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Commentary

Recent clinical failures in Parkinson's disease with apoptosis inhibitors underline the need for a paradigm shift in drug discovery for neurodegenerative diseases

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ABSTRACT

Understanding the mechanisms of neuronal death in concert with the identification of drugable molecular targets key to this process has held great promise for the development of novel chemical entities (NCEs) to halt neurodegenerative disease progression. Two key targets involved in the apoptotic process identified over the past decade include the mixed lineage kinase (MLK) family and glyceraldehyde phosphate dehydrogenase (GAPDH). Two NCEs, CEP-1347 and TCH346, directed against these respective targets have progressed to the clinic. For each, robust neuroprotective activity was demonstrated in multiple in vitro and in vivo models of neuronal cell death, but neither NCE proved effective Parkinson's disease (PD) patients. These recent clinical failures require a reassessment of both the relevance of apoptosis to neurodegenerative disease etiology and the available animal models used to prioritize NCEs for advancement to the clinic in this area.

1. Introduction

By the mid 20th century, the process of physiological cell death had been known for over a century, being an active research area for developmental biologists and histologists [1] but with a limited interest in cell death mechanisms from a therapeutic perspective. Studies of the breakdown of silkmoth abdomen intersegmental muscles that occurred at the end of metamorphosis however, triggered the concept of an active, regulated process termed 'programmed cell death (PCD)' [2] that could be modulated by pharmaceutical agents [3].

In 1972, Kerr et al. [4] described a type of active cell death characterized by chromatin condensation, cell shrinking, membrane blebbing and, finally phagocytosis of the remaining cell by neighboring cells, and coined the term, 'apoptosis' that is now widely used as a synonym for PCD.

Until recently, cell death was generally considered to be either necrotic or apoptotic despite concerns that the process might not be so simple [5]. Cell death can be divided into several distinct phenotypes, from caspase- dependent or-independent apoptosis, to autophagy, to programmed and passive necrosis. Elements of the different types of active cell death can also coexist in the same dying cell, with pathways partially overlapping and with one form of cell death transitioning to another depending on as yet poorly understood factors [5,6].

It was not until the early 1990s that the concept of a directed, well-regulated process of cell death was accepted.

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This was driven by discoveries that included: the identification of *bcl*2 as an anti-apoptotic gene; the pro-apoptotic functions of p53 and c-*Myc*; the death-mediating cellular receptor, Fas/Apo-1; the caspases [1] and the role of mitochondria in cell death [7]. It soon became apparent that apoptosis, in addition to being a programmed process for the controlled demise of single cells in response to developmental needs or cellular damage, could also represent a novel therapeutic target for a variety of human disease states. While the initial focus was on cancer, it was soon hypothesized that neurodegenerative processes could also be of interest [8–10], since these could result either from erroneously induced apoptosis (''death by mistake''), in response to serious tissue damage or as part of an over acceleration of the intrinsic aging process. In these latter instances, inhibition of apoptosis might allow the time for tissue self-repair thus providing an effective treatment for neurodegenerative diseases [11] including Alzheimer's disease (AD), Parkinson's Disease (PD) and amyotrophic lateral sclerosis (ALS) [12].

2. Anti-apoptotic drugs

By the mid-1990s, knowledge related to apoptotic mechanisms and signaling pathways had progressed to a level [9,12–20] where strategic intervention points to interfere with apoptosis could be defined. NCEs directed against targets like the pro-apoptotic caspases [21], p53 [22] and the JNK pathway [23] were sought and their anti-neurodegenerative potential extensively evaluated in various cellular and animal models [11,24]. Alternatively, e.g., in the case of the neuroprotective propargylamines, e.g. deprenyl (Fig. 1), apoptosis-relevant targets were identified [25]. Since safety studies failed to identify any problems that might theoretically be expected due to increasing the survival of unwanted cells, e.g., promotion of cancer growth, maintaining dysfunctional or malfunctioning cells, or interfering with the turnover of proliferating cells, two of these NCEs, the mixed lineage kinase inhibitor, CEP-1347, a K252a analog (Fig. 1; [26]) and the glyceraldehyde-3-phosphate (GAPDH) ligand, TCH346, a propargylamine derivative (Fig. 1; [27,28]), were advanced to clinical evaluation for the treatment of neurodegenerative diseases. Concurrently, considerable evidence accumulated for the presence of apoptotic processes in post-mortem brain tissue from patients with, or in animal models thought to represent, neurodegenerative diseases, e.g. signs of DNA nick-end labeling (TUNEL staining); changes in the expression levels of pro- or anti-apoptotic proteins of the *bcl*2 family, p53 or death receptors e.g. tumor necrosis factor 1 (TNFR1), etc., and activation of effector caspases, e.g. caspase-3, or initiator caspases like caspase-8 [11,24]. While still controversial and heavily disputed at that time, these findings increased interest and potential for the therapeutic efficacy of anti-apoptotic NCEs in neurodegenerative diseases.

2.1. *Neurodegenerative diseases and apoptosis*

The current view of the pathophysiology of PD is that dying dopamine (DA)ergic neurons show apoptotic features, but

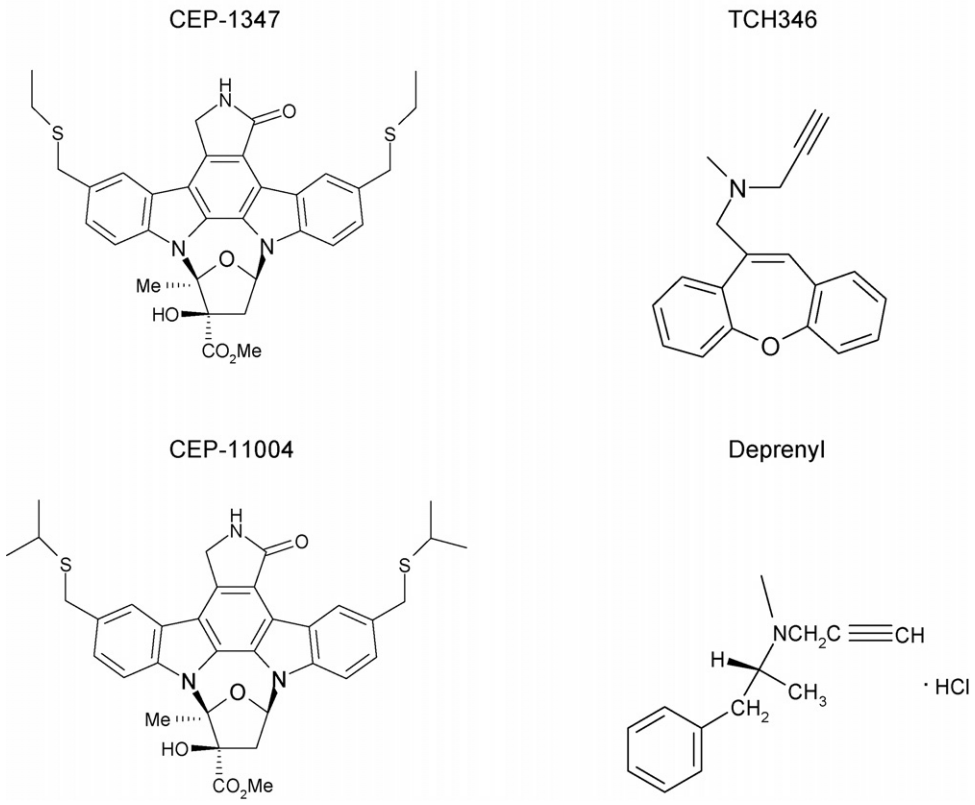

Fig. 1 – Structures of CEP-1347, TCH346 and related compounds.

that degeneration probably results from the convergence of several pathogenic factors, some from within, but others from outside those neurons [29,30]. It should be noted, however, that much of the evidence comes from acute, neurotoxin-induced disease models in rodents, and its relevance rests on the fidelity with which these models reflect the pathogenic mechanisms of human disease. With respect to ALS, cells from affected patient tissue exhibit apoptotic features, although not in a classical sense. As in PD, much of the evidence suggesting engagement of apoptotic pathways comes from animal models, in this instance SOD transgenic mice, and must therefore be considered with appropriate caution [31]. Likewise, the evidence for a role of apoptosis in AD and Huntington's disease based on post-mortem patient brain tissue is sparse and confusing. Supporting data from cellular and animal models are heterogeneous and sometimes contradictory depending on the model, the species and experimental conditions [32–35]. In the last 5 years or so, understanding of the pathophysiology of animal models of neurodegenerative diseases has progressed admirably–much more than that of the diseases themselves.

3. Anti-apoptotic agents and Parkinson's disease

In the research landscape described above, CEP-1347 and TCH346 entered clinical trials for the treatment of PD as their primary indication. Although historical reasons influenced this choice in the case of TCH346 (the original concept was to develop an NCE that shared the neuron rescuing/anti-apoptotic properties of the novel MAO (monoamine oxidase)-B inhibitor, deprenyl, but did not inhibit MAO and was also not metabolized to amphetamine-like compounds [36]), the principal reason was the observed efficacy of the two NCEs in mouse and primate MPTP models (Table 1) coupled with a strong belief that the cellular and anatomical pathophysiology observed in MPTP models was relevant for PD [11,37,38].

Data from large and well-controlled clinical studies in PD, and PD and ALS, respectively, for CEP-1347 and TCH346, showed both NCEs to be ineffective [39,40]. In the present commentary, the implications of these disappointing results for the future development of NCEs for the treatment of neurodegenerative disorders in general, and for those that interfere with PCD in particular are discussed.

Table 1 – In vitro and in vivo neuroprotective profiles of CEP-1347 [93–112] and TCH346 [36,88–90]

CEP-1347	TCH346
In vitro/cellular systems; rescues	*In vitro/cellular systems; rescues*
PC12 cells from toxicity by:	PC 12 cells from toxicity by:
Trophic withdrawal	Trophic withdrawal
β-Amyloid toxicity	β-Amyloid toxicity
Oxidative and UV-induced stress [88]	Rotenone
	Lactacystin (proteasome inhibitor)
Rat embryonic:	Rat cortical neurons from:
Cortical neurons from β-amyloid [89]	NMDA excitotoxicity
and arsenite toxicity [90]	
Motor [91], sensory [92] and symphathetic	Kainate excitotoxicity
neurons [93] from trophic withdrawal	
Sensory [94] and hippocampal [95] neurons	
from gp120 toxicity	
Cerebellar granule cells from low K+- [96] and	Cerebellar granule cells from toxicity by
colchicines-induced [97] death	cytosine arabinoside (ara C)
Mesencephalic DAergic cells from trophic withdrawal	Rat oligodendrocytes from AMPA excitotoxicity
[98], FeCl2+/METH toxicity [99] and facilitates survival	
following transplantation [98]	
	Rat embryonic mesencephalic DAergic cells
	from toxicity by MPP+/MPTP
Human SH-SY5Y cells from MPP+ [100]	Human neuroblastoma (PAJU) cells from toxicity by:
In vitro/cellular systems; blocks	Rotenone
Monocyte and microglial inflammatory responses induced	GAPDH overexpression
by LPS or $A\beta_{1-40}$; TNF, IL-1 or INFγ induced astrocyte	
production of NO, PG and IL-6 and decreases COX-2 expression [101]	
Active concentration range ≈ 10^{-9} *M*–10^{-6} *M*	*Active concentration range* ≈ 10^{-12} *M*–10^{-5} *M, with a maximum at* ≈ 10^{-9} *M*
Prevents neurodegeneration in the following animal models	*Prevents neurodegeneration in the following animal models*
SNB model of developmental PCD [102]	Unilateral carotid occlusion/transient hypoxia model
Mouse [80] and monkey [81] MPTP	Facial motor neuron axotomy model
NBM ibotenic acid lesion [103,104]	Progressive motor neuronopathy mice
NMDA lesion of the entorhinal cortex(unpub.)	MPTP model in mice
Malonate-induced striatal lesion (unpub.)	6-OHDA models in rats
Fimbria fornix transaction [105]	MPTP model in monkeys
Noise-induced haircell and neuron loss [106]	
Hypoglossal and sciatic (neonates) nerve axotomy (unpub.)	
Active dose range ≈ 0.01–10 *mg/kg s.c.*	*Active dose range* ≈ 0.003–0.3 *mg/kg p.o. or s.c.*

4. Possible reasons for the lack of effect of CEP-1347 and TCH346 in PD

At the time that the decisions were made to develop CEP-1347 and TCH346, a number of issues were already recognized as possible hurdles to NCE advancement and these remained of concern during development. However, in the absence of reliable information to judge their importance, and the lack of an already established clinical path forward for disease modifying as opposed to palliative agents, an awareness that risk is inherent to advancing novel approaches in drug discovery and confidence in the concept of inhibiting apoptosis necessarily prevailed over such concerns. With the negative outcome of both trials and the need to learn for future trials in this area, a number of issues require consideration. These include:

- Firstly, and perhaps the easiest to address, were NCE exposure and trial design adequate?
- Were the DA neurons that could have been rescued by inhibition of apoptosis in PD patients already dead, or in an advanced state of dying, when treatment began?
- Were the pathways targeted by CEP-1347 and TCH346 involved in only a minor proportion of cell death?
- If neurons cannot die by apoptosis, can they die by necrosis?
- Or, as a variation of this theme, are the death pathways in PD redundant, e.g., can cell death proceed by another programmed route if one pathway is blocked?
- Did the NCEs in fact rescue (some) cells, but fail to maintain them in a functional state?
- And finally, the most pressing and painful question: are the present animal models of PD relevant to studying NCEs that have the potential to interfere with disease progression? Is protection from the consequences of acute toxin exposure (as in the various PD models) an easier challenge for NCE efficacy than protection from ongoing damage as occurs in the human disease state?

Although many of these points obviously overlap, they will be discussed separately below in the interest of providing structure to the discussion.

4.1. *Adequacy of dosing and trials*

Before looking for reasons why the NCEs failed to work in the clinic, it is important to establish convincing evidence that the negative outcomes were not the result of inadequate dosage or flawed trial design and execution. A caveat in bridging plasma levels from preclinical to clinical studies is that the plasma levels do not reflect brain exposure. While brain levels can be measured relatively easily, ex vivo in animals, imaging is the only facile way that brain levels of NCEs can be assessed in patients [41]. While a biomarker of the dopamine transporter, (β-CIT) was used in conjunction with imaging in the PRECEPT (Parkinson Research Examination of CEP-1347 Trial) trial of CEP-1347, the results derived from these studies were inconclusive.

4.1.1. *CEP-1347*

Dose(s) of CEP-1347 used in the clinic were selected on the basis of achieving similar plasma levels in humans as those producing efficacy in animal models (20–200 ng/mL). In MPTP models, doses of CEP-1347 that were active ranged from 0.3 to 3.0 mg/kg, s.c. The multi-center Phase II/III study termed PRECEPT, was a randomized, double-blind, placebo-controlled trial, using 3 doses (10, 25, and 50 mg b.i.d.) of CEP-1347, that provided plasma concentrations of the NCE comparable to those providing efficacy in animal models with maximal duration of exposure. The trial enrolled 806 patients (approximately 200/arm) with early PD under the auspices of the Parkinson Disease Study Group (PSG). The trial duration was planned for each patient to receive CEP-1347 for a minimum of 24 months. The primary endpoint was the time to onset of disability sufficient to require dopaminergic therapy [42]. Further details on the trials will be forthcoming in publications from the PSG.

4.1.2. *TCH346*

The TCH346 trial consisted of a randomized, placebo-controlled, parallel-group, multicenter study of three doses (0.5, 2.5 and 10 mg daily). In the absence of a validated biomarker that could have been used in Phase I studies, doses were selected based on the effective dose range in MPTP and 6-hydroxydopamine (6-OHDA) models in mice, rats and monkeys (0.003–0.3 mg/kg b.i.d.) adjusted for differences in exposure (five times higher in humans than in animals). For a 70 kg individual this translated into a dose range of 0.3–3 mg daily. Early stage PD patients (75 per arm) were treated for 12–18 months after a pre-randomization phase of up to 2 weeks, followed by a 4-week withdrawal period. The primary endpoint was the time to need for symptomatic treatment; secondary endpoints were based on changes in UPDRS (sum scores parts II and III) after 4 weeks, at the end of treatment, and after the withdrawal period, and the percentage of patients needing symptomatic therapy after 12 months of treatment. On none of these parameters was there evidence of efficacy.

In both instances, the respective sponsoring companies after reviewing the data with independent data monitoring committees and other consultants considered the results as conclusive enough to abandon further development of CEP-1347 and TCH346 for a PD indication.

4.1.3. *Trial execution*

To the extent possible, both the trial for CEP-1347 and that for TCH346 were designed based on traditional clinical trial design in the area using opinion leader input. In the CEP-1347 trial, a biomarker (β-CIT) was used as a surrogate to the UPDRS and other traditional phenotypic assessments but, as noted, the results were inconclusive. No biomarker was used in the TCH346 PD trial. In hindsight, a major question is whether trials assessing a novel, non-DA modulator approach to PD should be conducted in a manner similar to all the symptomatic treatments (L-dopa, pergolide, etc.) that have gone before. Simply, is there a need for a paradigm shift in clinical trial design for neurodegenerative disorders with a more aggressive and urgent use of the translational medicine concept? The latter [43,44] encompasses a number of concepts: from initiatives for the pharmaceutical and biotech industries to partner with academic medical centers [44]; a need within the industry to better integrate the 'bench-to-bedside' concept of drug discovery by ensuring that exploratory clinical trials of drugs with novel mechanisms, like the two discussed in this Commentary, are undertaken as extensions in understanding and extending

preclinical research rather than as quasi-pivotal trials [45]; to an earlier focus in the life of a prospective NCE on mechanism of action and biomarkers for clinical use [46]. FitzGerald has also emphasized the better use of preclinical data "to inform human pharmacology" and a "more complex analysis of the factors (leading) to inter-and intra-individual differences in drug response" [44]. This would lead to expanded (and more expensive) Phase II trials but would in the long run, better control risks perhaps reducing the costs and time for Phase III trials. The need for an effective transitional medicine model is key to increasing success in the drug discovery process although the semantics and implementation currently leave much to be desired [43–46]. Also, while it has been optimistically suggested that "the physician-scientist is best placed to address the problems in drug discovery [46]", individuals like Roy Vagelos with a solid grounding in both the basic science and a full appreciation of its application to drug discovery are few and far between.

An additional confound in assessing the trial outcomes for CEP-1347 and TCH346 is the potential for heterogeneity in the patient cohorts, not only in terms of overt disease diagnosis but also the etiology or the degree of progression of the neurodegenerative process. While there are clear guidelines for PD diagnosis [47,48] the individual genetic contributions to the disease or defense reactions against it may be varied. The noxae ultimately leading to the symptomatology may not only differ between toxin- and drug-induced PD, Parkinsonism-dementia complex of Guam and PD associated with multiple system atrophy (Shy-Drager syndrome) and age-related idiopathic PD, but also within the latter group itself. Different noxae may indicate different pathophysiological mechanisms for disease cauality, which would imply the necessity for drugs addressing different targets. An obvious conclusion is that the anti-apopotic drugs discussed in this review that were robustly active in drug-induced models may be inactive in idiopathic PD due to basic differences in neurodegenerative etiology.

4.2. *Acute neuronal damage in animal models versus persistent, ongoing damage in the human disease state*

Current views on the pathogenesis of sporadic PD are that unknown etiological factor(s) perturb healthy DAergic neurons, triggering multiple transcriptional and biochemical events that can interact with one other and ultimately lead to neuronal death [29]. The relentlessly progressive nature of PD further indicates that whatever factor(s) induce the disease – the effect is chronic with a persistent assault on DAergic neuron viability over time, although there may be some pattern in this, as some DAergic neurons appear to be more vulnerable than others [49]. In general, animal models of PD do not mimic this situation, least of all the older ones, e.g., medial forebrain bundle transections or 6-OHDA treatment, but neither does acute or the majority of "chronic" versions of the MPTP model [50], which reflect multiple toxin administrations over a relatively limited time period. Furthermore the more recently developed chronic rotenone infusion model [51] appears to induce non-specific CNS and systemic toxicity and may be of doubtful value as a PD model [52,53].

Conversely, MPTP-induced degeneration of DAergic neurons in humans [54,55] or inflammatory lipopolysaccharide injections in rats [56] can progress long after the original insult has been removed, suggesting that such insults induce self-perpetuating processes that themselves might serve as the continuously present, yet hypothetical, noxae alluded to above. However, current evidence of recovery after cessation of MPTP treatment in most animal species including non-human primates [50] argues against the existence of such a disease triggering effect in these models.

For a valid disease progression model for PD, it may be irrelevant whether damage occurs via an acute insult in many DAergic neurons simultaneously or sequentially by a chronic insult in neurons, as long as the processes and pathways leading to cell death remain the same as in the disease.

4.3. *Were DA neurons already beyond rescue when treatment started?*

Although DAergic neurons are not the only neurons lost in PD, their disappearance is critical for a major part of the disease symptomatology. This is believed to begin when putaminal DA levels are reduced by 70–80% and when the loss of DAergic cell bodies in the substantia nigra pars compacta (SNpc) is in the order of 60% [31,57]. The greater loss in the projection than in the cell body area may be indicative of a retrograde degenerative process [30]. Not all nigral DA neurons appear equally affected: loss is more marked in the ventrolateral and ventromedial than in the dorsomedial part, a pattern opposite to the (much smaller) loss occurring during aging or in other basal ganglia diseases [49]. Loss appears to begin sequentially, caudorostrally through five pockets termed nigrosomes which contain approximately 40% of SNpc DAe neurons and stain poorly for calbindin; a parallel caudorostral loss was observed in the other, calbindin-rich SNpc areas called matrix [58,59]. This regional pattern of cell loss corresponds to that of the extent of DA depletion in striatal areas [60]. Of note, this regional degeneration pattern is mimicked by MPTP, a feature that has contributed to the putative predictive reputation of this PD model [61].

In addressing the large presymptomatic loss of DA neurons on the one hand, and the differential pattern of loss on the other, in perspective with the reported indices for apoptosis in the SNpc of deceased patients at the end stage of the disease [11,62], there are some obvious questions:

- Did all the DA neurons in the patients involved in the clinical trials of the two anti-apoptotic compounds die the same way, or were other death modes or pathways engaged in those that died earlier than in those that died later? Additionally, was there evidence for coordinated or sequential cell loss?
- Were those neurons that showed signs of apoptosis those that were the last to die?
- Do the signs of inflammation that appear to be typical of PD brain tissue [56,63] suggest that the neurons that died earlier in the course of the disease did so by necrotic mechanisms?

Moreover, one might also consider whether symptoms would not develop if it were possible to entirely stop DA neuron degeneration immediately before their appearance. In other words, are symptomatology and disease progression

indeed linked to progressive degeneration of the last third of remaining DA neurons, or perhaps a consequence of progressive exhaustion of mechanisms that compensated for the loss of the first two thirds of these neurons? If the answer to any of these hypothetical points were yes, treatment of the disease probably began too late to be useful in the PD trials with CEP-1347 and TCH346.

4.4. *Did CEP-1347 and TCH346 target the wrong pathways?*

There is considerable evidence that the intrinsic (mitochondrial) PCD pathway is recruited after MPTP intoxication, a consequence of the activation of the JNK and p53 pathway(s) triggered by DNA damage indirectly caused by the toxin, through generation of reactive oxygen species (ROS) and perturbation of energy metabolism and calcium homeostasis [64].

From literature data on samples from PD patients, arguments exist for participation of the extrinsic pathway additional to activation of the intrinsic pathway. Increased levels of TNFα, along with other cytokines, were found in nigrostriatal tissue, ventricular and lumbar CSF; levels of TNFR1 and soluble Fas were increased in nigrostriatal tissue [65]. Fas and Fas ligand immunoreactivities were reduced in both caudate/putamen and SNpc [66]. A significant decrease of the percentage of DA neurons immunopositive for Fas-associated protein with a death domain (FADD), a proximal adaptor protein for the TNF receptor family death pathway, in patient SNpc was reported which correlated with the selective vulnerability of nigral DA neurons [67], and an increased proportion of SNpc DA neurons exhibited caspase-8 activation [65]. Such findings were taken to suggest that DA neurons expressing the TNFR1 transduction pathway are particularly degeneration-prone in PD [68]. The role of Fas in MPTP toxicity appears controversial. Data exists for it playing both a cytotoxic and neuroprotective role, as mice lacking Fas have attenuated MPTP-induced SNc dopaminergic loss and microglial activation [69] and decreased Fas expression renders dopaminergic neurons highly susceptible to degeneration [70]. Evidence for a role of the TNFR transduction pathway in the MPTP [71] and 6-OHDA model [72,73] is limited. Considering the mechanism of action of TCH346, it is unlikely that this NCE interferes with the extrinsic pathway and there is no direct evidence of CEP-1347 interception with this pathway; however, MLK 3 appears to be involved in JNK activation following stimulation of TNFR, at least in fibroblasts [74].

4.5. *Dying by necrosis if apoptosis is blocked, or redundancy of death pathways?*

The possibility of changing death routes in response to tissue insults is not an unlikely scenario. In cellular models, cells can die by necrosis if the execution of apoptosis is blocked, e.g. by caspase inhibitors [75,76]. Moreover, initiation of apoptosis may actively suppress programmed necrosis since activated caspases inactivate proteins required for this process [77]. Additionally, the energy status of a cell can help define death pathways, since the execution of apoptosis requires energy. In the absence of sufficient ATP, necrosis may occur, and the cellular features of apoptosis, necrosis and autophagy often coexist [78]. In PD SN tissue in particular, DA neurons showing apoptotic features occur together with neurons exhibiting signs of autophagic degeneration [79], probably representing a (futile) rescue attempt [77]. Astrocytosis and microglial activation in PD SN tissue is also a well-documented [56,63] consequence of apoptosis, programmed necrosis or necrosis that may contribute to and further propagate cell death by providing stimuli that differ from, and engage different pathways than, those that originally started the process. On the other hand, these processes may also provide protection by releasing trophic factors [80]. Microglia chronically exposed to apoptotic neurons can change their gene expression and release pattern towards immunomodulatory and neuroprotective factors at the expense of pro-inflammatory molecules [81]. The exhaustion of this ability (neuroprotective tolerance?) resulting from long-term activation in PD, or its loss due to aging may contribute to the marked upregulation of pro-inflammatory factors [56,63]. Thus, there are several ways by which cells initially routed towards apoptosis can change direction with death being the ultimate outcome. It is not known whether treatment with NCEs like CEP-1347 or TCH346 can affect such routing processes. In cell culture or animals models, the time-course of cell death may have been too rapid for such directional changes to occur, but the conditions may be quite different in the diseased human brain in general and in the PD brain in particular, where cells may be in a traumatized, stressed state for prolonged periods of time [82]. Another open question that has not yet been addressed experimentally is whether damaged cells (neurons) routed towards apoptosis and exposed to agents interfering with an initially preferred cell death pathway can circumvent the NCE imposed roadblock within the apoptotic signaling system (for diagrammatic depictions see, [11,83,84]) if given sufficient time and if the intrinsic drive to die is strong enough.

4.6. *Neurons rescued, but not functional?*

It has been argued that preventing neurons from dying by apoptosis could result in the survival of dysfunctional cells. This makes a certain sense in the context of PD: the observation that reductions of DA striatal levels are more marked than losses of DA cell bodies in the SNpc, both in PD and in different versions of the MPTP model, gave rise to the view that DA neurons degenerate in a retrograde manner from the terminals to the soma (dying backwards) [30]. It is thus conceivable that anti-apoptotic agents only preserve somata with axonal stumps and are unable to sustain DAergic neurotransmission.

With CEP-1347, tyrosine-hydroxylase (TH)-positive neurons in the SNpc were only partially rescued (~50 and ~30%, respectively) in both the mouse [85] and non-human primate [85] MPTP models. In the latter model, CEP-1347 prevented the development of parkinsonian symptoms (as measured by the Laval score) even with a persistent MPTP insult. Increases in striatal DA levels were not observed but significant increases in homovanillic acid (HVA) levels occurred in both the caudate and putamen in CEP-1347 treated animals. CEP-1347 did not affect the loss of SNpc TH positive neurons induced by

transection of the median forebrain bundle (M. Saporito and J. H. Kordower, unpublished data). It was also inactive in the 6-OHDA model in adult rats (M. Miller, unpublished data) and was not evaluated in the 6-OHDA model in neonatal rats, but a closely related NCE (CEP-11004; Fig. 1) from both a structural and activity standpoint, was active in this model [87].

Although TCH346 only partially rescued TH-positive (DA) somata in the SNpc and did not preserve striatal DA levels in the mouse MPTP model, it prevented the behavioral deficits caused by intranigrally as well as intrastriatally administered 6-OHDA [88,89]. It also prevented deterioration of motor performance and loss of [^{18}F]-DOPA uptake in rhesus monkeys systemically lesioned with MPTP [90].

In the PD SNpc, but not in controls, there appears to be a pool of melanized neurons that do not stain for TH amounting to approximately 20% of the total of surviving melanized neurons [91]. This is corroborated by findings of reduced TH mRNA and protein in surviving PD DA cell bodies [92]. These might represent damaged former DAergic neurons that have not yet entered the degenerative stage, but have ceased to function as DAergic neurons [81]. If CEP-1347 and TCH346 prevented such neurons from dying, they might lead to an accumulation of non-functional cells, which could explain their clinical failure. However, a similar reduction of TH content was seen in monkeys subacutely or chronically lesioned with MPTP [93]. It is not known whether CEP-1347 or TCH346 affected this reduction of TH content per cell in the respective monkey MPTP experiments [72,86], but both NCEs attenuated the development of motor disability. Thus, despite reservations with respect to the validity of PD animal models (see below), these findings suggest that if DA neurons are preserved by these NCEs, at least some of them are in a functional state. Therefore, one would argue that non-functionality of preserved DA neurons is not a plausible explanation for the clinical failure of CEP-1347 and TCH346.

Curiously, neither CEP-1347 nor TCH346 provided full protection against the TH-positive (DA) neuron loss in the SNpc induced by MPTP. Is this because in the MPTP model there is a mixed mode of neuronal death? Full protection against cell death in animal models appears to be a necessary goal for the advancement of future compounds/treatments to the clinic.

4.7. *Are the present PD animal models appropriate?*

Information derived from post-mortem PD patient brain tissue reflects late/end stage processes, meaning that little is known about their relevance for the disease process in earlier phases. Changes in gene expression, translational or biochemical processes are seen in cells that were alive long after most of their companion cells had vanished. Do these changes forecast the imminent death of those remaining cells, or do they indicate why they survived? To assess this possibility would require large data sets from patients dying at various stages of the disease with well documented case histories, including very early ones where they were not yet recognized as suffering from PD, a major diagnostic challenge. While this may be possible in the future, with improved diagnostics, biomarkers etc., while urgent, it is not currently feasible. This explains why a greater amount of information perceived to be relevant for PD pathogenesis comes from the toxin-based, particularly MPTP, animal models [29].

Is it unfair to conclude that there is a self-fulfilling prophecy aspect as far as NCEs are concerned that are designed according to the 'disease development profile' these models provide? If such NCEs fail in the real disease, as has now happened with CEP-1347 and TCH346, this strongly suggests that the model(s) used to develop such agents have major limitations.

Thus rather than concluding that two distinct mechanistic approaches to the treatment of PD have failed or that inhibition of apoptosis is the wrong strategy for PD, there is an urgent need to systematically develop models which more closely resemble the time course of the disease pathophysiology.

5. Conclusions

The lack of clinical efficacy of two NCEs targeting different elements of the apoptotic pathway function that is thought to be involved in the death of DA neurons in PD, raises serious concerns as to the suitability of currently available models, e.g., the classical 6-OHDA and MPTP models of PD as a basis for the preclinical evaluation and prioritization of NCEs designed to slow or halt progression of PD based on novel cellular mechanisms and in vitro cellular activity. These concerns obviously extend to the validity of other animal models of neurodegeneration, including transgenics, and also highlight the need for the development of biomarkers that can act as surrogates to assess NCE activity at the defined molecular target as an urgent priority. Clearly, conducting drug discovery research in areas where there is a paradigm shift from a palliative to disease modifying approach requires a similar paradigm shift away from animal models that have been highly successful in identifying palliative NCEs, to those that more reasonably recapitulate the human disease state. In this regard, a more focused effort on translational medicine approaches [44] to better understand the pros and cons of newer animal models and their relevance to the human disease state is clearly required, assuming that the ability to develop NCEs for diseases, the existence of which can only be currently diagnosed at a stage when they are far advanced, becomes more tractable.

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