

# Degeneration and death of neurons in adult neurodegenerative diseases

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**Biochemical and biophysical events responsible for the degeneration and death of neurons in some common adult neurodegenerative diseases have been discussed. Critical analysis suggests that although aberrant aggregation of the diseased proteins represents an important factor in some instances, oxidative stress is probably the mother of most of the crucial events responsible for cell death, since it not only contributes to protein aggregation but also induces two other phenomena, viz. mitochondrial dysfunction and glutamate excitotoxicity, which play an important role in the death of neurons. Excellent reviews on oxidative stress<sup>1</sup>, protein aggregation<sup>2</sup> and mitochondrial dysfunction<sup>3</sup> have appeared recently. Here, the discussion is focused on the relative contribution and interrelationship between the critical events, which are responsible for the ultimate demise of neurons.**

**Based on the knowledge of the critical steps of the death pathway of neurons, current strategies for the development of potentially important multifunctional drugs, which are under clinical trial, have been reviewed. These new drugs target multiple steps in the cascade of the death pathway of neurons and are expected to be far more effective in dealing with these as yet incurable and inexorable diseases.**

**Keywords:** Multifunctional drugs, neurodegenerative diseases, neuronal death, oxidative stress, protein aggregation.

NEURODEGENERATIVE diseases in adults generally represent illnesses of the selective regions of the brain tissues controlling vital physiological functions such as learning and memory, posture and movement of nerves/muscles and coordination of these activities. These diseases are almost always associated with degeneration and death of neurons of the affected brain tissues. Mechanistic details of the selective vulnerability and death of these neurons are still unknown.

Recent investigations<sup>4-6</sup> on the effect of hypothyroidism in developing rat brain yielded two paradoxical observations similar to that in adult neurodegenerative diseases. First, in the developing brain, hypothyroidism down-regulated the expression of all three neurofilament (NF) proteins (NF-L, NF-M and NF-H); paradoxically this declined expression of NF was found to be associated with its abnormal accumulation in the hillock region of the hypothyroid axons.

Initially these two effects (down-regulation and abnormal accumulation of NF) were found to be similar to that observed in the motoneuron disease called Amyotrophic Lateral Sclerosis (ALS). Later, a broader survey of the literature revealed that aberrant aggregation of specific disease-related proteins represent a characteristic feature of some of the most common neurodegenerative diseases like Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and Prion disease (PrD). However, although protein aggregation is an important marker of these diseases, it is not the major issue in promoting the death of neurons. This review is focused on the role of protein aggregation and other important factors, viz. oxidative stress, mitochondrial dysfunction and glutamate excitotoxicity, which play a major role in the degeneration and death of neurons.

For most of the diseases, there is no definite preventive or curative treatment available at present. Drugs currently in use only retard the progression of these diseases. Research during the last few years signals the discovery of several new drugs which are anticipated to be highly superior to the existing formulations since these new multifunctional drugs target multiple steps of the neuronal death pathway compared to the currently available monofunctional drugs which generally target a single pathological step.

## Protein aggregation – a marker of common neurodegenerative diseases

Table 1 lists the genes, proteins and tissues affected in some common neurodegenerative diseases and demonstrates that aberrant aggregation of proteins is a characteristic feature in all of them. It may be noted that only a small fraction (~10%) of these diseases are of genetic or familial origin and the vast majority of them occur sporadically. Despite these differences with respect to origin, region of brain tissue and type of neurons affected, all the diseases are associated with aberrant aggregation of specific disease-related proteins, which are deposited as intra- or extra-neuronal inclusion in the form of plaques or tangles. Table 1 shows the location of aggregates of amyloid  $\beta$  (A $\beta$ ) for AD,  $\alpha$ -synuclein for PD, NF for ALS, huntingtin (Htt) with CAG repeats of >36 for HD and Prion scrapie protein (PrSc) for PrD.

X-ray diffraction<sup>7</sup> and NMR studies<sup>8</sup> revealed that these aggregates have a ribbon-like  $\beta$ -sheet structure formed by

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**Table 1.** Genes, proteins and tissues affected in some common neurodegenerative diseases

Neurodegenerative disease	Characteristic feature	Defective (disease) genes/proteins	Nature and location of aggregates
<b>Alzheimer's disease (AD)</b> Onset age ~ 65 yrs	Progressive loss of memory, task performance, speech and recognition of people and objects	<b>APP</b> for A $\beta$ , <b>presenilins</b> I and II and hyperphosphorylated <b>tau</b> , <b>Apo E</b> risk factor	Extracellular plaques of A $\beta$ <sub>42</sub> ( $\beta$ -amyloid protein); intracellular tangles of hyper-phosphorylated tau <b>Hippocampus, cortex</b>
<b>Parkinson's disease (PD)</b> Onset age ~ 65 yrs,  Early onset forms	Resting tremor, rigidity, slow movements, posture instability, loss of dopaminergic neurons Same as above	<b><math>\alpha</math>-synuclein</b>  <b>Parkin, DJ1 or Pink1</b>	Cytoplasmic aggregates called Lewy bodies containing $\alpha$ -synuclein and ubiquitin in <i>S. nigra</i> and <i>L. cereleus</i> .  Lewy bodies in lesser amounts
<b>Amyotrophic lateral sclerosis or motoneuron disease</b> Onset age: 55 yrs	Progressive motor weakness and paralysis	<b>Superoxide dismutase, neurofilament H (NF-H)</b>	Cytoplasmic inclusions of NF and ubiquitin in <b>spinal motoneurons/cortex</b>
<b>Huntington's disease</b> Onset age: 40–50 yrs	Involuntary jerky movement, motor impairment and dementia	<b>Huntingtin (Htt)</b> with CAG (glu) repeats of 36 or more on exon 1 form pathogenic aggregates	Intranuclear/cytoplasmic aggregates of N-terminal Htt and ubiquitin <b>cerebral cortex/striatum</b>
<b>Other CAG repeat diseases:</b> <b>SCA</b> <b>DRPLA</b> <b>SBMA</b>	Same as above	<b>Ataxin</b> <b>Atrophin-1</b> <b>Androgen receptor</b>	Intranuclear aggregates of disease proteins, <b>basal ganglia, brain stem, cerebellum and spinal cord</b>
<b>Prion diseases</b> <b>Kuru/CJD/GSS</b>	Imbalance, lack of coordination and intense irritation	<b>Prion scrapie (PrPSc)</b> Sporadic/genetic and infectious	Extra- and intracellular <b>amyloid plaques similar to AD</b>

In general, only about 10% of the diseases are of genetic/familial origin and the rest occur sporadically. Name of disease, genes/proteins affected and location of aggregates are shown in bold. Nature and location of aggregated proteins remain identical for the disease irrespective of its origin, viz. genetic or sporadic. Transgenic animal models are available for all the above diseases and are used extensively for pathological and therapeutic studies. Drugs presently in use only retard the progress of diseases. However, the situation is rapidly improving and some highly effective drugs are currently under human trial. Survival after the onset of the disease is only about 5 years for ALS and about 15–20 years for AD/PD.

Apo E, Apolipoprotein E; Htt, Huntingtin; APP, Amyloid precursor protein; CJD, Creutzfeldt–Jacob disease; GSS, Gerstmann–Strausler–Scheinker disease; SCA, Spino cerebellar ataxia; DRPLA, Dentatorubral and Pallido–Luysian atrophy; SBMA, Spino bulbar muscular atrophy.

$\beta$ -strands running perpendicular and hydrogen bonds running parallel to the long axis. The formation of such fibrillar aggregates involves an initial step of transformation of the native forms of the proteins from  $\alpha$ -helix/random coil to  $\beta$ -form followed by oligomerization, formation of proto-filaments and their lateral aggregation into fibrils. In AD brain, oligomers of A $\beta$  have often been seen in clusters spatially distinct from fibrillar A $\beta$  deposits<sup>9</sup>. Similar oligomeric intermediates have been observed for  $\alpha$ -synuclein and huntingtin<sup>10,11</sup>.

Mechanisms which trigger the abnormal aggregation of the disease-related proteins are not clear. However, an initial common step is conversion of the native protein from its  $\alpha$ -helical/random coil form to the aggregate-prone  $\beta$ -form. Factors promoting this step include: (a) covalent modification of proteins, such as nitration in case of  $\alpha$ -synuclein<sup>12</sup> in PD or phosphorylation of NF-H<sup>13</sup> in ALS or of tau<sup>14</sup> in AD; (b) increased gene dosage, e.g. triplication of the  $\alpha$ -synuclein gene<sup>15</sup> in PD or (c) abnormal proteolytic cleavage, such as that seen in the case of cleavage of the amyloid precursor protein, APP  $\rightarrow$  neurotoxic A $\beta$ <sub>42</sub> by  $\beta$ - and  $\gamma$ -secretase instead of normal cleavage by  $\alpha$ - and  $\gamma$ -secretase which produces non-amyloidogenic and non-aggregate-prone peptide<sup>16</sup>.

Other proteins commonly found in aggregates of the disease-related proteins are ubiquitin, chaperones and

proteasomes. Ubiquitin mediates the destruction of most of the toxic proteins in the cell by interacting with them and delivering them to the proteasome complex for proteolytic degradation<sup>17</sup>. Accordingly, most of these aggregates are often found to be associated with ubiquitin (Table 1) and are immunostainable not only with the respective antibodies against the disease-related proteins, but also with antibody to ubiquitin.

Many investigators have examined the correlation between protein aggregation and degeneration of neurons. Axonal aggregates of NF protein in ALS mice has been reported to interfere with vital axonal transport system leading to degeneration of neurons<sup>18</sup>. Aggregates of alpha-synuclein have been observed in the form of swelled neurites of the degenerating neurons in both familial and sporadic forms of PD<sup>19</sup>, but correlation between aggregation and cell death has not been established. Likewise, there is a poor correlation between the clinical symptoms of AD patients and the number of amyloid plaques observed in their brain tissues<sup>20</sup> or between cell death and Htt aggregates in HD<sup>21</sup>. Recent evidence suggests that compared to the mature aggregates, misfolded oligomers of  $\beta$ -conformation, found in the early stages of the process, are relatively more toxic<sup>22,23</sup>. Thus while protein aggregation may represent an important marker of degeneration of neurons in most neurodegenerative diseases, oxidative stress plays a major role in the death

of neurons not only because of its contribution in protein aggregation, but also in mitochondrial dysfunction and glutamate excitotoxicity – factors which contribute maximally to a cascade of events leading to induction of the caspases (cysteine-dependent, aspartate-specific proteases) and apoptotic cell death. Studies on transgenic animal and cell culture models suggest that apoptosis, rather than necrosis, is primarily responsible for the loss of neurons<sup>24,25</sup> and that caspases are the major executioners of apoptosis in common neurodegenerative diseases<sup>26,27</sup>. The role of apoptosis and involvement of caspases in neurodegeneration and aging has also been reviewed recently<sup>28</sup>.

### Oxidative stress – the major initiative factor in degeneration and death of neurons

The respiratory chain of mitochondria, where oxygen is reduced to water by a series of enzymes, is important as the vital source of ATP. Defects in the respiratory chain yield partially reduced free-radical products of oxygen, such as superoxide ( $O_2^\bullet$ ), hydrogen peroxide ( $H_2O_2$ ) and  $OH^\bullet$  radicals, which are highly toxic and damage normal cellular constituents by reacting with them, and hence they are called reactive oxygen species (ROS). A wide variety of ROS is found in the biological system, which differs in the sites of formation, function and biological half-life<sup>29</sup>.

Nitric oxide ( $NO^\bullet$ ) represents another important free radical, which plays a prominent role in the physiology of the central nervous system<sup>29</sup>. While under normal physiological conditions,  $NO$  acts as a vasodilator/antithrombic agent/neurotransmitter, overproduction due to excessive activity of nitric oxide synthase (NOS) is toxic. The harmful effects of  $NO^\bullet$  result from reaction of excess  $NO^\bullet$  with superoxide to form peroxynitrite ( $ONOO^-$ ), which is a highly reactive nitrogen species (RNS). Together with the ROS, RNS also contributes to oxidative stress and cell death.

Table 2 lists some the most common ROS and RNS, which are responsible for oxidative damage of biomolecules, and also antioxidants (enzymes and small molecules) which help to relieve stress by acting as scavengers of the molecules causing oxidative stress. Under normal physiological conditions, such as that in young and healthy animals,

there is a balance between generation and scavenging of ROS. Oxidative stress is evident only when the level of ROS/RNS increases above the threshold level or that of the antioxidants declines beyond the normal limits.

Depletion of glutathione (GSH) and/or accumulation of toxic free radicals (ROS/RNS) represent one of the major causes for oxidative stress<sup>30</sup>. A large body of evidence indicates that the concentration of GSH, the most important antioxidant, decreases and oxidative damage of DNA, protein and lipid increases with age due to enhanced accumulation of ROS/RNS. Peroxynitrite formed by reaction between superoxide and nitric oxide is responsible for the nitration of tyrosine and a high level of 3-nitro-tyrosine is found in cerebrospinal fluid of PD patients<sup>31</sup> and in spinal cord of ALS patients<sup>32</sup>. Such covalent modifications of disease-related proteins may lead to their conversion from normal  $\alpha$ -helical/random coil structure to the toxic  $\beta$ -configuration, which is prone to form aggregated fibres. The final aggregates, when accumulated in the perikaryon or in proximal region of axons, may disrupt the vital transport of nutrients across axons leading to strangulation of axons and death of the neuron. Alternatively, the more toxic intermediate oligomeric forms of disease-related proteins are often directly involved in the induction of p53 and generation of proapoptotic signals leading to mitochondrial dysfunction, cytochrome C release<sup>33,34</sup> and a series of reactions leading to cell death. Figure 1 shows the critical steps involved in the death pathway of neurons and the role of oxidative stress, mitochondrial dysfunction and glutamate excitotoxicity in the process.

### Mitochondrial dysfunction

Current evidence suggests that mitochondrial dysfunction (originating from oxidative stress or other events like induction of p53 by disease-related proteins) plays a crucial role in activating the cascade of reactions involved in the death of neurons (Figure 1). Two key initial events resulting from mitochondrial dysfunction are (a) disruption of energy metabolism leading to deficiency of ATP production and (b) opening of the mitochondrial permeability transition pore leading to loss of mitochondrial permeability with release of cytochrome C and other pro-apoptotic compounds. These later events, which are regulated by p53 and the Bcl-2 family of proteins (proteins which are characterized by their homology to the proto-oncogene *Bcl-2*), are shown in more detail in Figure 2.

Brain mitochondria are a primary target of oxidative stress (GSH depletion, accumulation of ROS/RNS, lipid peroxidation), excitotoxicity and  $Ca^{++}$  overload – factors leading to loss of membrane permeability. Experiments with transgenic animals and cultured neurons suggest that the pro-apoptotic proteins Bid, Bad, Bax and Bak and the anti-apoptotic protein Bcl-2 and Bcl-Xl play a major role in the loss of mitochondrial function, release of cyto-

**Table 2.** Some common reactive oxygen/nitrogen species causing oxidative stress and antioxidants which provides protection

Common biomolecules responsible for oxidative stress	Common antioxidants which protect from oxidative stress
Superoxide ( $O_2^\bullet$ )	Superoxide dismutase
Hydrogen peroxide	Catalase
$OH^\bullet$ radical	Glutathione (GSH)
Nitric oxide ( $NO$ )	GSH peroxidase
Oxidized glutathione (GSSG)	Lipoic acid
Malonaldehyde/hydroxyneonal	Tocopherol
Semiquinone radical	Dietary flavonoids
	Ascorbic acid

chrome C and apoptosis. Bax stimulates cell death in many types of neurons including ganglia, motoneurons, cerebellar and hippocampal neurons<sup>35</sup>. BCL-XL is essentially a negative regulator of the Bax mediated death pathway<sup>36</sup>.

Another important pro-apoptotic signal for mitochondrial dysfunction is the induction of p53. Miller *et al.*<sup>37</sup> reported that the induction of p53 mRNA could result from oxidative stress characteristic of most of the neurodegenerative disorders. Treatment of cultured neurons with neurotoxic amyloid beta peptides also led to the induction of pro-apoptotic transcription factor p53. As seen from Figure 2, p53 stimulated the expression of the two proapoptotic proteins, Bax and Bid, which are involved in the release of cytochrome C and apoptosis<sup>38</sup>. In addition, p53 activates Bax by direct interaction with the antiapoptotic protein BCL-XL, which is a negative regulator of Bax and normally disables Bax/Bid mediated pore formation and cytochrome C release<sup>39,40</sup>.

Mitochondria-mediated apoptosis can also be induced by the death receptor pathway, e.g. binding of the ligand Fas-L to its receptor Fas-R in the plasma membrane may lead to activation of caspase 8, which may directly activate pro-caspase 3 to caspase 3 in some cells; in others, caspase 8 cleaves Bid and the truncated Bid enters the mitochondria to interact with Bax, causing loss of mitochondrial outer membrane permeability, thus releasing cytochrome C and other pro-apoptotic agents (Figure 2). Plesnila *et al.*<sup>41</sup>

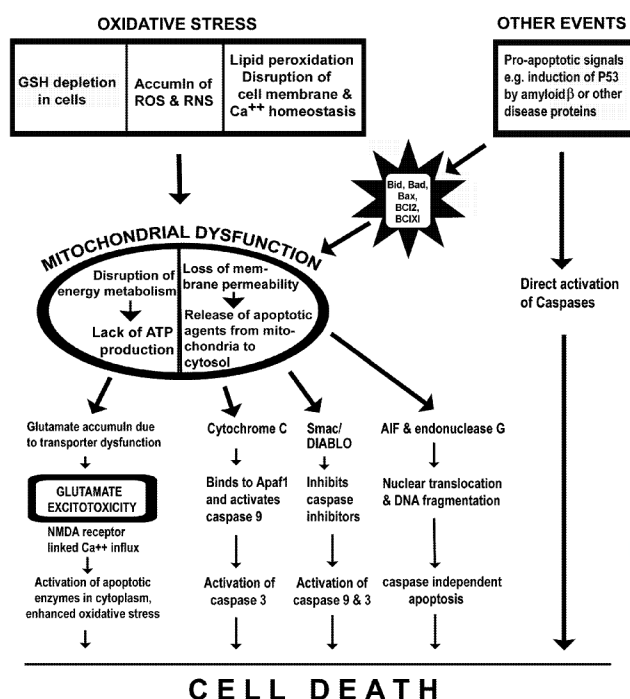
demonstrated that Bid-deficient mice offer protection against cytochrome C release and impair caspase 3 activation.

Excitotoxic  $\text{Ca}^{++}$  dephosphorylates and activates another BCL-2 protein Bad through  $\text{Ca}^{++}$ -dependent phosphatase calcineurin<sup>42</sup>. Bad regulates cytochrome C release in the neurons by inactivating anti BCL-2 proteins, which inhibit Bak/Bax<sup>43</sup>. Although the exact mechanism of action of these proteins is not clear, studies with double knockout mutants of Bax and its close relative Bak revealed that interaction of Bid with these multi-domain proteins (Bax/Bak) is essential for the release of cytochrome C and apoptosis<sup>44</sup>.

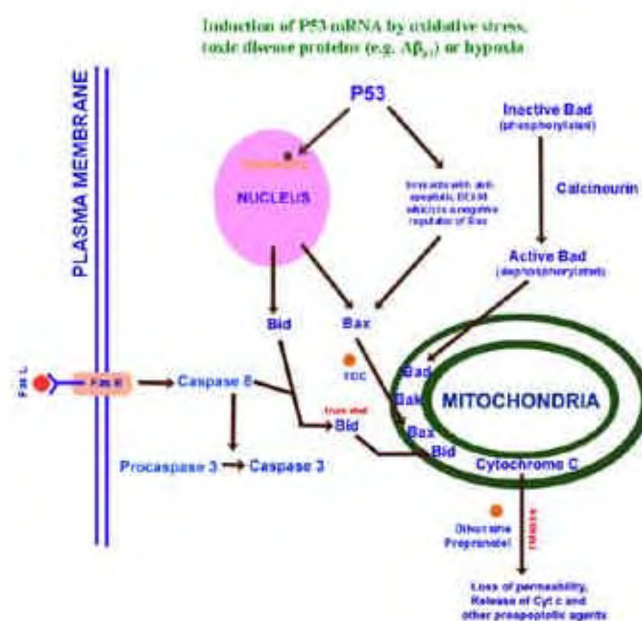
In neurons, Bax normally resides in the cytoplasm complexed with the 24 amino acid antiapoptotic peptide humanin<sup>45</sup>. Tauroursodeoxycholate prevents Bax translocation to mitochondria<sup>46</sup>, while dibucaine/propranolol inhibits Bax-induced cytochrome C release following translocation<sup>47</sup> (Figure 2). Bak, on the other hand, is a constitutive protein in mitochondria, which has a pro-death function. Considerable debate still exists concerning Bax-mediated cytochrome release<sup>3</sup>, but Shimizu *et al.*<sup>36</sup> demonstrated that Bax combines with the outer membrane voltage-dependent anion channel (VDAC) for the release of cytochrome C and other apoptotic agents.

## Glutamate excitotoxicity

One of the two major effects of mitochondrial dysfunction (Figure 1) is disruption of energy metabolism, since functional defects at the level of complex I in the respiratory chain lead to impaired ATP metabolism. Deficiency of ATP leads to dysfunction of glutamate transporters and accumulation



**Figure 1.** Critical events responsible for death of neurons. Bax, Bid and Bad, Proapoptotic proteins; BCL-2 and BCL-XL, Anti-apoptotic proteins; Smac, Second mitochondria derived activator of caspase; DIABLO, Direct IAP associated binding protein with low PI; IAP, Inhibitor of apoptosis; Apaf, Apoptosis protease activating factor; AIF, Apoptosis inducing factor.



**Figure 2.** Role of BCL-2 family of proteins in mitochondrial dysfunction and release of cytochrome C and other apoptotic factors. TDC, Tauroursodeoxycholic acid. Other abbreviations as in Figure 1.

of glutamate. This, in turn, leads to glutamate excitotoxicity due to excessive activation of NMDA-linked glutamate receptors followed by  $\text{Ca}^{++}$  influx, induction of  $\text{Ca}^{++}$ -induced enzymes, apoptosis and cell death.

Studies on postmortem AD and PD brains provided evidence not only for increased oxidative stress and mitochondrial dysfunction, but also of impaired uptake and increased accumulation of glucose and glutamate<sup>48</sup>. Likewise, increased levels of glutamate have also been reported in the cerebrospinal fluid of sporadic ALS patients<sup>49</sup>. Accordingly, reduced glutamate levels in spinal cord homogenates of ALS tissues are also considered to be due to impaired uptake of glutamate<sup>50</sup>. These results suggest glutamate excitotoxicity as the prime reason for the degeneration and death of neurons.

The intraneuronal concentration of glutamate is 10 millimolar and the extracellular concentration is approximately 0.6 micromolar; significant excitotoxic damage to neurons may occur when extracellular glutamate reaches a level of 2–5 millimolar. In normal cells, glutaminergic neurotransmission is finely tuned and glutamate levels are regulated by ATP-dependent, Na–K-coupled, high-affinity transporters of glutamate, referred to as excitatory amino acid transporters. ATP depletion due to mitochondrial dysfunction leads to failure of the glutamate transport system<sup>51</sup>.

Astrocytes play an important role in maintaining the level of extracellular glutamate since deficiency of ATP impedes the conversion of glutamate to glutamine in astrocytes by the ATP-dependent enzyme glutamine synthetase. This leads to intracellular accumulation of glutamate and its release in extracellular space. Furthermore, since glutamate transporters fail to deliver the glutamate to neurons, extracellular accumulation of glutamate continues.

Glutamate is an important excitatory neurotransmitter and its interaction with specific membrane receptors is linked with many important functions like learning, memory and sensation. Based on their agonists, the glutamate receptors are divided into three groups, viz. NMDA (*N*-methyl *D*-aspartate), AMPA (alpha amino 3-hydroxy 5-methyl 4-isoxalopropionate) and kainate.

Over-reactivity of glutamate with its receptors leads to prolonged opening of ion channels, permitting  $\text{Na}^+$  to enter in the post-synaptic neurons. The resulting depolarization and opening of NMDA-linked  $\text{Ca}^{++}$  channels leads to excessive  $\text{Ca}^{++}$  influx and a cascade of reactions, viz. activation of a series of  $\text{Ca}^{++}$ -dependent enzymes, including protein kinase, proteases, phosphatases, phospholipases, nNOS and xanthine oxidase (the last three enhancing oxidative stress) leading to apoptosis and cell death.

Figure 3 shows the interrelationship between oxidative stress and other events facilitating degeneration and death of neurons. It can be seen that while oxidative stress is one of the major factors responsible for mitochondrial dysfunction, the latter itself leads to increased production of ROS and RNS, and further enhances oxidative stress due

to inhibition of the respiratory chain enzymes<sup>52</sup>. Likewise, while ATP deficiency due to defects in the respiratory chain of mitochondria is largely responsible for glutamate excitotoxicity, brain mitochondria is the primary target of excitatory  $\text{Ca}^{++}$  overload, leading to loss of membrane permeability. Thus oxidative stress is not only related to protein aggregation but is closely interrelated with mitochondrial dysfunction and glutamate excitotoxicity. Finally, the process of cell death is contagious, since it is associated with release of additional factors like TNF- $\alpha$  and interleukin- $\beta$  that affect neighbouring cells<sup>53</sup>.

## Therapeutic strategy

Drugs currently available for treatment of some of the most common neurodegenerative diseases can only retard their progress rather than curing or arresting them. These drugs are monofunctional, since they generally target one of the many steps critical for the degeneration of neurons. However, based on advancement in our knowledge regarding the pathology of these diseases during the last few years, several new multifunctional drugs targeting multiple steps of the death pathway of neurons are under development. Table 3 lists some of the most important monofunctional drugs that are currently in use. Table 4 lists the new multifunctional drugs, most of which are under clinical trial. In view of the existence of some common pathological steps in the death pathway of neurons in various degenerating diseases and the wide spectrum of the multifunctional drugs, these new drugs are often active against more than one disease.

## Monofunctional drugs

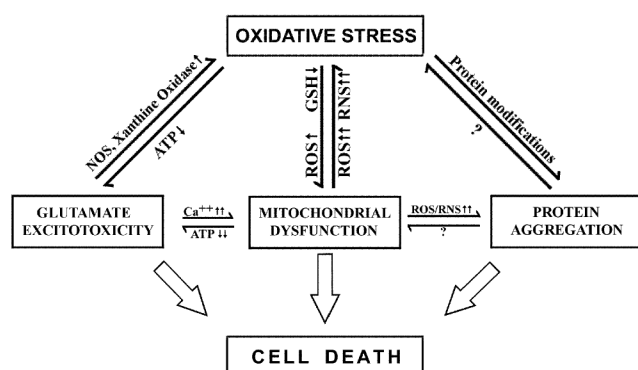
By far, the most common neurological disorder is AD, which is prevalent in as much as 1% of the adults at age 65, and increases<sup>54</sup> to almost 40% at age 90. Deficiency of acetylcholine (cholinergic deficit), which occurs due to degeneration and death of the cholinergic neurons is a prime factor responsible for the progressive loss of memory and attention associated with the disease. Based on this clinical symptom, a group of drugs currently in use are inhibitors of acetylcholine esterase. Treatment with these drugs is generally most effective within the first 6 months to a year, and the drugs appear to be less effective thereafter. Moreover, they show considerable side effects. The efficacy of three most popular cholinesterase inhibitors has been compared recently<sup>55,56</sup>; in a 6-month study the efficacy order was found to be as follows: rivastigmine > donepezil > galantamine.

Another group of drugs for AD target the NMDA receptors, which are involved in glutamate excitotoxicity. Memantine represents the most common NMDA receptor antagonist that is effective as first line monotherapy for

**Table 3.** Monofunctional drugs currently in use

Disease	Drug (s)	Target (s)	Remarks/side effects and reference (s)
AD	<b>Rivastigmine</b> Donepezil galantemine	<b>Inhibition of acetylcholine breakdown</b> and improvement of cholinergic function	These drugs are <b>acetyl choline-esterase inhibitors</b> ; treatment not specific for AD neurons and drug effect declines progressively <sup>55,56</sup> .
	<b>Memantine</b> deprenil	<b>To prevent NMDA-receptor mediated excitotoxicity</b>	These drugs are <b>NMDA receptor antagonists</b> . Memantine has minimal side effect due to selective blocking of NMDA receptor function <sup>57</sup>
	Lumiracoxib etoricoxib	<b>To prevent the activity of cyclogenase (Cox)</b>	<b>Cox 2 is inflammatory to AD neurons</b> <sup>58</sup>
PD	<b>Syndopa</b> Levodopa/carbidopa	<b>Dopamine substitutes used to replenish dopamine</b> which is reduced due to loss of dopaminergic neurons	<b>Effectiveness reduced 2–5 years</b> ; patients develop dyskinesia (involuntary movement ) and side effects like nausea and vomiting <sup>59</sup>
	Tolcapone and entacapone	<b>To extend the half-life of levodopa</b> by inhibiting its methylation and conversion to toxic homocysteine	<b>COMT (catecholamine methyl transferase) inhibitors</b> used as adjuncts to Levodopa to improve motor function <sup>62,63</sup>
	Pramipexole Pergolide ropinerole	<b>To mimic the action of dopamine</b> by activation of striatal neurons	<b>Dopamine agonists</b> used to achieve dopamine effect <sup>60,61</sup> .
	Selegiline	<b>To prevent MAO-B from breaking dopamine</b> and production of H <sub>2</sub> O <sub>2</sub>	Selegiline originally used as <b>MAO-B inhibitor</b> to reduce or prevent generation of ROS <sup>64</sup>
	Trihexiphenidyl and biperidine	<b>To reduce cholinergic neurotransmission</b>	<b>Anti-cholinergic</b> drugs which lessen tremor and drooling but are ineffective against brady kinesia and posture instability <sup>65</sup>
ALS	<b>Riluzole</b>	<b>To reduce excitotoxicity</b>	<b>Anti excitotoxic drug</b> which modestly increases survival <sup>66</sup>
PrD	Quinoline derivatives, Quinacrine/ chlorpromazine, amphotericin B	<b>To inhibit toxic PrScP formation</b>	<b>Inhibits transformation of normal Prion protein</b> to toxic PrSc in infected animals <sup>67</sup> .
HD	Juglone celastrol	<b>To prevent aggregation of Htt</b>	These drugs <b>reversed aberrant aggregation of Htt</b> in striatal neuronal cell line <sup>68</sup>

The most popular drugs and their targets are shown in bold.



**Figure 3.** Inter-relationship between oxidative stress and other events in facilitating neurodegeneration and death of neurons.

AD, and this drug is associated with minimal side effects<sup>57</sup>. A third group of drugs for AD are cyclogenase (Cox) inhibitors since Cox, the superfamily of prostaglandin synthase genes, potentiates or propagates inflammation in AD neurons. Preventive intervention for AD includes vitamins (especially vitamin E) and selenium as well as non-steroidal, anti-inflammatory drugs such as lumiracoxib and etoricoxib, which are inhibitors<sup>58</sup> of cox 2. Drugs currently in use for PD are dopamine substitutes<sup>59</sup> (syndopa and related ones) or dopamine agonists<sup>60,61</sup> like pramipexole. These drugs generally lose their effectiveness in 2–5 years, when patients

develop dyskinesia and other side effects. The COMT inhibitors<sup>62,63</sup> (tolcapone and entacapone), represent important adjuncts to levodopa treatment, since they enhance the duration of active life of levodopa. Inhibitors of MAO such as selegiline<sup>64</sup> and anti-cholinergic drugs<sup>65</sup> like trihexiphenidyl represent the other group of drugs currently used to combat PD.

Therapeutic strategy for ALS, PrD and HD remains to be improved. For ALS or motoneuron disease, riluzole<sup>66</sup> appears to be the only available drug. For PrD, the quinoline group of drugs<sup>67</sup> which prevent the conversion of the native prion protein (PrPC) to the toxic scrapie form (PrPSc) is generally employed and for HD, juglone/celastrol, which inhibit the aggregation of huntingtin<sup>68</sup> are the only drugs of choice.

### Multifunctional drugs

These drugs target more than one pathological event in the death pathway of neurons and hence are generally more powerful compared to the monofunctional drugs. Interestingly, rasagiline, originally discovered as a MAO-B inhibitor, has recently been identified as one of the most potent multifunctional drugs. Youdim *et al.*<sup>69</sup> showed that rasagiline

**Table 4.** Multifunctional drugs currently on trial

Disease	Drug (s)	Target (s)	Remarks/side effects and reference(s)
PD	<b>Rasagiline plus levodopa</b>	<b>To inhibit MAO B, to prevent cytochrome C release and apoptosis</b>	<b>Minimal side effects; anti-apoptotic effects are due to activation of Bcl-2, protein kinase C and inhibition of Bax and FAS</b> <sup>69</sup> .
AD	<b>Lipocrine</b> (hybrid of tacrine and lipoic acid)	<b>To inhibit acetyl choline esterase and generation of ROS by lipoic acid</b>	Bifunctional drug <b>aimed at increasing cholinergic function and reduce ROS</b> <sup>71</sup>
PD/AD	<b>Ladostigil</b> (carbamate plus the propargyl amine)	<b>To inhibit acetyl choline esterase activity and apoptotic activity as rasagiline</b>	<b>Neuroprotection achieved by inhibition of Mao B and due to anti-apoptotic activity</b> <sup>70</sup>
	<b>VK28 plus propargyl-amine</b> (HLA-20)	<b>To chelate iron and prevent apoptosis as rasagiline</b>	<b>Neuroprotection by preventing generation of toxic OH<sup>o</sup> by Fenton's reaction and apoptosis</b> <sup>70</sup>
ALS	<b>Riluzole plus rasagiline</b>	<b>To inhibit excitotoxicity and achieve all the other antiapoptotic effects</b>	<b>Should be potentially far more effective than riluzole now in use</b> <sup>72</sup>
ALS/ PD/HD	<b>Minocycline</b> (second generation tetracycline)	<b>To inhibit NOS, cytochrome C release from mitochondria and caspase 3-mediated apoptosis</b>	<b>Highly successful multi-functional drug now at phase III human trial</b> <sup>74</sup>
PD/HD/ ALS	<b>Stem cell replacement therapy</b>	<b>To replace the affected neurons in the respective tissues by stem cells</b>	<b>Partially successful in PD/HD; experiments still in progress for AD/ALS. Refinement of technology needed</b> <sup>83-86</sup>
PD	<b>Deep brain stimulation</b>	<b>Stimulation of dopamine release using micro-electrode insertion in subthalamic nuclei</b>	<b>Clinical improvement varies depending on precise location and volume of lesions; Surgical procedure needs further development</b> <sup>87,88</sup>
	<b>Intrastriatal trans-plantation of foetal mesencephalic tissue</b>	<b>Reinnervation by grafting dopaminergic neurons of embryonic tissue</b>	<b>Offers substantial benefits in the limited number of trials performed; procedure still under development</b> <sup>89,90</sup>

is not only an inhibitor of MAO-B, but it also prevented loss of mitochondrial permeability and cytochrome C release, and down-regulated the expression of the proapoptotic genes, *FAS* and *Bax*. In view of these important properties of this drug, clinical trial of a large number of multifunctional drugs containing rasagiline alone or in combination with a variety of other agents, are currently in progress.

Ladostigil (TV 3326), a multifunctional drug containing the propargylamine moiety of rasagiline and a carbamate moiety of rivastigmine as inhibitor of acetylcholine esterase has been found to inhibit MPTP-induced PD in mice and is currently under clinical trial<sup>70</sup>. Likewise, lipocrine, a hybrid of tacrine (an inhibitor of acetylcholine esterase) and lipoic acid (an antioxidant preventing the generation of ROS) is being tested as a promising anti-Alzheimer compound<sup>71</sup>.

Iron accumulation in dopaminergic neurons of PD patients or in the amyloid plaques and tangles of AD patients, is one of the common features of these diseases. For this reason, iron chelators like VK 28 have been used as neuroprotective agents since iron reacts with hydrogen peroxide to produce the highly toxic OH radical (Fenton reaction) and thus increases the level of ROS. A multifunctional drug containing VK28 and the propargylamine moiety of rasagiline, the MAO inhibitor, has recently been prepared and is being investigated for its neuroprotective effect<sup>70</sup>.

Riluzole, the only available antiexcitotoxic monofunctional drug for ALS patients who normally survive 4–5 years, can only extend the survival by about a year. Rasagiline in

combination with riluzole, now under clinical trial<sup>72</sup>, is certainly expected to yield a much better result.

A potentially important drug for ALS, now under phase III human clinical trial, is minocycline – a second generation tetracycline. Minocycline inhibits iNOS and directly inhibits the release<sup>73</sup> of cytochrome C and caspase 3. Neuroprotection with minocycline has been successful in mouse models of ALS, HD, PD and multiple sclerosis<sup>25</sup>. Minocycline has great expectation since it is orally bioavailable, crosses blood-brain barrier, proven to be safe in humans and is under clinical trials for ALS and HD patients<sup>74</sup>.

It may be conceivable that the therapeutic effects of drugs containing rasagiline or minocycline are based largely on their antiapoptotic properties. Several other therapeutic agents targetted against aberrant aggregation properties of the disease-related proteins or immunization of patients against these proteins, are also under development. One such agent is based on the common  $\beta$ -sheet/ $\beta$ -strand conformation of the toxic oligomeric forms of mutated disease-related proteins, viz. A $\beta$ 42,  $\alpha$ -synuclein and huntingtin in AD, PD and HD respectively. Indeed antibody prepared against a conformation-specific oligomeric precursor of A $\beta$ 42 recognized fibrils of unrelated sequences, including those of polyglutamine ( $\alpha$ -synuclein or lysozyme, i.e. those containing the  $\beta$ -sheet/ $\beta$ -strand structure), but not to globular, non-native aggregates of collagen, gelatin or elastin, which were free of  $\beta$ -conformation<sup>75</sup>. Some of the agents effective against aberrant aggregation of proteins are: Congo red (binds to all



$\beta$ -sheeted proteins and prevents their aggregation<sup>76</sup>; bis-acridine (inhibits conversion of PrP to PrScP)<sup>77</sup>; phthalocyanine sulfonates (inhibits aggregation of  $\alpha$ -synuclein<sup>78</sup>; and benzothiazoles (inhibits aggregation of polyglutamines<sup>79</sup>). The therapeutic efficacy of these agents *in vivo* is still under investigation.

Since most of the diseases discussed above are associated with the generation of toxic proteins of altered conformation, active or passive immunization against these proteins is another potentially important approach to combat these diseases. Attempts to immunize APP mutated transgenic mice<sup>80</sup> bearing A $\beta$ <sub>42</sub> or passive immunization with A $\beta$ <sub>42</sub> antibody<sup>81</sup> have been successful in clearing the A $\beta$  plaques; however, the adverse reaction of the antibody in patients (encephalitis) suggested that some refinement of immunization methods are essential<sup>82</sup>. Investigators in this field are aware that many drugs, successful in animal models, have proven to be ineffective in human. Nevertheless, like in the case of other diseases, successful preparation of an effective vaccine against the toxic proteins of the neurological diseases will have a major impact. This problem represents one of the most challenging and exciting areas of current research in this field.

Finally, recent investigations on stem cell research and their successful application in animal models of several neurodegenerative diseases have opened up a new direction for treatment of PD<sup>83</sup> and ALS<sup>84</sup>. The properties that make the foetal stem cells particularly useful for neural and other transplantation have been recognized<sup>85</sup>. It has also been realized that for successful treatment, the transplanted cells should not only be integrated with the host tissue by growing neurites and synapses, but also display functional properties with respect to metabolism of the appropriate neurotransmitters. However, despite some initial success in experiments with human foetal cells for PD and HD<sup>83,85</sup>, significant constraints still remain to be resolved for routine use of foetal cells for neural transplantation, particularly in the case of ALS<sup>84</sup>. Likewise, deep brain stimulation of subthalamic nuclei<sup>87,86</sup> and intrastriatal transplantation of foetal dopaminergic neurons<sup>89,90</sup> are two other recent surgical innovations which have yielded substantial benefits to PD patients. However, several critical issues remain to be resolved in both these procedures before they can be employed routinely as an effective and safe therapy.

## Conclusions

During the last few years, there has been considerable progress in our knowledge about the degeneration and death of neurons in common neurodegenerative diseases. Oxidative stress appears to be the most important factor, since it induces several other key events like protein aggregation, mitochondrial dysfunction and glutamate excitotoxicity, which together contribute to the death of neurons. Among these, mitochondrial dysfunction plays a pivotal role in

cell death. First, disruption of energy metabolism leads to glutamate excitotoxicity and enhances oxidative stress further. Second, Bax and BCL-2 family proteins alter mitochondrial outer-membrane permeability, resulting in release of cytochrome C and other proapoptotic signals activating the killer caspases. Advancement in our understanding of the process of neuronal death has led to the development of several novel multifunctional drugs targeting multiple steps of the death pathway of neurons. These drugs, most of which are under human clinical trial, are expected to be far more effective compared to the monofunctional drugs currently used to combat the incurable diseases.

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