Inherited neurodegenerative diseases: the one-hit model of neurodegeneration

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The clinical manifestations of inherited neurodegenerative diseases are often delayed for periods from years to decades. This observation has led to the idea that, in these disorders, neurons die from cumulative damage. A critical prediction of the cumulative damage hypothesis is that the probability of neuronal death increases with age. However, we recently demonstrated, in 17 examples of neurodegeneration, that the kinetics of neuronal death appear to be exponential. These examples include both monogenic disorders, such as photoreceptor degenerations, as well as others that are partly or entirely acquired (such as the clinical phase of parkinsonism and retinal detachment). Exponential kinetics indicate that (i) the risk of death is constant, (ii) death occurs randomly in time and (iii) the death of each neuron is independent of other neurons. We use the term ‘one-hit model’ to refer to the single catastrophic intracellular biochemical event, analogous to radiative decay, which leads to neuronal death in the diseases we analyzed. Here, we examine the major features and implications of the one-hit model and provide preliminary evidence that amyotrophic lateral sclerosis also appears to fit this model. We also discuss a testable biochemical hypothesis, the mutant steady-state active decay, which leads to neuronal death in the diseases we analyzed. One explanation of the cell death in inherited neurodegenerations is that the neurons gradually accumulate damage, secondary to the mutation, which ultimately overwhelms cellular homeostasis (11,12); this is the cumulative damage hypothesis. One of the mechanisms most frequently proposed to underlie cumulative damage is oxidative stress (12–15), in which an imbalance between the production of reactive oxygen species and cellular antioxidant mechanisms results in chemical modifications of macromolecules, thereby disrupting cellular structure and function. Mechanisms that have been specifically postulated to lead to photoreceptor apoptosis include oxygen toxicity (16,17), the accumulation or mislocalization of mutant proteins within the cell (18), and the constitutive activity of the phototransduction cascade (19–21).

A key prediction of the cumulative damage hypothesis is that the probability that any individual neuron will become committed to apoptosis increases as damage accrues within it. A mutant neuron in an older patient will have accumulated a greater amount of damage and will therefore be more likely to die than in a younger patient. Consequently, early in the course of disease, the chance of a cell containing a sufficient amount of damage to initiate apoptosis is small, and the rate of cell loss is correspondingly low. However, as the amount of intra-

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cellular damage increases, the chance that a cell will die also increases. Thus, cumulative damage will produce a neuronal death curve with a sigmoidal shape (Fig. 1), similar to population mortality curves.

THE ONE-HIT MODEL OF NEURONAL DEGENERATION

To determine whether cumulative damage, accompanied by increasing risk of death, is responsible for the initiation of cell death, we recently determined whether the probability of cell death increased over time in 17 examples of neurodegeneration, including 13 inherited disorders and four acquired conditions. The inherited diseases included 11 examples of photoreceptor degeneration (the clinical phase of idiopathic pathogenesis), a chemically induced murine model of Parkin’s disease, cultured hippocampal neurons undergoing excitotoxic degeneration, and experimentally induced retinal detachment (22). Interestingly, we also found that four examples of acquired neurodegeneration (the clinical phase of idiopathic Parkinson’s disease, chemical and experimental induction of Parkinson’s disease, and experimentally induced retinal detachment) also exhibited exponential cell death kinetics (Fig. 2C), indicating that this feature of neurodegeneration is not restricted to monogenic diseases. Recently, in preliminary analyses, we have found that in patients with amyotrophic lateral sclerosis (ALS), the decrease in evoked motor potentials (24), an indirect measure of the number of motor units, also exhibits kinetics consistent with both a constant ($R^2 = 0.993$, $P < 0.001$) and an exponentially decreasing risk ($R^2 = 0.998$, $P < 0.001$) of neuronal death (Fig. 2D).

THE MUTANT STEADY STATE (MSS) HYPOTHESIS

To provide a biochemical explanation for the major features of the one-hit model, we proposed that the mutant neurons in delayed onset neurodegenerative diseases enter a near-normal homeostatic state, the MSS. The principal features of the MSS are, first, that the living mutant neurons function very well, in fact virtually normally, for years or decades, as indicated by clinical assessment of affected patients. Secondly, a predominant feature of the mutant neurons is that they are at a constant (or exponentially decreasing) risk of death (22).

We suggested that the MSS is characterized by subtle alterations in the activity or function of only a few genes, proteins, or metabolites, which were named, respectively, mutant response genes (MuRGs), proteins (MuRPs), and metabolites (MuRMs). One or more of the MuRGs, MuRPs or MuRMs of the mutant neuron must be pathogenic and confer the constant risk of cell death. For example, the altered expression or activity of a MuRP may increase the concentration of a critical pre-apoptotic compound, X, in the MSS. As outlined in Figure 3, the constant risk of death is conferred by the elevated basal concentration of compound X, which brings it closer to a critical level above which it initiates apoptosis. This critical level would be attained only rarely, due to random fluctuations in cell metabolism, accounting for the random and infrequent death of neurons in neurodegenerations.

The different rates of neuronal cell death associated with different loci (Fig. 2) therefore reflect the diverse effects that different MuRPs have on the risk of cell death; mutations that bring a cell closer to the ‘cell death threshold’ will exhibit a

Figure 1. Schematic representation of the kinetics of cell loss predicted by a cumulative damage model versus the one-hit model (22). The cumulative damage hypothesis predicts that the temporal pattern of cell loss should be qualitatively similar to well-documented sigmoidal mortality curves of human populations. Initially, neurons exhibit a low risk of apoptosis and a corresponding slow decline in population size (early phase). As intracellular damage accumulates over time, an acceleration in the rate of neuronal death produces a steep downturn in the cell loss curve (acceleration phase). As further damage accumulates, the risk of apoptosis increases, but the rate of cell loss eventually declines as the number of neurons available to die is reduced (late phase). These events lead to a sigmoidal cell loss curve when plotted linearly (left panel), or in a straight line on semi-log plots (right). This behaviour indicates that the time at which an individual neuron dies is independent of that of any other neuron.

mice (discussed below), an increasing probability of cell death could be firmly excluded (22,23). These results indicated that an escalating risk forced by cumulative damage was not responsible for cell death.

In contrast, we found that neuronal death exhibited exponential decay kinetics in the examples we studied (Fig. 2A) (22). Exponential kinetics, which also describe radioactive decay, indicate that in the neurodegenerations we examined, the risk of cell death is constant throughout the life of the neuronal population (in some cases, as described below, an exponentially decreasing risk of death was equally acceptable) (Fig. 2A and B). Secondly, cell death occurs randomly in time and, thirdly, the death of one cell is independent of that of any other cell. Based on the analogy to radioactive decay, we suggested the term ‘one-hit’ to describe the catastrophic event that initiates apoptosis in the neurodegenerations we analyzed (22,23).

Interestingly, we also found that four examples of acquired neurodegeneration (the clinical phase of idiopathic Parkinson’s disease, a chemically induced murine model of Parkinson’s disease, cultured hippocampal neurons undergoing excitotoxic degeneration, and experimentally induced retinal detachment) also exhibited exponential cell death kinetics (Fig. 2C), suggesting that this feature of neurodegeneration is not restricted to monogenic diseases. Recently, in preliminary analyses, we have found that in patients with amyotrophic lateral sclerosis (ALS), the decrease in evoked motor potentials (24), an indirect measure of the number of motor units, also exhibits kinetics consistent with both a constant ($R^2 = 0.993$, $P < 0.001$) and an exponentially decreasing risk ($R^2 = 0.998$, $P < 0.001$) of neuronal death (Fig. 2D).

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higher risk of cell death. Clearly, there is no requirement that this change in intracellular concentration involves a single molecule. In theory, the commitment a neuron makes to the initiation of a cell death cascade may require that specific concentrations or activities of several compounds be achieved simultaneously.

Recently, a novel biochemical mechanism consistent with the one-hit model was presented by Perutz and Windle (25), to
apoptotic threshold equals the risk that any cell will commit to apoptosis. In this figure, the area under the normal curve that is greater than the random fluctuations to 7 U/ml, which would initiate apoptosis, are now more probable. In this figure, the area under the normal curve that is greater than the apoptotic threshold equals the risk that any cell will commit to apoptosis.

Figure 3. The MSS hypothesis. Individual neurons may commit to apoptosis as a consequence of a normal fluctuation in chemical concentration. (A) In a normal neuron, the usual concentration of compound X is 3 U/ml. Compound X will initiate apoptosis only if its concentration exceeds a threshold, for example 7 U/ml, which would rarely if ever occur in a normal cell. (B) In the MSS, the mean concentration of X might be 5 U/ml, which is not disruptive per se. However, random fluctuations to 7 U/ml, which would initiate apoptosis, are now more probable. In this figure, the area under the normal curve that is greater than the apoptotic threshold equals the risk that any cell will commit to apoptosis.

account for the exponential decline in cell number seen in the clinical phase of HD. They proposed that the initial nucleation of molecular aggregates of the mutant huntingtin protein (with an expanded polyglutamine tract) is responsible for the pathogenesis of HD, in contrast to the growth and accumulation of aggregates. According to their hypothesis, the exponential cell death kinetics arise from the fact that the nucleation of an intracellular aggregate, the one hit, is random in time, and dependent on the interaction of a sufficient number of molecules. As such, this proposal represents a one-hit mechanism that complements the MSS hypothesis. However, whether or not the nuclear aggregation of polyglutamine-tract-expanded proteins is actually required for neuronal death in HD and related disorders is currently a matter of debate (26).

Our observation of an exponential decline in neuron number was not a novel one. For example, idiopathic parkinsonism (27–29), the loss of granule cells in the brains of PcdΔ mice (30) and cultured hippocampal neurons undergoing excitotoxic cell death (31) had all previously been observed to exhibit exponential cell death kinetics. However, few explanations had been put forward to account for these observations. One interpretation was provided by Massof and colleagues (32,33) after observing an exponential decrease in visual field size in patients with RP. They suggested that mutation results in a predisposition to retinal degeneration, and a second, random systemic or environmental factor determines the time of disease onset. However, a random process that is responsible for the initiation of disease cannot account for the fact that the time of death of any individual photoreceptor is random, as we have demonstrated (22). Similar suggestions concerning the pathogenesis of idiopathic parkinsonism (28,29) have been addressed elsewhere (34).

SIX UNRESOLVED ISSUES RELATING TO THE ONE-HIT MODEL

The one-hit model of neuronal death articulates a common principle that appears to underlie many types of neurodegeneration. However, several issues that appear to challenge the validity of this model must be closely examined, and weaknesses in the data on which it is based must be identified. Here, we review six unresolved issues of relevance to the one-hit model and suggest additional research that may be required to address them.

**Constant versus exponentially decreasing risk of death**

For eight of the 18 examples of neuronal cell death that we analyzed (22; Fig. 2), the kinetics of neuronal loss could be fit equally well to models utilizing a constant or an exponentially decreasing risk of death. However, a constant risk model was the most parsimonious model and, therefore, the behaviour we favored, for two reasons. First, a constant risk fit very well to all 18 examples we analyzed (22). Secondly, in the eight examples that also fit an exponentially decreasing risk of death, there was no significant difference in the fit between a constant risk and a decreasing risk. For example, in Rom1 mice, 99.3% of the variation in the cell death kinetics was accounted for by a constant risk model, versus 99.5% by an exponentially decreasing risk model (22). Only a more detailed analysis of cell death kinetics, with additional data points, may allow a distinction to be made between these two alternatives.

If an exponentially decreasing risk of neuronal death actually occurs in some systems, the underlying processes must differ from those responsible for constant risk of death (22). Exponentially decreasing risk indicates that the probability of neuronal death declines in direct proportion to the number of cells remaining in the affected population (22). In this case, therefore, the risk of death of an individual neuron is dependent on the death of surrounding cells, whereas with constant risk kinetics the death of a cell is independent of the cells around it. An exponentially decreasing risk of death could occur, for example, if the reduced neuronal population is exposed to increasing amounts of a neurotrophic factor. Conversely, if dying neurons release a cytotoxic substance into their environment, then the concentration of that factor will decrease as more neurons die, causing a concomitant decline in the risk of cell death. Evidence in support of this latter possibility is provided by experiments with dying retinal progenitor cells in culture. When healthy photoreceptors are cultured in media conditioned by exposure to dying retinal precursors, the photoreceptors show an increase in the rate of apoptosis, suggesting that the dying cells produce a diffusible, toxic factor capable of inducing apoptosis in the otherwise healthy photoreceptors (35).
Death of normal photoreceptors in chimeric mice

In chimeric mice, retinas may contain both normal and mutant photoreceptors. Two studies have demonstrated that the rate of death in the normal photoreceptors is comparable to that observed in the mutant cells (36,37). Since wild-type photoreceptors die in the presence of mutant cells, we suggest that only the increased risk of death associated with mutant photoreceptors, but not the apoptotic signal, is transmitted to the normal cells. The increased risk may be conveyed through signaling mediated by diffusible factors (36,37). Because only the risk of death is transmitted [for example, by increasing the concentration of compound X (Fig. 3) in the wild-type cells], the time of death would be random, just as it is in the mutant photoreceptors. No analysis of the kinetics of photoreceptor death has been performed in chimeric animals, but the one-hit model predicts that both the wild-type and the mutant cells would exhibit a similar constant probability of undergoing apoptosis, and that the time of death of any photoreceptor in these animals would be random.

Interestingly, the rate of photoreceptor attrition in the chimeric retinas was noted to be slower than that observed in the retinas of non-chimeric mutant mice (36,37). This observation indicates that the wild-type cells have a reciprocal effect on the risk of death of the mutant neurons. Again, intercellular communication may mediate this phenomenon.

Geographic distribution of photoreceptor death in the human retina

In patients with RP, photoreceptor death usually commences within the mid-peripheral retina (2,3), indicating that photoreceptors in some parts of the retina have a greater risk of apoptosis. This observation might seem to belie the prediction of the one-hit model that apoptosis occurs randomly in the photoreceptor population. However, this inconsistency can be reconciled by suggesting that local geographic factors may increase the risk of death in photoreceptors in the mid-peripheral retina compared to photoreceptors located elsewhere. The nature of the local risk factors is unknown, but they may, for example, be molecules whose distribution varies across the retina, such as basic fibroblast growth factor (38).

The common rate of visual field contraction in patients with RP

A clinical method used to gauge the progress of photoreceptor degeneration in RP is to evaluate the size of the visual field, a measure of the area of the retina that is still capable of responding to light. One important study compared the rates of visual field contraction of patients with different genetic and pathophysiological forms of RP and found, unexpectedly, that these rates were identical among all subtypes (32,33). This result appears to conflict with the fact that the rate of cell death in animal models of photoreceptor degeneration is widely variable (Fig. 2). However, visual field measurements may not distinguish variations in the absolute number of photoreceptor cells in a retina, due to a compensatory effect in the response of surrounding photoreceptors to light. If this is the case, patients with different types of RP may still exhibit inter-individual variations in the rate of loss of photoreceptors, similar to the widely documented variations seen in animal models (Fig. 2).

Exponential death kinetics during the clinical phase of HD

We reported that the risk of neuronal death is constant in the clinical phase of both HD and idiopathic parkinsonism (22; Fig. 2). However, data on neuronal death from the clinical phase of these disorders may not accurately describe the kinetics of cell death that occurred in such patients prior to the clinical onset of the condition. Although it seems reasonable to suggest that the preclinical cell death kinetics of these diseases would be identical to the kinetics observed in the clinical phase, such preclinical data must be collected to evaluate this concept, since other explanations are possible.

For example, HD usually manifests between the ages of 35 and 50 years, although significant neuronal death has been shown to occur in the decade prior to clinical onset (39). Our observation of an exponential decline in the metabolic activity of the caudate nucleus in clinically affected HD patients (22) does not constitute definitive proof that neuronal death observes exponential decay over the lifetime of a patient. Instead, our observation is consistent with at least three possibilities. First, there may be no neuronal death in HD until, by some unknown initiating mechanism, the mutant cell population begins to die in accordance with exponential kinetics (Fig. 4, delayed exponential kinetics). Secondly, the kinetics of cell death in HD may indeed be exponential throughout life (Fig. 4, exponential kinetics), as is the case with the other animal models we examined (22; Fig. 2). However, if this is the case, one must ask why substantial cell loss in the preclinical years has not been detected. The explanation may be that current non-invasive measures of cell number in the living human brain are insensitive. For example, measures of brain volume may be a poor reflection of neuronal number, since substantial cell death may have to occur before a reduction in volume can be detected. Thirdly, cumulative damage and sigmoidal cell death kinetics may actually occur preclinically in HD (Fig. 4, sigmoidal kinetics). In this case, our identification of exponential cell death kinetics in HD may simply reflect the fact that the late phase of a sigmoidal curve can resemble exponential decay, as can be seen in Figure 1. Which of these possibilities is correct will undoubtedly be revealed by the quantitative analysis, from birth, of affected neuronal populations in animal models of HD.

Data quality

The ability to differentiate between constant and increasing risk depends critically on the quality and completeness of available data sets. For example, the kinetics of photoreceptor degeneration in the mouse mutant retinal degeneration (Rd−−) was consistent with either a constant or an increasing risk of apoptosis (22). Consequently, it is unclear whether the death of photoreceptors in this example adheres to delayed exponential or sigmoidal kinetics (Fig. 4). Clarification of this issue will require that we obtain additional data points from early in the period of degeneration. This larger data set will allow us to determine whether photoreceptor loss curve of Rd−− mice displays an acceleration phase (i.e. a ‘shoulder’) (Fig. 1), a characteristic of sigmoidal kinetics and cumulative damage, or whether the loss of cells is truly exponential, without a shoulder.
Figure 4. Three possible explanations for the appearance of exponential kinetics of neuronal death in the clinical phase of HD. (i) Delayed exponential kinetics: there may be no cell death early on, until the impact of some unknown initiating mechanism, after which the kinetics are exponential. (ii) Exponential kinetics: neuronal loss may follow exponential kinetics throughout the life of the patient, but cell attrition may not be detected pre-clinically. (iii) Sigmoidal kinetics: cumulative damage may actually occur in HD, producing the expected delay in cell death that characterizes sigmoidal kinetics. However, if only post-clinical cell death kinetics are studied, they would mimic exponential kinetics (i.e. note the similar shape of the post-clinical curve of sigmoidal kinetics to the curve of exponential kinetics).

CLINICAL IMPLICATIONS OF THE ONE-HIT MODEL

The one-hit model of neuronal death has several important implications for the treatment of neurodegenerative disorders (22). First, the absence of cumulative damage that increases the risk of cell death indicates that neurons that are still alive in older patients should be as readily rescued as the neurons of a younger patient. Secondly, if pathogenic MuRGs can be identified (for example by microarray comparisons of wild-type and mutant tissues), the normalization of the level of such MuRGs should reduce the risk of cell death. Thirdly, since the death rate of mutant photoreceptors is reduced by the presence of wild-type photoreceptors in a chimeric retina (36,37), then the constant risk of death of mutant photoreceptors should be reduced by the correction of the genetic defect of some fraction of them, by retinal gene therapy (40).

CONCLUSIONS

Our observation of one-hit kinetics of cell death in 18 diverse examples of inherited and acquired neurodegeneration suggests that the one-hit model may represent a fundamental principle of neuronal degeneration. Although the one-hit model can apparently be applied widely, as our analysis of six types of neurons affected by various genetic and environmental insults suggests, the degree to which it can be generalized will require the analysis of cell death kinetics in a much wider sample of diseases and experimental contexts. It will also be of great interest to determine whether exponential kinetics also characterize the death of other cell populations undergoing apoptosis, as occurs, for example, in some developmental processes. All of this research will require mathematical models that accurately translate cell death data into risk estimates.

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