive for tau dephosphorylation) are important issues that now need to be addressed. Nevertheless, it is becoming clear that direct or indirect tau hyperphosphorylation via at least two proline-directed kinases may accelerate or cause neurodegeneration and NFT formation.

The importance of dephosphorylation in tauopathy is highlighted by the ability of the phosphorylation-dependent prolyl isomerase (Pin1) to provide relative protection from age-dependent neurodegeneration [28]. Pin1 recognizes specific phosphorylated serine or threonine residues in tau that are followed by proline residues and catalyzes a critical conformational change that allows dephosphorylation at these residues to occur. Pin1 expression is inversely correlated with markers of neurofibrillary pathology in human AD brains, and Pin1 mice develop tau hyperphosphorylation, NFTs, and age-dependent neurodegeneration [28]. This pathology is very similar to that caused by misexpression of either mutant or wild-type tau in other models [31,32], emphasizing that multiple pathways affecting tau expression levels, dephosphorylation [33], and phosphorylation may...
coalesce in a common phenotype characterized by tau hyperphosphorylation, aggregation, and NFT formation. Current evidence shows that the known FTDP-17-causing tau mutations either disrupt MT binding or affect tau splicing, leading to tau isoform imbalance. Therefore, one feature potentially in common with the human mutations and animal models of tauopathy is an increase in free tau unbound to microtubules. Since tau polymerization into filaments is concentration-dependent \textit{in vitro}, this provides an obvious common mechanism for tau aggregation and NFT formation \textit{in vivo} (Figure 6).

2.7 Oxidative stress

Oxidative stress is a phenomenon associated with pathogenetic mechanisms of several diseases including atherosclerosis, neurodegenerative diseases, such as AD and Parkinson’s disease, cancer, diabetes mellitus, inflammatory diseases, as well as psychological diseases or aging processes. Oxidative stress is defined as an imbalance between production of free radicals and reactive metabolites, so-called oxidants, and their elimination by protective mechanisms, referred to as antioxidative systems. This imbalance leads to damage of important biomolecules and organs with potential effects on the whole organism. Oxidative and antioxidative processes are associated with electron transfer influencing the redox state of cells and the organism. The changed redox state stimulates or inhibits activities of various signal proteins, resulting in a changed ability of signal pathways to influence the fate of cells [34].

Oxidative stress (OS) has been implicated in the pathogenesis of AD [35] by the finding of several characteristics, such as enhanced lipid peroxidation, in specific areas of the brain in presenilin conditional knock-out mice [36]. Several investigators detected an increase in the activity of catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase in the hippocampus and amygdala (Figure 7) [37,38]. The suggestion that OS causes oxygen radical formation with resultant neurodegeneration and possibly plaque formation in the CNS was supported by the study of Pratico \textit{et al.} [39]. Moreover Pappolla \textit{et al.} provided evidence for the hypothesis that \textbeta-amyloid protein, the major constituent of the senile plaque, is neurotoxic and that such toxicity is mediated by free radicals \textit{in vitro} and in a transgenic mouse model of AD [40].

Free radicals are molecules with an unpaired electron in their outer orbit. Free radicals have very important role in origin of life and biological evolution, having beneficial effects on the organisms [41]. Oxygen radicals are involved in many biochemical activities of cells such as signal transduction, gene transcription and regulation of soluble guanylate cyclase activity. NO is an important signaling molecule that essentially regulates the relaxation and proliferation of vascular smooth muscle cells, leukocytes adhesion, platelet aggregation,
angiogenesis, thrombosis, vascular tone and hemodynamics [42]. Humans are constantly exposed to free radicals created by electromagnetic radiation from the manmade environment such as pollutants and cigarette smoke. Natural sources such as radon and cosmic radiation, as well as cellular metabolisms (respiratory burst, enzyme reactions) also add free radicals to the environment. The most common reported cellular free radicals are hydroxyl (OH), superoxide (O$_2^-$) and nitric monoxide (NO). Even some other molecules like hydrogen peroxide (H$_2$O$_2$) and peroxynitrite (ONOO$^-$), which are not free radicals, are reported to generate free radicals through various chemical reactions in many cases [43]. Cells exposed to environments fortified with oxygen continuously generate oxygen free radicals (OFR). Antioxidant defense systems co-evolved along with aerobic metabolism to counteract oxidative damage from OFR [44]. The human body produces OFR and other reactive oxygen species as by products of numerous physiological and biochemical processes. Oxygen related free radicals (superoxide and hydroxyl radicals) and reactive species (hydrogen peroxide, NO, peroxynitrite and hypochlorous acid), are produced in the body, primarily as a result of aerobic metabolism. At the same time, antioxidants, such as glutathione, arginine, citrulline, taurine, creatine, selenium, zinc, vitamin E, vitamin C, vitamin A and tea polyphenols help to regulate the reactive oxygen species (ROS) thus generated. Antioxidant is further supported with antioxidant enzymes, for example superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase that exert synergistic actions in removing free radicals [45].

It has been reported that epigallocatechin-3-gallate (EGCG), the main polyphenolic constituent of green tea, reduces A$\beta$ generation in both murine neuron-like cells (N2a) transfected with the human ‘Swedish’ mutant APP and in primary neurons derived from Swedish mutant APP-overexpressing mice (Tg APPsw line 2576). In concert with these observations, it was reported that EGCG markedly promotes cleavage of the alpha-C-terminal fragment of APP and elevates the levels of the N-terminal APP cleavage product, soluble APP-alpha. These cleavage events are associated with elevated alpha-secretase activity and enhanced hydrolysis of TNF-$\alpha$-converting enzyme, a primary candidate alpha-secretase [46].

Results from Obregon et al. suggests that a disintegrin and metalloprotease 10 (ADAM10) activation is necessary
for EGCG promotion of non-amyloidogenic (alpha-secretase cleavage) APP processing. Thus, ADAM10 represents an important pharmacotherapeutic target for the treatment of cerebral amyloidosis in Alzheimer disease [47].

It has also been reported that the citrus bioflavonoid luteolin reduces Aβ peptide generation in both human 'Swedish' mutant APP transgene-bearing neuron-like cells and primary neurons through decrease in amyloidogenic gamma-secretase APP processing, and promotion of presenilin-1 (PS1) carboxyl-terminal fragment (CTF) phosphorylation [48].

2.8 ROS-mediated neuronal damage

ROS are particularly active in the brain and neuronal tissue as the excitatory amino acids and neurotransmitters, whose metabolism is factory of ROS, which are unique to the brain and serve as sources of oxidative stress. ROS attack glial cells and neurons, which are post-mitotic cells and therefore, they are particularly sensitive to free radicals, leading to neuronal damage [45]. It has been reported that deleterious effects of ROS on human cells may end in oxidative injury leading to programmed cell death, that is apoptosis [49].

Neuronal biochemical composition is mainly susceptible to ROS since it involves a pool of unsaturated lipids those are labile to peroxidation and oxidative modification. Double bonds of unsaturated fatty acids are hot spots for attack by free radicals tat initiate cascade or chain reactions to damage neighboring unsaturated fatty acids [50]. Brain contains high levels of fatty acids, which are more susceptible to peroxidation than other lipids, which consumes an inordinate fraction (20%) of total oxygen consumption for its relatively small weight (2%). In addition, it is not particularly enriched in antioxidant defenses. Brain is lower in antioxidant activity in comparison with other tissues having, for example, about 10% of the level of activity in liver. Moreover, human brain has higher levels of iron in certain regions and in general has high levels of ascorbate. As is evident from the above data, neural cells are considered to be more susceptible to oxidative damage as compared with other body tissues [51].

2.9 Mitochondria and Alzheimer's disease

Mitochondria, organelles found in all eukaryotic cells, are involved in a multitude of cellular processes that are essential to both survival and death [52]. Earlier it was thought that this organelle had a single role as the powerhouse for cellular function. Later it was observed that it also carries out other essential activities, including regulation of the levels of second messengers such as calcium ions and ROS. Only one decade ago it was observed that mitochondria integrate inductive signals of cell death and/or survival at the level of the regulation of the permeability of their membranes [53], since the...

Figure 7. The use of oxygen by cells with aerobic metabolism generates potentially deleterious reactive oxygen metabolites. The cell has the ability of degrade free radicals. The enzyme superoxide dismutase (SOD) transforms activated oxygen (O₂⁻) into hydrogen peroxide (H₂O₂) that could be converted by the enzyme catalase to harmless water (H₂O) and oxygen (II). A second detoxification pathway involves the enzyme glutathione-peroxidase (GPx), which converts hydrogen peroxide to water using the co-enzyme glutathione (GSH). Oxidized glutathione (GSSG) could be recycled by glutathione-reductase (GSRed).
Neurobiological aspects of Alzheimer’s disease

induction of apoptotic programs in cell-free extracts requires the presence of dATP and cytochrome c [54]. This process seems to be the point of no return in the apoptotic signaling cascade since, once released, these proteins recruit and activate other signaling cascades that will inevitably lead to irreversible death of the cell. As such, mitochondria are being considered the main link between initial cellular stress signals activated during the acute and chronic nerve cell injury events and the event of execution of nerve cell death [55].

Neuronal activity is extremely energy dependent; as such neurons are particularly sensitive to mitochondrial function changes. Neuronal activities such as synaptic transmission, axonal/dendritic transport, ion channels and ion pump activity are energy taxing processes [56]. Also the maintenance of calcium homeostasis is critical for neuronal synaptic function [57] and controlled by mitochondria at the synapse [58].

In animal models, mitochondrial dysfunction and energy metabolism deficiencies are recognized as one of the early events and correlate with impairments of cognitive abilities in AD [59]. Polysaturated-fatty-acid-rich membranes found in neurons are sensitive to oxidative stress produced by mitochondria [60]. Damage to both the components and the structure of mitochondria as well as increased oxidative stress are extensively reported in AD [61,62].

Cell death in AD usually occurs by apoptosis, more commonly by the intrinsic mitochondrial pathway than by the extrinsic cell-signaling pathway. The intrinsic pathway controls activation of caspase 9, through the adaptor molecule apoptotic peptidase activating factor 1 (Apaf-1), by regulating the release of cytochrome c from the inter membrane space to the cytosol. Proapoptotic and antiapoptotic members of the B cell leukemia/lymphoma 2 (Bcl-2) family, and also stress and survival signals, regulate the release of cytochrome c in the cytoplasm. Proapoptotic signals can also release proteins such as second mitochondria-derived activator of caspase (Smac)/direct IAP binding protein with low P (DIABLO) and Omi/high temperature requirement protein A2 (HTRA2), which block inhibitor of apoptosis proteins (IAP) to activate cell death caspases. However, in the intrinsic pathway of apoptosis, mitochondria are not merely passive containers capable of leaking cytochrome c. Rather, their life-supporting functions are clearly linked to their death-promoting activity. These modulating factors include i) The respiratory chain activity, with the unavoidably associated generation of ROS; ii) mitochondrial fusion and fission; iii) calcium homeostasis; iv) lipid composition of the mitochondrial membranes; v) the mitochondrial permeability transition. Mitochondrial damage due to extracellular Aβ could be caused by 4-hydroxy-2-nonenal (HNE), generated as a result of Aβ-induced lipid peroxidation at the plasma membrane [61]. Aβ has also been shown to induce HNE formation in synaptosomes to concentrations high enough to result in significant depletion of ATP [63]. Aβ caused a significant reduction in state 3 and state 4 mitochondrial respiration in isolated rat brain mitochondria.

Importantly a variety of naturally occurring precursor of cofactors for mitochondrial enzymes with high antioxidant properties are showing positive effects on AD-related mechanisms. For example, lipoic acid, through the activation of choline acetyltransferase increases acetylcholine levels [64], severely reduced in AD. Lipoic acid is also a potent chelator of redox-active transition metals and a potent ROS scavenger [65]. Furthermore, it has been shown to be effective at mitigating oxidative and apoptotic markers in vitro using AD patients’ fibroblasts [66] and effective on cognition and AD related pathology in AD animal models [67].

2.10 Inflammation and Alzheimer’s disease

In addition to direct toxic effects, Aβ may also promote neurodegeneration by parallel mechanisms including the activation of microglial cells and astrocytes. The induction of a microglia-driven inflammatory response results in the release of various inflammatory mediators including a whole array of neurotoxic cytokines [68]. Once activated, microglia cells may also recruit astrocytes that actively enhance the inflammatory response to extracellular Aβ deposits (Figure 8). This neuroinflammatory component of AD is further characterized by a local cytokine-mediated acute-phase response, activation of the complement cascade and induction of inflammatory enzyme systems such as iNOS and the prostanooid generating COX-2. Several lines of evidence suggest that all of these factors can contribute to neuronal dysfunction and cell death, either alone or in concert [69,70].

2.11 Cellular components of neuroinflammation in Alzheimer’s disease

Microglia cells represent the brain innate immune system and hence the first line of defense when challenged by bacterial, viral or fungal infection. Although these functions are of major importance and beneficial, it has become clear that microglial activation may also be evoked by endogenous proteins and can significantly contribute to neuronal damage. Along with microglia, astrocytes and even neurons are directly reacting and contributing to the chronic neuroinflammatory changes in AD.

2.11.1 Microglia

Microglia cells constitute around 10% of all cells in the nervous system. They represent the first line of defense against invading pathogens and serve as specialized sensors for brain tissue injury [71]. Under pathological situations, such as neurodegenerative disease, stroke, traumatic injury and tumor invasion, these cells become activated, migrate to and surround damaged or dead cells, and subsequently clear cellular debris from the area, similar to the phagocytic active macrophages of the peripheral immune system [72]. Activated microglia upregulate a variety of surface receptors, including the MHC and complement receptors [73]. They also undergo dramatic morphological changes from a resting ramified phenotype to motile activated amoeboid cells. Once immunostimulated
these microglia cells release a variety of proinflammatory mediators including cytokines, ROS, complement factors, neurotoxic secretory products, free radical species and NO, all of which can contribute to neuronal dysfunction and cell death, ultimately creating a vicious cycle [74].

Several amyloid peptides and APP can act as potent glial activators [75], and disruption of the APP gene and its proteolytic products delay and decrease microglial activation [76]. Microglial cells have been suggested to be preferentially associated with certain amyloid plaque types, indicating that plaque development and the degree of microglial reaction are interrelated. Aβ stimulates a NF-κB-dependent pathway that is required for cytokine gene transcription [77], activated microglia and reactive astrocytes. Not only Aβ, but also the carboxy-terminal 100 amino acids of APP (CT100), which is also present in senile plaques, can induce astrogliosis and neuronal death.

It has been reported that interaction of CD40 with CD40L enables microglial activation in response to Aβ, which is associated with AD-like neuronal tau hyperphosphorylation in vivo. Transgenic mice overproducing Aβ, but deficient in CD40L, showed decreased astrogliosis and microgliosis associated with diminished Aβ levels and beta-amyloid plaque load [78,79].

But from the reports of Schenk et al. [80] and Bard et al. [81] it has to be considered that not all forms of brain innate immunity are deleterious. Aβ-directed ‘immunotherapy’ activates microglia in a productive way to clear amyloid plaques. Transgenic animals were immunized with Aβ42, either before the onset of AD-type neuropathologies (at 6 weeks of age) or at an older age (11 months). Immunization of the young animals essentially prevented the development of beta-amyloid-plaque formation, neuritic dystrophy and astrogliosis. Treatment of the older animals also markedly

Figure 8. Beta-amyloid plaques induce neuroinflammation as characterized by glial activation and elevation in local pro-inflammatory cytokine production. Recent experiments have reported that transgenic overexpression of human IL-1β restricted to the mouse hippocampus is associated with neutrophil recruitment and increased clearing of plaques, highlighting a benefit of the neuroinflammatory response.
Neurobiological aspects of Alzheimer’s disease

reduced the extent and progression of these AD-like neuropathologies showing that immunization with Ab may be effective in preventing and treating AD. On a similar note, it was recently shown that blocking TGF-beta-small and motherepase against decapentaplegic (Smad) 2/3 signaling on innate immune cells promotes a beneficial form of innate immune cell phagocytosis/clearance of cerebral amyloid [82].

2.11.2 Astrocytes

Astrocytes participate in β-amyloid clearance and degradation, provide trophic support to neurons, and form a protective barrier between Ab deposits and neurons [83]. The presence of large numbers of astrocytes associated with Ab deposits in AD suggests that these lesions generate chemotactic molecules that mediate astrocyte recruitment. It has been shown that astrocytes throughout the entorhinal cortex of AD patients gradually accumulate Ab1-42-positive material, and the amount of this material correlates positively with the extent of local AD pathology. Ab1-42 within these astrocytes appears to be of neuronal origin, possibly accumulated by phagocytosis of locally degenerated dendrites and synapses, especially in the cortical molecular layer [84]. In support of this finding, experimental evidence suggests that astroglial cells are able to phagocytize Ab peptides, a process which may depend on their apolipoprotein E (ApoE) status, suggesting that ApoE polymorphisms may influence the risk of developing AD by affecting astroglial Ab phagocytosis. Although astrocytes serve as a constant and important source of neurotrophic factors under physiological conditions, in vitro and in vivo experiments suggest that chronically activated inflammatory astrocytes may not generate significant amounts of these molecules [85].

2.11.3 Neurons

Experimental evidence suggests that neurons themselves are capable of producing mediators. Thus, neurons can serve as source of complement, COX-2-derived prostaglandins [86,87] and several cytokines including IL-1β, IL-6 and TNF-α [88]. Although COX-2 expression is driven by physiological synaptic activity, it is possible that neurons themselves may exacerbate local inflammatory reactions and thus contribute to their own destruction in AD. Expression of the inflammatory induced enzyme iNOS has been associated with degenerating neurons in AD brains [89], and compelling evidence exists for iNOS-related long-term NO release and NO-dependent peroxynitrite formation. Glial- and neuronal-derived NO and peroxynitrite have been demonstrated to cause neuronal dysfunction and cell death in vitro and in vivo [90].

2.12 Insulin and Alzheimer’s disease

Both insulin and IGF-1 play an important role in the development of AD. Both insulin and IGF-1 stimulate Ab release from neurons and IGF-1 exerts a stimulatory effect on brain amyloid clearance. So, abnormal function of the insulin–IGF-I axis may be another putative mechanism in AD. In addition, insulin and IGF-I levels are altered in Alzheimer’s patients and, probably cell sensitivity towards insulin and possibly IGF is decreased in these patients. Insulin exerts a double-sided effect on brain Ab. It stimulates neuronal release of Ab and at the same time contributes to extraneuronal accumulation of Ab by competing for insulin-degrading enzyme. The net action of insulin is therefore to increase brain Ab. IGF-I decreases brain levels of Ab and increases the plasma levels of Ab complexed to transport proteins. Thereby IGF-I stimulates clearance of brain Ab [91].

Insulin and IGF-I might also influence the development of neurofibrillary tangles. In AD, tau becomes hyperphosphorylated, which is likely to impair its microtubule-stabilizing role. Hyperphosphorylated tau is believed to misfold, undergo net dissociation from microtubules, form abnormal filaments (paired helical filaments) and aggregate into neurofibrillary tangles. It has also been reported [92] that insulin and IGF-1 transiently increase tau phosphorylation on specific amino acid residues in human neuroblastoma cells, suggesting precise regulation of the phosphorylation of tau by insulin signaling. Furthermore, disruption of insulin or IGF-1 signaling increases tau phosphorylation in the brains of insulin-receptor-substrate 2 (IRS-2)-knockout mice [93]. These results indicate that insulin and IGF-1 could play a pivotal role in regulating tau protein phosphorylation and assembly in neurons.

In this context, decreased insulin and/or IGF-1 levels could elevate brain Ab burden and, thus, indirectly increase tau pathology. Further investigation to explore this hypothesis thoroughly is required.

2.13 Lipids and cholesterol in AD

Lipids and cholesterol are transported through the bloodstream by lipid-protein particles, lipoproteins that can be classified into different subsets according to their density: high-density (HDL), medium-density (IDL), low-density (LDL), and very-low-density (VLDL). LDL has an elevated fat proportion and participates in lipoprotein transport from the bloodstream to peripheral tissues. HDL has a larger protein fraction with respect to lipids, and takes part in reverse cholesterol transport from peripheral tissues to liver where conjugation and excretion occur. Cholesterol is transported in the plasma predominantly as cholesteryl-esters associated with lipoproteins. Brain cholesterol is mainly synthesized locally within the CNS. It is estimated that during CNS development, neurons synthesize most of the cholesterol needed for their growth and synaptogenesis.

Earlier reports suggested that cholesterol plays an important role in the formation and/or progression of senile plaques. Two different fluorometric-staining techniques (filipin staining and an enzymatic technique) for the determination of cholesterol in brains of postmortem-confirmed AD patients and in nondemented, age-matched histopathologically
normal controls were employed. AD patient brains showed abnormal accumulation of cholesterol in congophilic/birefringent dense cores of senile plaques that was essentially absent in histopathologically normal controls. To determine whether increased senile plaque-associated cholesterol occurred generally in all plaques or was restricted to a specific subset, quantitative analysis was performed. Data indicated abnormal accumulation of cholesterol in cores of mature plaques but not in diffuse or immature plaques. Additionally, transgenic mice that overexpress the ‘Swedish’ amyloid precursor protein (Tg APPsw, line 2576) exhibited a similar pattern of abnormal cholesterol accumulation in mature, congophilic amyloid plaques at 24 months of age that was absent in their control littermates or in 8-month-old Tg APPsw mice (an age prior to amyloid deposition) [94].

Several findings indicate that lowering cholesterol levels might be of beneficial use for treating AD. Epidemiological studies with a mixed outcome showed a potential link between cholesterol lowering compounds, especially statins [95], and a strongly decreased prevalence or incidence for dementia, which may indicate that targeting lipid metabolism in humans may be a potential strategy for AD prevention. ApoE is one of the most prominent transport proteins in the brain and, besides others; the LDL-receptor-related protein (LRP) and the VLDL receptor are the main receptors for ApoE in the brain. ApoE is an important factor in the metabolism and distribution of cholesterol and triglycerides within many organs in the human body [96].

The precise mechanisms by which ApoE participates in AD pathogenesis remain largely undefined. Several hypotheses have been proposed to explain this. These include i) the proposed role of ApoE in mediating neuroinflammation, ii) its participation in the regulation of the cholinergic neurotransmitter system, iii) its role in neuronal signaling, and iv) ApoE’s maintenance of the integrity of the blood–brain barrier. However, the most prominent hypotheses of ApoE function is its key role as a mediator of Aβ metabolism. ApoE binds Aβ, affects the deposition and clearance of Aβ, and is required for amyloid deposition. Furthermore, ApoE affects amyloid deposition in an allele-specific manner. However, the exact pathophysiological process is yet to be elucidated [97].

The role of cholesterol is further brought to the fore by the fact that intracellular cholesterol may regulate APP processing by directly modulating secretase activity or by affecting the intracellular trafficking of secretases and/or APP. Cholesterol loading increases γ-secretase activity and the amyloidogenic pathway while low intracellular cholesterol favors the non-amyloidogenic pathway. There are also genetic factors linking cholesterol metabolism and AD, though ApoE is the only gene with replicable evidence, several candidate genes involved in lipid metabolism like alpha-2-macroglobulin (A2M), LRP, insulin degrading enzyme (IDE), ATP-binding cassette transporter (ABCA1), acyl-CoA cholesterol acyl transferase (ACAT) and cytochrome P450, family 46 (CYP 46, which converts cholesterol to 24S-hydroxycholesterol) are being investigated for putative roles with mixed results [98].

3. Therapeutics

The mainstays of conventional pharmacotherapy for AD are compounds aimed at increasing the levels of acetylcholine in the brain, thereby facilitating cholinergic neurotransmission through inhibition of the cholinesterases. These drugs, known as ACE inhibitors (ACIs), were first approved by the FDA in 1995. In 2004 the FDA approved Memantine, an NMDA antagonist, for treating dementia symptoms in moderate to severe AD cases [99]. The current strategies include direct activation of PPARγ receptor by NSAID’s, which has shown the promising result in clinical trials. Neurotrophic factors (including hormone replacement therapy and drugs acting on insulin signal transduction) and anti-amyloid agents (including cholesterol-lowering therapy) are in Phase III clinical trials [100]. Herbal drugs like berberine and curcumin have shown potential effects in preclinical studies. In recent years the proclivity of cannabinoids to exert a neuroprotective influence has received substantial interest as a means to mitigate the symptoms of neurodegenerative conditions. These approaches hold promise for disease modification and have a potential to be used as combination therapy for cognitive enhancement (Table 1).

4. Animal models of Alzheimer’s disease

The neuropathological changes associated with AD have been studied using four different experimental animal models; pathologies associated with normal aging, to produce lesion using neurotoxins, using surgical methods to administer chemicals directly into brain and the transgenic models.

4.1 Age-related pathologies as models

Many of the cognitive impairments observed in patients with AD also occur to a somewhat lesser degree in normal aging. Initially there is a slight amnesia that progresses gradually over a period of many years as general intellectual functions also decline. However, the syndrome described for AD clearly differs from normal aging. For example, in non-pathological aging there is no loss of basal forebrain cells and only a moderate development of senile plaques. Usually the differences in the pathological, biochemical and cognitive changes between AD and non-pathological normal aging are in degree rather in their nature [101].

Rats also show age-related changes in cholinergic and many non-cholinergic biomarkers throughout the brain, including complex alterations in the levels of 5-hydroxytryptophan and the catecholamines [102]. However, unlike in AD, rats, humans and nonhuman primates do not show age-related changes in cholinergic markers, including choline acetyltransferase, high-affinity choline uptake and many pre and postsynaptic cholinergic receptors, although the presence or
Expert Opin. Ther. Targets (2011) 15(S)

4.2.1 Choice of lesion locations

Discrete regions of the brain can be selectively destroyed by injection of specific neurotoxins or by the application of electrical current. Recent animal models of the pathology associated with AD have involved lesions of either the basal forebrain cholinergic system, noradrenergic locus ceruleus, serotonergic raphe nuclei or some combination of these systems. Each of these neural systems may degenerate in AD.

4.2.2 Choice of neurotoxins

The neurotoxins that are typically used are restricted analogs of the endogenous amino acid neurotransmitter glutamate. These compounds destroy cell bodies, but leave fibers of passage and efferent terminals into the injection region relatively intact. The toxins that have been used include ibotenic (IBO), kainic (KA), quinolinic (QN) and quisqualic (QA) acids and N-methyl-D-aspartate. These five excitatory amino acids have been used to produce brain lesions that reproduce specific components of the pathology associated with AD. Each toxin affects a slightly different population of neurons within the injection site. Cytotoxicity is probably determined by the specific subtype of glutamate receptor that the neuron expresses. For example, KA is a potent agonist at the kainite subtype of glutamate receptors and is far less potent at sites that are sensitive to IBO or QA. QA and QN are effective neurotoxins in the basal forebrain and destroy many cholinergic and noncholinergic neurons throughout the ventral pallidum/substantia innominata. However, neither acid effectively destroys neurons in the medial septal area. KA is a potent neurotoxin that destroys cells near the site of its injection. KA also produces a significant amount of cell loss in the hippocampus when it is injected into the basal forebrain. This nonspecific injury can be overcome by pretreatment with an anticonvulsant immediately following surgery.

4.2.3 Aluminum-induced pathology

A role for aluminum in the etiology of AD has arisen primarily because of three independent laboratory findings: first, aluminum administration can induce neurofibrillary changes in the neurons of experimental animals; second, aluminum exposure can produce neurological and biochemical changes that lead to impaired memory and cognitive function that is similar to that observed in the early stages of AD; and third, aluminum levels in the brain tissue of AD patients exceeds the levels normally found in age-matched controls.

Aluminum salts injected intrathecally into susceptible species induce neurofibrillary abnormalities in the perikarya and dendrites of neurons in the brain stem and spinal cord. These neurofibrillary aggregates are composed of normal neurofilament triplet proteins in contrast to the paired helical filaments associated with AD. The cause of this accumulation may be related to an abnormality in the synthesis, processing, or transport of the neurofilaments within axons and dendrites. Indeed, the aluminum-induced neuropathological preparation may be a better model of impaired neurofilament homeostasis and pathology than a model of the etiology of AD.

### Table 1. Therapeutic strategies for Alzheimer’s disease.

<table>
<thead>
<tr>
<th>Therapeutic targets</th>
<th>Ligands</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholinergic insufficiency</td>
<td><strong>Cholinesterase inhibitors:</strong> First generation Tacrine Second generation Donepezil Galantamine Rivastigmine (patch) Dimebon, Xaliproden (in clinical trials)</td>
</tr>
<tr>
<td>Excitotoxicity</td>
<td>Memantine Dimebon, Xaliproden (in clinical trials)</td>
</tr>
<tr>
<td>Inflammation</td>
<td>NSAIDs</td>
</tr>
<tr>
<td>Oxidative stress</td>
<td>Curcumin, resveratrol, green tea, Vitamin E, selegeline, furelic acid, lipoic acid etc</td>
</tr>
<tr>
<td>Amyloid cascade</td>
<td>Statins, secretease effectors, Aβ vaccination (passive and active therapies)</td>
</tr>
<tr>
<td>Tau phosphorylation</td>
<td>Kinase inhibitors</td>
</tr>
<tr>
<td>Hormonal</td>
<td>Nerve growth factor, estrogen, insulin</td>
</tr>
</tbody>
</table>

Absence of age-related changes in cholinergic markers may be related to the particular strains of rats that have been investigated. The lack of changes in cortical cholinergic markers does not correlate with an age-related cell loss in the basal forebrain. Furthermore, behaviorally-impaired aged rats often have the most severe pathological changes in the basal forebrain and the greatest decline in normal sleep patterns, brain biochemistry and brain glucose metabolism. Aged rats demonstrate a decrease in the activity of specific energy-metabolizing enzyme systems, in particular those related to glycolysis and energy production, similar to that seen in normal human aging and AD. Rats also demonstrate an age-related decline in peripheral sympathetic function and their ability to regulate blood glucose levels that may be related to central mnemonic processes. The sympathetic response to cognitive stimuli and glucose regulation and utilization are also impaired in patients with AD.

4.2 Lesions as an interventional approach

Although young animals do not develop pathological or biochemical changes similar to those seen in AD, it is possible to reproduce a subset of these changes experimentally. One approach is to produce lesions in discrete brain regions.
4.2.4 Surgical method (intracerebroventricular administration)

4.2.4.1 Atropine

Each rat is pretreated with atropine to prevent excess secretions that might impair respiration during surgery and then anesthetized. The rat is placed in the stereotaxic apparatus, the scalp is shaved, incised, and retracted, and holes are drilled in appropriate locations in the skull with a dental drill. The coordinates for multiple injections of a neurotoxin into the nucleus basalis of Meynert (NBM) are as follows: 0.4 and 0.8 mm posterior to Bregma, 2.6 mm lateral (bilaterally) from the midline, and 6.8 mm below the dorsal surface of the neocortex or 6.9 mm below the dura. A single aliquot of the solution is thawed on ice immediately prior to use and kept cold and protected from light during surgery. The surgical cannula or syringe is filled immediately prior to injection to prevent excess warming of the solution and spontaneous oxidation. Each NBM injection site receives 0.4 – µl of IBO (the precise volume may vary depending on the concentration of each neurotoxin) injected over a period of 5 min to prevent widespread diffusion. The coordinates for injection of toxin into the medial septal area/vertical limb of the diagonal band region are as follows: 0.8 mm anterior to bregma, on the midline, and 5.8 mm below the dura. Usually only 0.6 µl of IBO is injected per site. It is important to avoid destroying neurons within the lateral hypothalamic feeding centers or injecting large volumes of the neurotoxin. If the neurotoxin reaches the lateral hypothalamus, the rat may stop eating, and if the neurotoxin reaches the third ventricle, the rat usually dies during surgery. Although the precise cause has not been investigated rigorously, death may be owing to destruction of the vegetative centers in the floor of the fourth ventricle. The survival rate for this surgery can be greatly enhanced if the lesions are produced in two stages. In the first stage, the excitotoxin is injected into the basal forebrain in one or two places unilaterally. The second stage of the surgery is performed 1 week later; the neurotoxin is then injected into the contralateral basal forebrain. This procedure significantly decreases the mortality rate, but does not comprise the overall effectiveness of the lesions. It is especially valuable in behavioral studies of the retention of an acquired memory, particularly when a great deal of time and effort have been invested in the initial training [112].

After the injection is made, the needle or cannula should be withdrawn slowly to avoid drawing the neurotoxin to the cortical surface by capillary action. The neurotoxin might destroy intracortical cells and confound the behavioral and biochemical studies.

4.2.4.2 Streptozotocin

Rats are anesthetized with a suitable anesthesia. The scalp is shaved, cleaned and cut to expose the skull. The head is positioned in a stereotaxic frame and a midline sagittal incision is made in the scalp. Burr holes are drilled in the skull on both sides over the lateral ventricles by using the following coordinates: 0.8 mm posterior to bregma; 1.5 mm lateral to sagittal suture and 3.6 mm beneath the surface of the brain [113]. Streptozotocin (3 mg/kg, intracerebroventricular) is injected bilaterally in two divided doses on the first and third day. The concentration of streptozotocin in artificial cerebrospinal fluid (ACSF) is adjusted so as to deliver 10 µl of the solution. The skin is sutured after the second injection followed by daily application of antiseptic powder. Intracerebroventricular injection of streptozotocin in subdiabetogenic dose in rats causes reduced energy metabolism/oxidative stress leading to cognitive dysfunction by inhibiting the synthesis of ATP and acetyl-CoA [113].

4.2.4.3 Okadaic acid

Rats are anesthetized with a suitable anaesthesia and placed in a stereotaxic instrument with the incisor bar set 2 mm below the ear bars. After the scalp is incised (5 – 8 mm), the skull is cleaned and two holes (diameter 1.0 mm) are made for injection at the co-ordinates anterior-posterior (AP) 21.4, lateral (L/R) 22.5, vertical (V) 27.0 (in mm from bregma and dura, flat skull) according to the stereotaxic atlas of Paxinos and Watson [114]. A sterilized needle connected to a 5 ml syringe is stereotaxically placed into the nucleus basalis of Meynert (NBM). The rats receive bilateral injections of 0.2 mmol/l Okadaic acid (OA). OA is a most commonly used selective protein phosphatase inhibitor with highest inhibitory activity toward PP2A, moderate toward PP1 and weak toward PP2B [115]. It has been reported previously that OA produced significant depletion of endogenous tissue acetylcholine (ACh) and this depletion is not the result of ACh release. It was observed using cultured hippocampal slices that OA treatment led to the reduction of choline acetyltransferase (ChAT) activity [116]. OA caused hyperphosphorylation of tau and other cytoskeletal proteins, such as neurofilaments (NF) and microtubule-associated protein 1b (MAP1b) in various cell types [117].

4.2.4.4 Aluminium

Neurofibrillary changes can be produced by intracerebroventricular (ICV) injections of aluminium salts into rabbits, cats and rats. Neurofibrillary tangles usually develop in frontal and occipital cortex; the concentration of aluminium directly correlates with the number of neurofibrillary tangles in each affected region. ICV injections of aluminium salts are usually given as two injections of 5 moles into each lateral ventricle or one 50 pl injection of a 1% (w/v) solution [118]. Sterile aluminium lactate is prepared in deionized water, and then filtered (0.22 µm filters) under sterile conditions. The LD50 for aluminium using this exposure route is about 1600 µmoles/kg/injection.

4.2.4.5 Colchicine

Rats are anesthetized with a suitable anaesthesia and placed in a stereotaxic instrument with the incisor bar set 2 mm below the ear bars. After the scalp is incised (5 – 8 mm), the skull is...
Neurobiological aspects of Alzheimer’s disease

cleaned and two holes (diameter 1.0 mm) are made for injection at the co-ordinates 0.8 mm posterior to bregma, 1.8 mm lateral to sagittal suture and 3.6 mm beneath the cortical surface. The rats receive bilateral injections of 7.5 µg colchicine in ACSF [119]. Colchicine, a microtubule-disrupting agent, produces marked destruction of hippocampal granule cells, mossy fibers and septohippocampal pathways (SHC; a cholinergic link between medial septum and the vertical limb of the diagonal band). It induces neurofibrillary degeneration by binding to tubulin, the principal structural protein of microtubules, which is associated with loss of cholinergic neurons and decrease in choline acetyltransferase, thereby resulting in impairment of learning and memory [120].

4.3 Transgenic models

Transgenic organisms are generated by one of two general strategies. In the first, a genetic modification is introduced on top of the existing genetic makeup of the organism. In the second, the homologous gene of interest is modified selectively in its normal chromosomal position; this process is called gene targeting. In mice, both approaches are highly developed. In the first approach, a 1-cell embryo at the pronuclear stage is administered a transgene by injection. The transgene contains the coding region, often in the form of a cDNA of the protein of interest, coupled to a promoter that drives expression. The promoter is typically not the promoter of the native gene but rather a heterologous promoter chosen because of its strength or pattern of expression. However, transgenes may also consist of segments of genomic DNA that contain the gene of interest, often in the form of bacterial artificial chromosomes or P1 artificial chromosomes, in which case the transgene is driven by its native promoter and enhancers. The injected transgene integrates randomly, typically in multiple copies at a single site. Because no corresponding allele exists on the homologous chromosome opposite the integration site, these mice are usually called hemizygous. Because the heterologous promoters chosen are typically strong, the transgenic protein is often expressed at levels higher than would be present physiologically. In addition, because the mouse typically contains its own endogenous version of the gene, the transgene is generally expressed on top of the background expression of the mouse’s endogenous gene, and this leads to further overexpression.

4.3.1 Relevance as models of Alzheimer’s disease

Transgenic mouse models now exist that mimic a range of AD-related pathologies. Table 2 contains a summary of some of the more widely studied mouse models. These models have suggested new insights into the pathophysiology of this condition as well as novel therapeutic approaches. The models, however, raise a number of issues as well. First, it is clear that the success of transgenic mouse models has depended on the overexpression of APP transgenes containing FAD-associated mutations at levels that are not physiological. Indeed, many proteins, if overexpressed at sufficient levels, will become toxic at some point. It might be argued, therefore, that it is hardly surprising that overexpressing a naturally amyloidogenic protein would lead to amyloid deposits. However, interestingly, even though a number of APP wild transgenic lines have been created, only one transgenic mouse line overexpressing wild-type APP has been described that develops plaques. Thus, in general, overexpression of wild-type APP in mice does not induce plaque pathology, and the pathology that is seen seems to require the presence of an FAD mutation [121].

5. Expert opinion

Molecular pathogenesis of AD has grown enormously over the past few decades, and is now leading to the development of a range of intervention strategies, from which, hopefully, successful disease-modifying treatments will arise. Aβ is thought to be the most significant target for the therapy, but it is not a necessary initiator for the disease process. The lack of specificity of senile plaques and tangles highlight the need to look beyond Aβ for early pathogenesis. Tau pathologies play an important role, and their development offers additional targets for therapeutic intervention. In particular, the protein kinases, which cause the characteristic over-phosphorylation of tau protein in the neurofibrillary tangles, are viewed as tractable drug targets. A major hurdle for this approach is the unsolved question as to which of the different phosphorylation sites in the tau protein are essential for the transformation of native tau to its pathologic form and which protein kinase accomplishes this phosphorylation step. The proline-directed protein kinases GSK3b and Cdk5 have been described as having important tau-phosphorylating activity.

Neuroinflammation, especially activated microglia, is a consistent feature of lesions associated with AD. The generation and secretion of proinflammatory mediators may interact at multiple levels with neurodegenerative mechanisms. Thus, several proinflammatory cytokines not only induce neuro-pathic mechanisms and thereby contribute to neuronal death, but are also able to influence classical neurodegenerative pathways such as APP processing. The concomitant release of anti-inflammatory mediators may partly antagonize this action, ultimately contributing to the chronicity of the disease. Oxidative damage of macromolecules is markedly increased in AD. However, there are signs of systemic alterations in oxidative balance, the major site of damage is the cell bodies of neurons at risk of death in AD. This localization is consistent with an abnormality in the neuronal cytoplasm. Increased oxidative damage may be a result of altered turnover of damaged mitochondria, leading to increased redox-active metals. The extent of the abnormalities, as well as their chronic nature, suggest that a fundamental metabolic compromise is crucial to disease development.

Epidemiological data shows that lowering cholesterol by intake of statins reduces the risk of developing AD. Furthermore, studies in cell cultures and in transgenic mouse
models have demonstrated that hypercholesteremia can increase the production of Aβ peptides and amyloidosis. However, clinical studies that show a clear therapeutic effect of statins in AD patients are still lacking. While all these factors may be of little help for those where the disease has already struck, they may offer some incentives for those at risk, and this may well be everybody beyond a certain age, to minimize their personal risk. Nevertheless, it is evident that effective therapeutics are urgently needed, and it is hoped that anti-amyloid strategies will offer a significant step towards a causal therapy.

**Declaration of interest**

The authors state no conflict of interest and have received no payment in preparation of this manuscript.
Neurobiological aspects of Alzheimer’s disease

Bibliography

Papers of special note have been highlighted as either of interest (*) or of considerable interest (**) to readers.


* This paper is important in explaining the pathophysiology of Alzheimer’s disease.


* These results are important in explaining the primal role of amyloid-beta (Abeta) peptide in the development of Alzheimer’s disease.


Neurobiological aspects of Alzheimer’s disease

cytokines IFN-gamma and IL-12 and down-regulation of IL-4 in cerebral cortex regions of APPSwe transgenic mice. J Neuroimmunol 2002;126:50-7

This paper is important in explaining the pharmacotherapy of Alzheimer’s disease.


**This publication is important in explaining the rat brain in stereotoxic coordinates.**


Affiliation
Kanwaljit Chopra1, Shubham Misra & Anurag Kuhad
1Author for correspondence
Pharmacology Research Laboratory, University Institute of Pharmaceutical Sciences, UGC Centre of Advanced Study, Panjab University, Chandigarh-160014, India
Tel: +91 172 2534105; Fax: +91 172 2544142; E-mail: dr_chopra_k@yahoo.com