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Interrupting apoptosis in neurodegenerative disease: potential for effective therapy?

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Current treatment options for neurodegenerative diseases are limited and mainly affect only the symptoms of disease. Because of the unknown and probably multiple causes of these diseases, they cannot be readily targeted. However, it has been established that apoptosis contributes to neuronal loss in most neurodegenerative diseases. A possible treatment option is to interrupt the signaling networks that link neuronal damage to apoptotic degradation in neurodegeneration. The viability of this option depends upon the extent to which apoptosis accounts for neuron loss, whether or not interruption of apoptosis signaling results in recovery of neurological function and whether or not there are significant downsides to targeting apoptosis. Several compounds acting at different sites in known apoptotic signaling networks are currently in development and a few are in clinical trial. If an apoptosis-targeted compound succeeds in slowing or halting neurological dysfunction in one or more neurodegenerative diseases, a new era in the treatment of neurodegenerative diseases will begin.

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▼ Neurodegenerative diseases (ND) are responsible for a major portion of the economic burden of ill health and the burden will increase greatly as the proportion of the elderly increases in the population. The magnitude of the medical and socioeconomic problems presented by ND is considerable and is illustrated by the figures provided in Box 1 and Table 1. Current treatments mainly ameliorate the symptoms of disease and the treatment effects are generally moderate, with the exception of Parkinson's disease (PD). Therefore, the development of drugs with a capacity to delay the neurological deficits associated with ND is an urgent and important goal. One approach that has the potential to achieve this goal is the use of small molecules that interfere with apoptosis signaling mechanisms thought to contribute to neuronal loss in ND.

Neuronal loss, dysfunction and regrowth in neurodegeneration

Progressive neuronal loss is a key feature of ND. Different ND are recognized by the neuronal phenotypes that are primarily lost and the neurological defects that accompany this loss. For example, cortical and spinal motoneuron loss causes spasticity and reductions in muscle power and bulk in amyotrophic lateral sclerosis (ALS); hippocampal, septal and cortical neuron loss contributes to reductions in short term memory and cognitive functions in Alzheimer's disease (AD); and nigrostriatal and locus coeruleus neuronal loss results in poverty and slowness of movement with muscle stiffness in PD. Neuronal loss alone does not always fully explain the neurological deficits found in ND. Decreased neuronal cell body size, atrophy of neuronal dendrites and reductions in axonal terminal fields have all been identified in ND [1]. These changes appear to reduce the complexity of interneuronal connections and the capacity of affected neurons to support their electrophysiological and synaptic functions. Therefore, it is a combination of progressive neuronal loss and neuronal dysfunction that underlies ND [2]. Clinical progression in ND is further complicated by the fact that as some neurons in a given population become dysfunctional and die, others appear to compensate for the loss by expanding their individual connective interactions and functional capacities [3]. Therefore, neuronal circuits relating to specific neurological functions are lost, pruned, expanded and reorganized as ND progresses. Compensatory neuronal circuit reorganization could be partially responsible for observations that neurological deficits only become clinically

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or neurophysiologically detectable when 50–70% of the neurons in a functional population have been lost. Those neurons remaining become all the more important for maintaining an albeit limited function.

Causes of neurodegenerative processes

Data generated by examination of post-mortem nervous tissue and studies of animal or tissue culture models indicate that there are a variety of different causes that could trigger ND. Suggested causes include inadequate provision of trophic molecules by neighboring neurons or glia, impaired axonal transport, glutamate receptor overactivation, excessive levels of reactive oxygen species (ROS), compromised metabolic pathways, reduced mitochondrial energy production, increased formation or inadequate degradation of inappropriately folded proteins, inflammatory processes, virus or prion infection, loss or gain of functions of specific protein or lipid moieties as a result of nuclear or mitochondrial DNA mutations or deletions and improper RNA or protein processing and combinations of and interactions among these processes [4–16]. Ideally, one would like to protect neurons from ND by eliminating one or more basic causes. However, the causes of ND cannot be definitively stated and therefore cannot be targeted directly. Familial forms account for a small percentage of ND causes and although defects in genes and their products have been identified for some of these forms, it is not known precisely how the defects translate into neuronal dysfunction, atrophy or death.

Potential treatment approaches

Given the dynamic progression–compensation processes and the late clinical emergence of ND, a variety of treatment approaches might be undertaken: (i) supporting reduced neuronal function(s), particularly neurotransmission; (ii) halting or slowing the progression of neuronal loss by eliminating causative factors; (iii) interfering with crucial steps in neuronal death processes; (iv) inducing recovery of surviving but dysfunctional neurons; and (v) replacing lost neurons by transplantation or induction of pluripotential progenitor cells. This review will consider the first three treatment approaches.

Box 1. Care costs of neurodegenerative diseases and the potential impact of medications that inhibit disease progression

Annual costs of care for Alzheimer's disease (AD) in the US is approximately $100 billion, making it the third most costly disease after cardiovascular disease (approximately $180 billion) and cancer (approximately $110 billion) [93]. Combining the equivalent values for the top seven grossing drug markets (US, Japan, Germany, France, UK, Spain and Italy; total population approximately 2.5-times the US population), and taking into account that care costs per patient might be lower in some of these countries, gives an estimated cost of close to $200 billion in the top seven markets. Delaying onset of AD by one year would reduce prevalence by 10%, which would result in care cost savings of about $20 billion. Delaying onset of AD by five years would reduce prevalence by 50%, resulting in care cost savings of approximately $100 billion [94]. With prevalence of the disease projected to increase between 3 and 7% annually [93], these values will have to be adjusted accordingly. Annual costs of care for Parkinson's disease and amyotrophic lateral sclerosis in the top seven markets are estimated to be $25 billion and $20 billion, respectively, based on prevalences (Table 1) and figures found in the Internet (http://www.medscape.com/viewarticle/424386_4; http://www.abivest.com/research/whitepapers/880ABALSCompanion.pdf).

Table 1. Statistics concerning major neurodegenerative diseases[a]

Disease	Total prevalence in top seven markets (US, Japan, Germany, France, UK, Spain and Italy)		
	2002	2012 (predicted)	Age-dependence
Alzheimer's disease	5.2 million	6.7 million	1 in 10 at age ≥ 65[c]
			1 in 2 at age ≥ 85[c]
Parkinson's disease	2.8 million	3.4 million	1 in 100 at age ≥ 65[d]
			1 in 50 at age ≥ 85[d]
Amyotrophic lateral sclerosis	87000	108000	Risk increases with age up to 74[e]
Huntington's disease	30000–50000[b]	Unavailable	Unavailable

[a]Sources EPI database 2003; DR DB7,2003, DR Cognos 2002
[b]Estimated from indications on several websites of Huntington's disease organizations
[c]http://www.alz.org/ResourceCenter/FactSheets/FSAlzheimerStats.pdf
[d]http://www.departmentofmedicine.ualberta.ca/pdf/halfday/ahd_01-23-03-2.pdf
[e]http://www.neuro.wustl.edu/neuromuscular/spinal/als.htm

Supporting neurotransmission

Current treatment for ND predominantly deals with the symptoms of diseases and is aimed at improving neurotransmission. PD patients can be managed reasonably well for 5–15 years [17] by the activation of dopamine receptors by dopamine metabolized from levodopa or by dopamine receptor agonists [18]. Acetylcholinesterase inhibitors can provide some benefit for patients with mild to moderate AD for up to three years [19]. For all other conditions that are within the category of ND [e.g. ALS or Huntington's disease (HD)] there is no effective symptomatic pharmacotherapy [20].

Eliminating causative factors

Numerous attempts have been made to combat several of the proposed causative factors, including agents that reduce production or levels of ROS, agents that improve mitochondrial ATP production by facilitating respiratory chain activity and agents that reduce intracellular calcium levels through blocking of calcium channels or by reducing excitatory neurotransmission. Although some of these approaches have appeared promising based on cellular and animal models of acute or chronic ND, the promise has not been fulfilled clinically [20], with the exception of three compounds, which could provide some limited benefit. One such compound is the glutamate release inhibitor, riluzole, which extends survival of ALS patients by ~3 months, but does not significantly improve muscle strength [21]. Another compound, the non-competitive N-methyl-D-aspartate (NMDA) antagonist, memantine, improves cognition and is approved in the European Union, and recently also in the US, for the treatment of moderate to severe AD [22]. Coenzyme Q10, an electron acceptor for mitochondrial complexes I and II and an antioxidant, has been suggested to slow functional decline in PD [23].

Interfering with neuronal death processes

Neurons are thought to die via two broadly defined processes – necrosis and so-called 'programmed cell death' (PCD). Necrosis involves rapid swelling and subsequent rupture of cells as a result of combinations of energy failure, excessive transmembrane ion fluxes and/or ROS formation. Uninvolved, adjacent cells can undergo secondary death as a result of inflammatory responses to the release of intracellular contents from primarily affected cells. The rapid time course of necrosis largely makes intervention in the process difficult and necessitates a therapy that prevents its initiation.

In contrast to necrosis, PCD occurs more gradually, is an energy-requiring process and usually involves single cells rather than groups of adjacent cells. Two forms of PCD have been recognized – apoptosis (PCD1) and autophagy (PCD2). In apoptosis, multi-organelle signaling networks induce cell degradation, a process that includes cytoplasmic and nuclear shrinkage, nucleic acid and protein lysis, outer membrane blebbing and marking of the membranes to facilitate macrophage engulfment. Autophagy involves the regulated degradation of intracellular proteins and organelles through lysosomal pathways [24]. During autophagy macromolecules are converted into energy substrates and amino acids are recycled. Autophagy and apoptosis are not exclusive processes because they can share signaling elements and can occur simultaneously in a particular tissue or cell [25]. For schematic representations of current views on apoptosis signaling pathways the reader is referred to [26–28].

Caspases and caspase inhibitors in neurodegeneration

As many as 14 different cysteine-aspartate proteases (caspases) have been identified in mammalian nervous tissue and separated into apoptotic initiators, apoptotic executioners and inflammatory mediators [29]. The activation of caspases has been observed in stroke (caspase-3 [30]), PD (caspase-3 [31] and caspase-8 [32]), ALS (caspase-3 [33]) and AD (caspase-9 [35] and caspase-3 [36]). Caspase inhibitors are protective in some cellular and animal neurodegenerative models [37] but not in others [38]. In the case of spinal muscular atrophy (SMA) it has been proposed that there is a connection between neurodegeneration and failed caspase inhibition. In severe SMA, the neuronal specific inhibitor of apoptosis (NAIP), a member of the inhibitor of apoptosis (IAP) family, is often dysfunctional because of missense and truncation mutations [39]. NAIP potently inhibits caspase-3 and -7 [40], suggesting that NAIP mutations could permit unopposed developmental apoptosis to occur in sensory and motor systems, resulting in lethal muscular atrophy. Conversely, adenovirally-mediated overexpression of NAIP [41], or the X chromosome-linked IAP (XIAP) [42], reduces the loss of CA1 hippocampal neurons following transient forebrain ischemia. Therefore, selective caspase inhibition could reduce neuronal apoptosis in ND [43].

Role of mitochondria in neurodegeneration

Mitochondria often mediate a pivotal decision step in apoptosis signaling. Increases in mitochondrial membrane permeability can cause the release of intra-mitochondrial factors that induce the degradation phase of apoptosis [26]. One or more of at least seven factors can be released from mitochondria, including cytochrome-c, caspases-2 and -9, apoptosis inducing factor (AIF), Smac/DIABLO and endonuclease G, which act through activated caspase dependent signaling (e.g. caspase-3 and caspase-6) or caspase independent signaling to degrade the cellular cytoskeleton, nuclear

scaffold and DNA-associated proteins, and nuclear DNA. The relocation of the pro-apoptotic proteins Bax, Bak and Bid to mitochondria facilitates the permeability increases whereas mitochondrial anti-apoptotic proteins Bcl-2 and Bcl-X_L oppose permeability increases [26]. Heterodimerization of particular factors (e.g. Bad) with Bcl-2 or Bcl-X_L prevents their capacity to maintain impermeability and thus promotes permeability increases and apoptotic degradation. Increases in levels of neuronal Bax have been identified in AD, PD, and ALS [31,44–46], which suggests that increases in mitochondrial membrane permeability are associated with these diseases.

A number of signaling pathways, that are upstream to mitochondria and some of which interact with each other, can increase or decrease the mitochondrial membrane actions of the pro- or anti-apoptotic Bcl-2 protein family factors and, therefore, are themselves either pro- or anti-apoptotic [26–28]. The levels of the tumor suppressor protein p53 can be increased by a variety of events, including hypoxia, DNA damage, excitotoxins and inadequate proteosome activity, and this has been implicated in PD [47], AD [48] and ALS [49]. Bax upregulation and mitochondrial relocation is induced by p53, which might also upregulate and induce the nuclear relocation of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which in turn decreases several anti-apoptotic and protective factors, including Bcl-2, Cu^{2+}/Zn^{2+} and Mn^{2+} superoxide dismutases, glutathione peroxidase and heat shock protein 70 as well as affecting the availability of some trophic and growth factors [50]. Phosphorylation of kinases [e.g. jun N-terminal kinase (JNK)] causes the phosphorylation of factors such as c-jun that promote increased mitochondrial membrane permeability in AD and ALS [51–53]. Tumor necrosis factor-α (TNF-α) and tumor necrosis factor receptor superfamily member 6 (FAS) death receptors activate caspase dependent cascades that induce apoptotic degradation (in part through Bid and Bax) and have been suggested to play a role in PD and AD [36,48,54]. Catecholamine receptors (D2 dopamine receptor and α_2-adrenoceptors), ovarian steroid receptors and trophic factor receptors act in part on the phosphoinositol-3-kinase (PI3K)–protein kinase B (Akt) pathway to induce changes in gene transcription or protein phosphorylation, which promote the maintenance of mitochondrial membrane impermeability by Bcl-2 or Bcl-X_L while opposing increased permeability mediation by Bax or Bad [28,55–57].

The dust settles on the controversy over the role of apoptosis in ND

Initial studies of apoptosis in ND depended on evidence of nuclear DNA fragmentation in post-mortem brain tissue obtained using terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL). These studies differed markedly in terms of locating degraded neuronal nuclei in affected or control brains, probably as a result of the marked variability of the TUNEL technique [26,28]. The inconsistencies in the TUNEL-based studies fostered controversy regarding the role of apoptosis in ND. However, recent studies on human post-mortem brain tissue have shown changes in apoptosis signaling factors that are appropriately localized to different neuronal phenotypes to explain the neuronal loss typical of a particular ND. Although the studies of apoptosis signaling factors strongly support a role for apoptosis in ND, they do not establish the extent to which apoptosis contributes to neuronal loss or whether or not an interruption of apoptosis would provide clinical benefit or what the potential downsides of anti-apoptosis therapy might be.

Anti-apoptotic compounds in development for the treatment of ND

In terms of drug development for the treatment of ND, apoptosis intervention is a relatively new area. As a result, although numerous companies have research programs encompassing, among others, caspase inhibitors, JNK or other kinase inhibitors, gene therapy and antisense oligonucleotide approaches, the number of small molecule, orally bioavailable and brain penetrating anti-apoptotic compounds that has reached or could reach the stage of clinical exploration is rather limited. Here, the principal classes of compounds under investigation in the pharmaceutical industry are discussed, insofar as corresponding information is available in the literature or via competitor information services like ADIS R&D Insight (http://library.dialog.com/bluesheets/html/bl0107.html) or Pharmaprojects (http://library.dialog.com/bluesheets/htmlaa/bl0128.html). Examples of more advanced prototypic compounds are given in Figure 1a–e.

Caspase inhibitors

According to competitor information services, various biotech companies have ongoing preclinical caspase inhibitor projects [58]. Only one compound (IDN-6556, a caspase-8 inhibitor of unknown structure) is listed as being in Phase II trials as a hepatoprotective, whereas multiple sclerosis has been suggested as a possible neurological indication. Isatin sulfonamides have been described as potent and selective non-peptidic caspase-3 and -7 inhibitors [58], but no development has as yet been reported.

The second-generation tetracycline antibiotic minocycline (Figure 1a), sometimes referred to as a caspase inhibitor, reduces caspase-1 and -3 activities, presumably by interfering

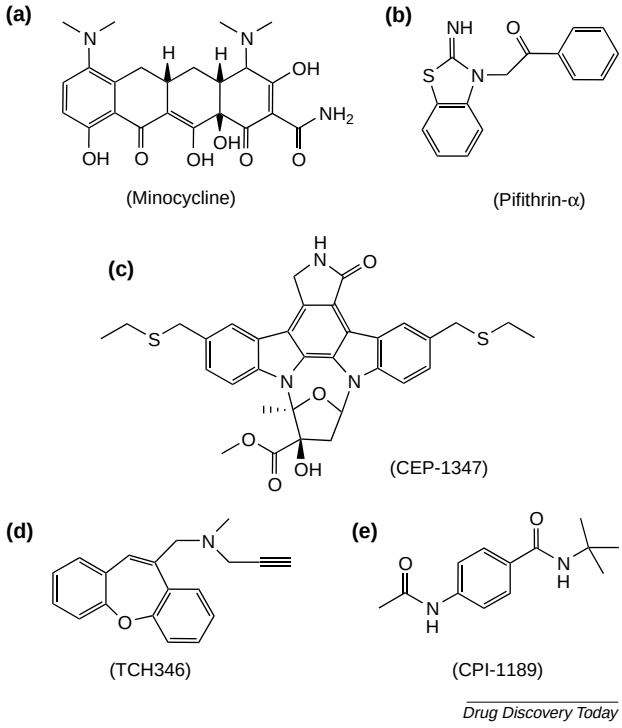

Figure 1. (a) Minocycline. The tetracycline antibiotic minocycline inhibits immunologic processes, for example, matrix metalloproteinases, reactive oxygen species release from neutrophils, inducible nitric oxide synthase and reduces the activities of p38-mitogen activated protein kinase and caspases-1 and –3, presumably by interfering with upstream activation. **(b)** Pifithrin-α. The p53 inhibitor pifithrin-α is protective in models relating to apoptosis. No development is reported in ND indications. **(c)** CEP-1347. The staurosporin derivative CEP-1347 inhibits the jun-N-terminal kinase pathway by inhibition of mixed-lineage kinases. **(d)** TCH346. TCH346 prevents apoptosis induced in a variety of cell lines caused by stimuli such as trophic withdrawal, rotenone, cytosine arabinoside or 1-methyl-4-phenyl-pyridinium. It binds to, and prevents upregulation and nuclear translocation of glyceraldehyde-3-phosphate dehydrogenase, known to be involved in the mediation of p53-dependent apoptosis. This is considered to be responsible for prevention of apoptosis-related downregulation of Bcl-2, mitochondrial relocation of Bax and reduction of mitochondrial membrane potential. **(e)** CPI-1189. It has been suggested that CPI-1189 might oppose apoptotic effects of tumor necrosis factor-α via Bcl-2 induction. The development of CPI-1189 in neurodegenerative diseases was stopped and rerouted to neuropathic pain.

with the upstream activation of these enzymes. Minocycline also prevents mitochondrial permeability transition-mediated cytochrome-c release [59]. These two inhibitory activities of minocycline support the proposal that neuroprotection by this antibiotic is related to inhibition of apoptosis. Minocycline delays disease progression in R6/2 transgenic HD mice and G93A transgenic ALS mice, and was reported to be protective in rodent 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) PD models [60]. However, a recent study suggests that minocycline aggravates damage to the dopaminergic system caused by MPTP [61]. An uncontrolled Phase II trial in HD was promising, a Phase III trial in ALS is ongoing [61].

p53 inhibitors

Pifithrin-α (Figure 1b), a synthetic inhibitor of p53-induced transcriptional activation, was originally designed to protect non-cancerous cells from genomic stress inflicted by cancer therapy, but was shown to be neuroprotective in a variety of models relating to apoptosis caused by DNA damage, excitotoxicity, ischemia and β-amyloid peptide exposure. It is also protective in the mouse MPTP PD model [62] and inhibits wildtype and mutated presenilin-2-triggered caspase activation and apoptosis [63]. A series of analogs of similar or higher potency was recently reported [64]. In view of the anti-cancer functions of p53 [65], the long-term application of p53 inhibitors as a therapy (as required for diseases such as PD or AD) could prove problematic, and pifithrin-α has indeed been shown to promote genomic instability [66]. However, such compounds might prove useful for short-term administration in treating apoptosis that accompanies stroke or nervous system trauma.

JNK inhibitors

Competitor information services list at least four companies that have active research programs at preclinical stages on prototypic synthetic JNK inhibitors of unrevealed chemical structures and varying specificities for JNK 1, 2 and 3. These compounds are aimed at various peripheral indications, as well as neurological indications such as MS. The only compound presently in clinical development is CEP-1347 (Figure 1c), an orally active staurosporin derivative that inhibits the JNK pathway through inhibition of mixed-lineage kinases [67]. CEP-1347 promotes survival of PC12 cells and various chick or rat primary neurons after challenges, including DNA damage, trophic withdrawal or oxidative stress. *In vivo*, it prevents developmental death of chick lumbar motoneurons, reduces developmental death of spinal motoneurons in postnatal female rats, prevents death of rat hypoglossal neurons after axotomy and protects dopaminergic neurons from MPTP-induced death in mice and monkeys. CEP-1347 also shows potential for the treatment of AD: it increases choline acetyltransferase (CAT) activity in cultured embryonic septal neurons, antagonizes β-amyloid-induced JNK activation and death in cultured cells, protects cholinergic neurons after fimbria-fornix lesions, partially preserves CAT activity and prevents associated behavioral deterioration after excitotoxic lesions of the nucleus basalis magnocellularis. A combined Phase II and III trial in PD is ongoing.

GAPDH ligands

The glycolytic enzyme GAPDH has several functions that are unrelated to its role in energy production, one of which is its implication in p53-dependent apoptosis. The damage-sensor protein p53 effects upregulation and translocation of the pro-apoptotic factor Bax to mitochondria, which result in mitochondrial membrane permeabilization, loss of mitochondrial membrane potential and release of apoptogenic proteins (e.g. cytochrome-c and AIF. Concomitantly, p53 mediates upregulation and nuclear translocation of GAPDH [68], which effects downregulation of the anti-apoptotic protein Bcl-2 and other protective factors [26,27,50], thus corroborating the pro-apoptotic effect of Bax induction.

The tricyclic propargylamine derivative TCH346 (previously known as CGP 3466B; Figure 1d) inhibits apoptosis in PC12, cerebellar granule or PAJU neuroblastoma cells or embryonic mesencephalic dopaminergic cells, and promotes neuronal survival in several animal ND models. TCH346 binds to GAPDH, stabilizes the dimeric form of the protein and prevents apoptosis-related upregulation and nuclear translocation of GAPDH in association with reduced apoptosis and prevention of increased mitochondrial membrane permeability, as indicated by the maintenance of mitochondrial membrane potential [69–72]. It is protective in mouse and monkey MPTP PD models, facial motor neuron axotomy and global ischemia models, and in progressive motor neuronopathy mice. Following a small Phase II clinical trial in ALS, TCH346 is currently undergoing more extensive Phase II trials in PD and ALS. Other propargylamine derivatives [71,73,74], which act by inhibiting monoamine oxidase type B (MAO-B), demonstrate neuroprotective properties in some models.

Dopamine, noradrenaline and ovarian steroid receptor agonists

D2 dopamine receptors, α_2-adrenoceptors and ovarian steroid receptor agonists have reduced levels of apoptosis in a variety of culture and animal nervous system models [75–77]. Based on 2-β-carboxymethoxy-3-β-(4-iodophenyl)tropane (β-CIT) or ^{18}F-DOPA positron emission tomography (PET) imaging studies, the rate of striatal dopamine terminal loss was claimed to be less in PD patients chronically treated with D2 dopamine receptor agonists in comparison with those treated with levodopa. However, the interpretation of these data was challenged, and clinical outcome measures favored levodopa over the D2 dopamine receptor agonists in the same studies. Thus, there is no direct clinical or pathological evidence for D2 dopamine receptor agonist neuroprotection [78]. As yet, α_2-adrenoceptor agonists have not been tested in ND and ovarian steroids have not proved to be effective, despite their potent effects in model systems.

Opposing inflammatory mediators

CPI-1189 (Figure 1e) was synthesized as a novel antioxidant related to the spin-trapping agent phenyl-N-tert-butylnitrone, but could have anti-apoptotic properties that are unrelated to this. CPI-1189 reduces weight loss, impairment of performance in Morris maze, ventricle enlargement and the number of apoptotic cells in rats chronically infused icv with TNF-α. In brain cells, it reduces apoptosis caused by TNF-α, by the HIV envelope glycoprotein gp120, by neurotoxic factors from activated macrophages or microglia of patients with AIDS dementia and necrosis caused by quinolate. In addition, CPI-1189 inhibits IL-1β-induced p38-MAPK phosphorylation and enhances activation of extracellular-signal-related kinase (ERK) by TNF-α. It has been proposed that ERK activation induces Bcl-2, which could explain the protection by CPI-1189 against the apoptotic effects of TNF-α [27,79]. Initially, CPI-1189 was in clinical development for the treatment of AIDS dementia, AD and PD. However, a Phase II clinical trial in AIDS dementia showed no clinical efficacy of CPI-1189 [80], and, according to commercial competitor information services, clinical development in this neurological indication, as well as AD and PD, was stopped after the acquisition of Centaur by Renovis (http://www.renovis.com).

Outlook

The value of current ND models for predicting therapeutic success is not known

The predictive value of the ND animal models available for the preclinical evaluation of anti-apoptotic compounds is not known. Although current animal models reproduce some behavioral and pathological features of the respective human disease phenotypes, it is less certain whether or not the apoptosis signaling pathways in animal models and human diseases are identical or even similar. Our knowledge of signaling pathways in human ND comes from the examination of post-mortem material, generally from patients at the end-stage of their disease, which might not be representative of the status of neurons that were lost earlier in the disease process. ND animal models typically involve a relatively acute damage, but in chronically progressing diseases neurons could be chronically exposed to disease initiating stimuli. Prolonged exposure could enable redundant apoptosis signaling pathways to circumvent drug-induced interruption of apoptosis. Ultimately, clinical trials must confirm, or deny, the utility of anti-apoptotic agents in specific ND.

Clinical trials are difficult, long and costly

Demonstration of clinical efficacy of anti-apoptotic drugs in any neurodegenerative disease is a challenging and costly task. Because they probably will not produce immediate

clinical benefits, the duration of a study must allow for sufficient disease progression to ensure the reliable detection of a treatment effect (i.e. up to one year for conditions such as ALS and possibly 3–5 years for slowly progressive conditions). Furthermore, the variability and nonlinearity of disease progression rates means that large patient numbers are a necessity that requires the participation of numerous medical centers, which potentially introduces another variable. The choice of appropriate clinical endpoints and trial designs are crucial and need careful coordination with drug regulatory agencies to ensure differentiation of disease-modifying from symptomatic effects.

Importance of biomarkers

Development of anti-apoptotic drugs could be markedly expedited and streamlined by using suitable biomarkers of different types [81]. Type I biomarkers serve to establish proof of mechanism and effective dose-range in humans in phases I and/or IIa of clinical trials and can, but need not be, disease-related. For anti-apoptotic compounds, a type I biomarker would ideally be the target protein itself, a protein downstream in the same signaling pathway, or an apoptosis marker (e.g. annexin V) [82]. However, at present, this is barely an emerging area of research.

Type 0 biomarkers reflect the natural history of a disease and correlate longitudinally with clinical indices. Type II biomarkers are surrogate endpoints predicting clinical benefit. The use of these biomarkers would speed up Phase IIb trials (proof of concept and dose-finding) and optimize planning of Phase III trials. In addition, type 0 biomarkers would facilitate identification of presymptomatic patients, thus enabling early treatment to stabilize the disease before symptoms appear.

Potential type 0 and II biomarkers currently under investigation, particularly in relation to AD and PD, include proteins and biochemical parameters that reflect conditions such as oxidative damage in body fluids, structural and functional magnetic resonance imaging (MRI) and proton magnetic resonance (MR) spectroscopy to assess changes in brain volume or functionality of neurons, PET and single photon emission computed tomography (SPECT) to obtain a measure for density of protein deposits or neurons and metabolic activity or microglial activation, among others [83–89]. Surrogate endpoints for AD (e.g. MRI volumetry [88]) and PD (^{18}F-DOPA and dopamine transporter PET/SPECT [89]) appear within reach, but have yet to be validated. Surrogate endpoints could provide further evidence for disease-modification, but how they will affect development timelines remains to be seen. None of the numerous type 0 markers investigated thus far appear to show promise for effective drug evaluation.

The current knowledge on death modes (apoptosis, autophagy and necrosis) and pathways makes predictions as to which therapeutic targets are more promising than others difficult, which has a negative impact on the chances of success for corresponding R&D projects. The availability of validated markers would reduce development times and costs, and attrition rates at later development stages, thus greatly increasing the attractiveness of apoptosis as a target for anti-neurodegenerative drugs in the pharmaceutical industry. This in turn would be in the interest of patients, because the chances of finding an effective anti-apoptotic treatment for one or more neurodegenerative diseases increases with the number of targets tested.

Possible risks

The proposed risks of using anti-apoptotic agents are at this time theoretical because there is limited information concerning the actual effects of treatment with anti-apoptotic drugs on apoptotic processes occurring pathologically or physiologically. Will anti-apoptotic treatment preserve dysfunctional cells? If so, what are the potential clinical consequences of this? Or, if apoptosis is prevented, will necrosis ensue and aggravate the targeted pathology by causing inflammatory reactions? For example, caspase inhibition does not always lead to functional recovery, and could switch the mode of cell death from apoptosis to another mechanism [37]. However, there is evidence for functional improvement in animal neurodegenerative models, and no evidence for a switch of mode of cell death with compounds such as TCH346 or CEP-1347 [67,90,91]. These findings support the view that dysfunctional cells and a switch to necrosis are not obligatory consequences of apoptosis prevention. These risks might be determined by the mechanism of action of the drug (i.e. the location and specificity with which it interferes with apoptotic signaling pathways).

With respect to the concern that anti-apoptotic compounds might induce or promote cancer, which relates to the fact that several apoptotic signaling factors are dysregulated in some cancers [92], it could be argued that the drugs targeted to those factors could cause malignant replication. Conversely, it could be argued that, if the targeted pathways are already dysregulated, a drug designed to act on that pathway might have little or no impact. Several of the compounds listed in Figure 1a–e (e.g. CPI–1189 and TCH346) have passed long-term animal toxicity studies and show no evidence for tumorigenic potential, and long-term carcinogenicity studies will clarify the issue further. However, for the development of new compounds acting on different targets, these findings have no prognostic value. Each mechanism targeted could have its own potential

risks that warrant full safety and toxicity evaluation of each new moiety.

Impacts and consequences in case of success

Most ND are diseases that manifest in mid- to late-life. In an optimum scenario, the ability to delay progression of a ND by a few years could push the onset of neurological disability beyond the normal life expectancy or, in a more conservative scenario, postpone or reduce the period of neurological disability through the most productive years of life. These therapeutic gains would result in a significant reduction in the financial burden borne by patients, their relatives, and health care systems and, most importantly, preserve the quality and dignity of life.

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