Lysosomal enhancement with microbial hydrolases: a novel strategy for removing protein aggregates

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Summary
All neurodegenerative diseases are associated with the intracellular accumulation of substances that impair cellular function and viability. Reversing this accumulation may thus be valuable, but has proven challenging, doubtless because substances resistant to cellular catabolism are inherently hard to degrade. I suggest a radically new approach: augmenting humans’ natural catabolic machinery with microbial enzymes. Many highly recalcitrant organic molecules are naturally degraded in the soil. Since the soil in certain environments – graveyards, for example – is enriched in human remains but does not accumulate these substances, it presumably harbours microbes that degrade them. The enzymes responsible could be identified and engineered to metabolise these substances in vivo.

Introduction
The steady rise in life expectancy seen over the past 50 years in the developed world has resulted from the postponement of the major age-related causes of death, especially cardiovascular disease and cancer. A consequence of this is that aspects of age-related decline which have not been similarly delayed are increasingly widespread. Arguably the most serious of these, in terms of its quality-of-life and economic impacts, is Alzheimer's disease (AD), which now afflicts over four million people in the USA alone. A similar proportional increase, though with fewer people affected in absolute terms, has occurred for various other age-related neurodegenerative diseases, notably Parkinson's disease (PD).

Since the landmark results of Schenk et al. in 1999, much excitement has surrounded the very real possibility that the senile plaques found in AD can be eliminated by stimulating their uptake and degradation by microglia. Though initial clinical trials of this approach were aborted due to severe side-effects in a minority of patients, such data as was forthcoming from that trial encouraged many researchers to persist with this approach and new vaccines are being aggressively developed.

No similar progress has yet occurred, however, concerning intracellular aggregates. Two things make intracellular aggregates harder to treat: firstly their location in the cell body means that phagocytosis by other cells is not an option, and second, the greater sophistication of intracellular than extracellular proteolytic systems means that any material which accumulates inside cells must necessarily be extremely refractory to degradation. (Fibrillar amyloid beta is degraded in microglia, albeit slowly.) Some early studies reported encouraging hints that certain antioxidants could disperse some intraneuronal aggregates, but these results have not stood the test of time (though such an approach remains worthy of further consideration).

Bioremediation
The soil is home to an immense variety of bacterial and fungal life. Competition between these species is inevitably intense and it is no surprise that evolution's ingenuity is apparent in abundance in this ecosystem. Some microorganisms derive their selective advantage from "bran"—simply being able to outpace their competitors in terms of growth rate. Many,
however, secure an ecological niche by "brain"—by possessing the ability to digest (and derive energy from) material in their environment which their competitors cannot degrade. In 1952 Gayle published this concept and gave it the memorable moniker "the microbial infallibility hypothesis". Its validity has been proven repeatedly in the interim, giving rise to a flourishing subfield of environmental decontamination termed bioremediation.

The essence of bioremediation is that in any environment containing a substance that is (a) organic, (b) energy-rich and (c) undegradable by most life forms, selective pressure will attract and/or cause the evolution of microbes that can degrade that substance and live off it. Hence, a strategy for removing such substances from land (for the purposes of making it usable for housing, for example) is to isolate microbes from a sample of the contaminated soil, identify ones that can degrade the offending substance, and expand those bacteria in the laboratory to quantities that, when returned to the contaminated soil, will in a reasonably short period remove enough of the contaminant to allow the site to be used for the desired purpose. Compounds for which this strategy has succeeded range from explosives such as TNT to dioxins and polychlorobiphenyls.

When we consider how we might isolate microbes that can metabolise the highly recalcitrant substances that accumulate within the human body (and specifically the brain) during life, therefore, the only clear requirement is to identify an environment that is enriched in human remains. An obvious choice is graveyards. Accordingly, the first pilot study of this concept was conducted using soil taken from Midsummer Common in Cambridge, UK, a site where plague victims were buried in the 17th century. The target substrate used in this study was artificial lipofuscin, a convenient substance synthesised by UV-irradiating a crude mitochondrial fraction of tissue overnight. Artificial lipofuscin has the fluorescence characteristics of bona fide lipofuscin and is also entirely resistant to degradation by cells that internalise it in culture. Bacteria were readily isolated that demonstrated, at least in this very preliminary pilot study, the ability to grow using this material as the sole energy source. A promising target for AD may be 7-ketocholesterol, which may impair lysosomal function by inhibiting lysosomal acidification.

**Delivery options**

There are broadly three available approaches to introducing xenoenzymes into the neuronal lysosome. The first is to introduce the genes encoding them into the neuron by somatic gene therapy. With this approach, the gene must first be altered so that its encoded product is targeted to the lysosome, presumably either by the mannose 6-phosphate pathway or by chaperone-mediated autophagy. The second option is to introduce the gene into cells *ex vivo* which are then transplanted into the patient. This has the clear advantage that the cells can be screened after transfection to ensure that only cells with the desired modification are introduced. The third alternative for neurotherapeutic enzyme administration is to emulate the well-established techniques of lysosomal enzyme replacement therapy just mentioned. In this system the enzyme is synthesised *in vitro*, and then enzyme is simply injected into the recipient and taken up by the desired cell type as a result of appropriate *in vitro* glycosylation.

**Conclusion**

Exploitation of the principles of bioremediation for removal of the aggregates that accumulate in neurodegenerative diseases is a novel but promising therapeutic modality. A more detailed account of this strategy is in press.

**References**