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Positive Lysosomal Modulation As a Unique Strategy to Treat Age-Related Protein Accumulation Diseases

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Abstract

Lysosomes are involved in degrading and recycling cellular ingredients, and their disruption with age may contribute to amyloidogenesis, paired helical filaments (PHFs), and α-synuclein and mutant huntingtin aggregation. Lysosomal cathepsins are upregulated by accumulating proteins and more so by the modulator Z-Phe-Ala-diazomethylketone (PADK). Such positive modulators of the lysosomal system have been studied in the well-characterized hippocampal slice model of protein accumulation that exhibits the pathogenic cascade of tau aggregation, tubulin breakdown, microtubule destabilization, transport failure, and synaptic decline. Active cathepsins were upregulated by PADK; Rab proteins were modified as well, indicating enhanced trafficking, whereas lysosome-associated membrane protein and proteasome markers were unchanged. Lysosomal modulation reduced the pre-existing PHF deposits, restored tubulin structure and transport, and recovered synaptic components. Further proof-of-principle studies used Alzheimer disease mouse models. It was recently reported that systemic PADK administration caused dramatic increases in cathepsin B protein and activity levels, whereas neprilysin, insulin-degrading enzyme, α-secretase, and β-secretase were unaffected by PADK. In the transgenic models, PADK treatment resulted in clearance of intracellular amyloid beta (Aβ) peptide and concomitant reduction of extracellular deposits. Production of the less pathogenic $A\beta_{1–38}$ peptide corresponded with decreased levels of $A\beta_{1–42}$, supporting the lysosome's antiamyloidogenic role through intracellular truncation. Amelioration of synaptic and behavioral deficits also indicates a neuroprotective function of the lysosomal system, identifying lysosomal modulation as an avenue for disease-modifying therapies. From the *in vitro* and *in vivo* findings, unique lysosomal modulators represent a minimally invasive, pharmacologically controlled strategy against protein accumulation disorders to enhance protein clearance, promote synaptic integrity, and slow the progression of dementia.

Introduction

Lysosomes provide broad degradation pathways. Catabolic processes involve cooperation between autophagic trafficking and enzyme delivery for efficient degradation and turnover of proteins and other material. Altered protein-processing capability in lysosomes has been suggested to affect brain function during normal aging as well as in age-related diseases. Lysosomal instability is a distinct feature of brain aging,[1,2] resulting in gradual changes and increased risk for protein accumulations and aggregated protein stress responses. Such occurrences contribute to the neurodegeneration of protein accumulation diseases (PADs) and are related to the aberrant protein and glycoconjugate aggregates of devastating lysosomal storage disorders (LSDs). Disruption of key clearance mechanisms is believed to underlie many neurodegenerative events of PADs and LSDs, leading to abnormal brain development in LSDs, dynamic changes to synapses in both, and associated cognitive deterioration.

Some cellular accumulations are shared by LSDs and PADs,[3,4] and evidence suggests that lysosomal disturbances play a role in both. Lysosome dysfunction is associated with the early-onset LSDs and also appears to play a role in later-onset diseases including: (1) Alzheimer disease and related tauopathies (frontotemporal dementia), (2) Lewy body disorders and synucleinopathies such as Parkinson disease, (3) Huntington disease and other polyglutamine expansion disorders, and (4) prion encephalopathies (reviewed in ref. 5). Related to PADs, the aged brain exhibits the risk factor of

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compromised cellular pathways that remove damaged and misfolded proteins. Recently, clearance rates for amyloid beta (Aβ) peptides were found to be slower in Alzheimer disease as compared to the rates in cognitively normal individuals,[6] implicating an imbalance in Aβ production versus clearance as a plausible mechanism for Aβ dysregulation. Perhaps related, lysosomes have a propensity for age-related free radical formation through aberrant products from incomplete catabolism, and their membranes can be targeted by oxidative damage. This leads to disruption of the important balance between protein synthesis and breakdown. Resultant overloading of lysosomal capacity and decay of efficient catabolic functions likely contribute to the aging risk factor for neurodegenerative PADs and many aspects of LSD pathology.

Experimentally induced lysosomal disturbances in a brain slice model cause distinct pathogenic changes, including protein deposition, reduced staining of synapses, dystrophic neurites, cytoskeletal breakdown, and eventual cell death. In fact, a striking resemblance is apparent between the hippocampal slice model of protein accumulation and the Alzheimer disease brain regarding the loss of synaptic integrity as well as axonopathy and associated transport failure.[7–9] One of the earliest events linked to lysosomal disturbances is the reduction in pre- and postsynaptic markers. Synapses also experience dynamic changes in Alzheimer disease, with synaptic loss well established as the best neurobiological correlate of the cognitive deficits.[10] Understanding the functional synaptic dynamics as well as synaptopathogenic cascades elicited by protein accumulation will be critical in the fight to offset progressive cognitive decline. Promoting the clearance of pathogenic accumulations is the key commonality regarding therapeutic development for the wide range of PADs and LSDs. Here, we will summarize the findings showing that the lysosomal system can be functionally modulated by a variety of agents, and, with selective compounds, that positive lysosomal modulation in *in vitro* and *in vivo* proof-of-principle studies provides neuroprotection against protein accumulation pathology. Positive modulators of clearance mechanisms represent a strategy against protein accumulation disorders, taking into consideration, of course, the pathogenic stage at which treatment is to be administered.

Synaptic Vulnerability and Lysosomal Compromise

Destabilization and loss of central synapses and cerebral activity occur during aging and in age-related dementias,[10–13] indicating that synaptic pathology is a key contributor to cognitive decline. Associated with related protein accumulation events, integrity of synapses, axons, and neuronal communication exhibit distinct vulnerability during periods of lysosomal dysfunction.[5,14] Lysosomal disruption has been implicated in the development of amyloidogenic oligomers and paired helical filament tau (PHF-tau) that forms neurofibrillary tangles. In Alzheimer brains, transgenic mouse models, and the hippocampal slice model, amyloidogenic species have a tendency to accumulate intracellularly at synaptic connections, and this is associated with signs of synaptic pathology.[15,16] In the models and in Alzheimer tissue, synaptic markers and their expression are reduced in neurons containing tau species that aggregate as PHFs and neurofibrillary tangles.[7,17] Lysosomal dysfunction in *in vitro* and *in vivo* experimental models recapitulates many pathological features of age-related PADs, including the link between protein deposition and synaptic deterioration. The induced lysosomal dysfunction produced intracellular oligomeric species and protein aggregation, causing a characteristic cascade of microtubule destabilization, transport failure, and synaptic compromise. Such a pathogenic cascade is likely responsible for loss of synapses and associated cognitive decline. Both pre- and postsynaptic markers are reduced at the protein and message levels, and synapse function is compromised as evidenced by the gradual decrease in evoked excitatory responses. Note that in the slice model, the degree of synaptic decline correlates with lysosomal dysfunction-induced protein accumulation. Increased understanding of the cellular changes that lead to synaptic and cognitive dysfunction will help develop new protection strategies.

Positive Lysosomal Modulation

Lysosomes and autophagolysosomes are thought to be activated for the clearance of toxic accumulation events. Potential compensatory changes to the lysosomal system, as indicated by the upregulation of catabolic enzymes, are evident during normal aging and are a characteristic feature of the Alzheimer brain.[8,17–19] Enhanced levels of lysosomal enzymes are particularly apparent in familial Alzheimer disease and in related transgenic mouse models.[20,21] Evidence of lysosomal changes has also been found among LSDs. Metabolically compromised animals comparable to Niemann–Pick disease and Gaucher disease exhibit elevated activities of several lysosomal hydrolases during the cellular accumulation of lipids. In addition, mannose 6-phosphorylated glycoproteins and hydrolases are elevated in the brain in juvenile neuronal ceroid lipofuscinoses.[22] The increased lysosomal enzyme activities likely indicate cellular responses aimed to compensate for accumulating material. The examples of endogenous lysosomal enhancement illustrate survival tactics of the obstinate brain.

Protein oligomerization and aggregation may trigger a lysosomal response in an attempt to prevent the formation of intracellular deposits and, in doing so, slow associated neurodegeneration. This is supported by the fact that message, protein, and activity levels of lysosomal cathepsins are increased in brain tissue and a neuronal cell line when exposed to the Alzheimer disease A$\beta_{1–42}$ peptide.[20,23] Aβ can target lysosomes and produce detrimental effects regarding their functionality,[24] and a recent study has reported higher plasma cathepsin B levels in persons with Alzheimer disease compared to healthy individuals.[25] The lysosomal response occurred in correspondence with protein accumulation during Aβ exposure as well as during episodes of induced lysosomal dysfunction.[23,26] Lysosomal stress in cultured brain tissue not only caused gradual amyloidogenic changes and tau accumulation, but it also markedly upregulated a broad spectrum of lysosomal hydrolases. Thus, it is clear that protein accumulation events can elicit positive modulation of lysosomal enzyme expression, although the modulatory responses only have a small or negligible influence on the lysosome's ability to process the accumulating material. It could also be the case that, because lysosomal activity can

contribute to oxidative damage under certain circumstances,[27] the compensatory hydrolase increases may augment lysosomal stress and actually reduce processing capability in particular cellular states.

Using chloroquine, a weak base that attenuates protein processing by disrupting the lysosomal pH gradient through proton trapping, the hippocampal slice model of protein accumulation established that the resultant lysosomal response involves a range of hydrolases (Table 1). The broad lysosomal activation involves cysteine proteases cathepsins B and L, the aspartic acid protease cathepsin D, β-glucuronidase, and likely other components of the lysosome. An indication that the lysosomal response is part of compensatory signaling comes from the fact that β-glucuronidase is among the endosomal–lysosomal components highly expressed in Aβ-resistant neurons.[28] Cathepsin B has also been implicated in the cleavage of $A\beta_{1-42}$ into less amyloidogenic species.[20,29] A wide variety of agents are listed in Table 1 that have been found to elicit positive lysosomal modulation. Chloroquine and bafilomycin A1, the latter being an adenosine triphosphatase (ATPase) blocker that also disrupts lysosomal pH, both cause lysosomal stress resulting in hydrolase responses. The lysosomal stress produced by the two agents is associated with synaptic marker decline,[30] indicating an overall compromise of protein clearance and cellular homeostasis.

Protein accumulation stress produced by Aβ peptides also upregulates the message, protein, and activity levels of cathepsin B and cathepsin D (see Table 1). Uniquely elicited lysosomal stress may be involved in the cellular response to $A\beta_{1-42}$, a peptide that is taken up by neurons and stably sequestered in lysosomes,[15,24,31] perhaps allowing a modest route of Aβ detoxification. As mentioned, cathepsin B appears to be among a small group of Aβ-degrading enzymes, including neprilysin, insulin-degrading enzyme, and endothelin-converting enzyme, which may be responsible for Aβ homeostasis in the brain.

Among the diverse agents in Table 1, several inhibitors of lysosomal hydrolases are listed that elicit a paradoxical effect of positive lysosomal modulation, upregulating the same hydrolases targeted by the inhibitors as well as unrelated hydrolases. For instance, leupeptin, the broad-acting inhibitor of serine, cysteine, and threonine proteases, used at up to 200 mg/kg, caused the expected inhibition of cathepsins B and L in different tissues. However, after clearance of the administered leupeptin, not only did the cathepsin activities recover to control levels they increased more than two-fold.[32,33] E-64c, a potent and irreversible cysteine protease inhibitor also known as Ep-475, not only increased the mature forms of cathepsins B, H, and L by two- to four-fold, but also caused two- to three-fold increases in β-glucuronidase, β-galactosidase, β-hexosamidase, and arylsulfatase activity.[34] Also listed are two examples of glycohydrolase inhibitors that act as pharmacological chaperones for potential LSD treatments: (1) the β-hexosaminidase inhibitor M-31850 that increased β-hexosaminidase A activity in fibroblasts from adult-form Tay–Sachs disease and infantile Sandhoff disease,[35] and (2) isofagomine, which raised the level of β-glucocerebrosidase in Gaucher disease fibroblasts, as did two noncarbohydrate-based inhibitors of β-glucocerebrosidase.[36] The β-glucocerebrosidase inhibitors enhanced lysosomal transport of the enzyme, resulting in increased functional β-glucocerebrosidase in lysosomes of the treated fibroblasts.

Table 1. Positive Lysosomal Modulation Initiated by Protease Inhibitors and Other Factors

Agent	*Modulation effect*	*References*
Protease inhibitors:		
DAME	Increased proCD (brain slice)	23
E-64	Increased intCL, transient increase in proCL (fibroblasts)	33
E-64	Increased proCD (brain slice)	26
E-64c (20 mg/kg)	Increased matCB, matCH, matCL (liver)	34
E-64c (20 mg/kg)	Increased A-Sul, β-gal, β-gluc, β-hex activity (liver)	34
GFGas	Increased proCD (brain slice)	23
Leupeptin (200 mg/kg)	Delayed increase in CB activity (liver, heart, muscle)	32
Leupeptin	Increased proCD (brain slice)	26
Pepstatin A	Increased proCD (brain slice)	26
PADK	Increased intCL (long-term), increased proCL (fibroblasts)	33
PADK	Increased intα-man, intβ-gluc (*Dictyostelium discoideum*)	52
PADK	Increased matCB, CD, CS, elastase (brain slice)	8, 23, 38
PADK (18–20 mg/kg)	Increased matCB, intCD, CB activity (brain)	29
Glycohydrolase inhibitors (pharmacological chaperones):		
Hex inhibitor M-31850	Increased lysosomal Hex A activity (ATSD fibroblasts)	35
Isofagomine	Increased lysosomal GCase (GD fibroblasts)	36
Peptides and proteins:		
Aβ1–42	Increased matCD (brain slice)	8, 23
Aβ1–42	Increased CB mRNA, activity (neurons)	20
Aβ1–40 (aggregated)	Increased CB activity (neurons)	20
Huntingtin	Increased intCD, CL (striatal x57 cells, PC12 cells)	41
Ionotropic agents:		
Chloroquine	Increased matCB, CD, CL, β-gluc (brain slice)	8, 23, 26
Bafilomycin A1	Increased matCD (neurons)	50

Agents reported to increase the proform (pro), intermediate (int), and mature forms (mat) of enzymes or their activity levels are listed, with organ location or model utilized provided. Dosages of *in vivo* studies are shown.

α-man, α-mannosidase; A-Sul, arylsulfatase; β-gal, β-galactosidase; β-glu, β-glucosidase; β-gluc, β-glucuronidase; β-hex, β-hexosamidase; CB, cathepsin B; CD, cathepsin D; CL, cathepsin L; CS, cathepsin S; DAME, diazoacetyl-DL-2-aminohexanoic acid methyl ester; GCase, β-glucocerebrosidase; GFGas, glycyl-phenylalanyl-glycine-aldehyde semicarbazone; Hex, β-hexosaminidase A; PADK, Z-Phe-Ala-diazomethylketone.

M-31850 and two related β-hexosaminidase inhibitors selectively raised the enzyme activity in the lysosomal fraction of treated cells, likely by stabilizing the proper folding of the mutant β-hexosaminidase, thereby promoting endoplasmic reticulum export and subsequent trafficking to the endosomal–lysosomal compartment. Although effective as pharmacological chaperones when administered at low

levels, high concentrations of the compounds produced nonselective inhibitory effects, leading to cellular toxicity. Adverse cellular changes also occur with high levels of the cathepsin B and L inhibitor *N*-Cbz-L-phenylalanyl-L-alanyl-diazomethylketone (PADK; also known as ZPADK, ZPAD, and Z-Phe-Ala-CHN_2) in brain tissue.[30,37] However, low PADK concentrations dramatically increase cathepsin isoforms and other hydrolases without producing any indications of cellular or synaptic pathology after extended treatment.[8,21,23,29,30,38] Different active cathepsin forms were upregulated with evidence of enhanced trafficking of the hydrolases to lysosomes, whereas the low concentration PADK had no influences related to axonopathy, axonal initial segment atrophy, or somatofugal transport failure.

Broad lysosomal stress through inhibition of multiple hydrolases, as with the actions of chloroquine, bafilomycin A1, and leupeptin, may be adept at triggering a feedback response, perhaps related to the feedback mechanism referred to when continuous proteasome inhibitor treatment was found to increase proteasome components and inhibitor resistance.[39] On the other hand, compared to the broad lysosomal stressors, a much higher degree of positive lysosomal modulation is produced by PADK, the selective inhibitor of two thiol proteases, as well as by the specific cathepsin D inhibitor diazoacetyl-DL-2-aminohexanoic acid methyl ester and the cathepsin B inhibitor glycyl-phenylalanyl-glycine-aldehyde semicarbazone.[23] Distinct mechanisms may be involved because chloroquine and PADK are dissimilar in their ability to produce an additive lysosomal response with $A\beta_{1-42}$.[8] PADK does not appear to influence proteasome markers, and its lack of influence on lysosomal marker lysosomal-associated membrane protein 1 (LAMP1) levels or the number of cathepsin-positive organelles would appear to rule out any broad effect on lysosomal biogenesis.[23,29] Table 1 shows that lower PADK concentrations increase the expression of cathepsins B, D, L, and S and enhance active intermediate forms of a variety of hydrolases. As mentioned, cathepsin B is a suspected Aβ-degrading enzyme that is increased in Alzheimer disease and related transgenic mice.[19,20] Increases in cellular levels of hydrolase intermediates by modulatory concentrations of PADK may signify enhanced lysosomal transport of the enzymes and more efficient maturation, thereby improving clearance of material delivered to lysosomes.

Lysosomal Enhancement to Promote Cellular Recovery

Knowing the link between lysosomal dysfunction and selective pathogenesis, one logical step toward therapeutic intervention is the enhancement of enzymatic activity in lysosomes. Age-related PADs and developmental LSDs may be slowed or reversed by the positive modulation of the lysosomal system. Many studies suggest that lysosomal activation occurs with age and in diseased brains,[8,17,18] but not to the necessary extent that would prevent the gradual loss of neuronal integrity and brain function. Enhancement of lysosomal function has been proposed as a plausible strategy to reduce protein accumulation events in age-related disorders, including those events in Alzeimer disease, Parkinson disease, and Huntington disease.[8,20,23,29,40–46] Several of the studies indicate that induction of protein degradation processes is an attempt to clear amyloid peptides and tau species, as well as α-synuclein and mutant huntingtin. Another potential therapeutic strategy that may involve the endosomal–lysosomal system is the disaggregation of extracellular Aβ peptide, with the idea that the disaggregation would promote uptake of monomers and small oligomers into neurons and microglia where they are trafficked to lysosomes for degradation by cysteine proteases, including cathepsin B.[15,29,47] Note that besides the disaggregation of intra- and extracellular Aβ to facilitate clearance in this manner, extracellular proaggregation of soluble Aβ oligomers has been proposed as protective,[48] whereby the formation of large nontoxic complexes reduces the smaller oligomeric species that are responsible for synaptic pathology and cognitive deterioration.

Of the list of lysosomal modulatory agents in Table 1, only PADK, diazoacetyl-DL-2-aminohexanoic acid methyl ester, glycyl-phenylalanyl-glycine-aldehyde semicarbazone, and bafilomycin A1 have been reported to elicit protection against protein accumulation pathology under appropriate low-dose conditions, and of these all but bafilomycin A1 are selective cathepsin inhibitors. Low-dose bafilomycin A1 (0.1—1 nM) was found to be cytoprotective against chloroquine-induced accumulation pathology in human neuroblastoma cells, the protection being in part through bafilomycin A1's ability to maintain the autophagy–lysosomal pathway.[49] Synaptic protection against chloroquine-mediated pathology in hippocampus was also produced by 1 μM of the specific cathepsin D inhibitor diazoacetyl-DL-2-aminohexanoic acid methyl ester, and similar results were found with the cathepsin B inhibitor glycyl-phenylalanyl-glycine-aldehyde semicarbazone.[23] PADK, a mild inhibitor of cathepsins B and L, is probably the most studied agent regarding lysosomal modulation and the protective clearance of toxic proteins in models of protein accumulation pathology.

Low-level PADK produces apparent enhancement of hydrolase trafficking and maturation, thereby improving the clearance capacity of lysosomes (Fig. 1A). The brains of PADK-treated animals exhibited increased levels of cathepsin proenzyme forms.[8,29] In addition, the mature forms of cathepsin B increased much more as compared to the PADK effect on the proform of the enzyme. Thus, besides enhanced expression, these findings implicate trafficking and maturation as major components of the PADK effect because inactive procathepsin forms are processed to the active forms in late endosomes and lysosomes. PADK-mediated changes in trafficking/maturation were also supported by effects on the Rab5a marker of early endosomes in brain slice cultures.[29] Positive modulation of trafficking and maturation of enzymes would increase the lysosomal content of active hydrolases and enhance their clearance capacity for protection against protein accumulation pathology.

First tests of the idea of protective lysosomal modulation used the hippocampal slice model of protein accumulation pathology. Slice cultures treated with the lysosomal inhibitor chloroquine exhibited increased levels of tau isoforms and other aggregation-prone proteins found to accumulate in the aged brain and age-related diseases.[7,26,30,50] Evident in the model was a pathogenic cascade consisting of PHF-tau accumulation in neurons, tubulin breakdown, microtubule destabilization, transport failure, and gradual synaptic decline that

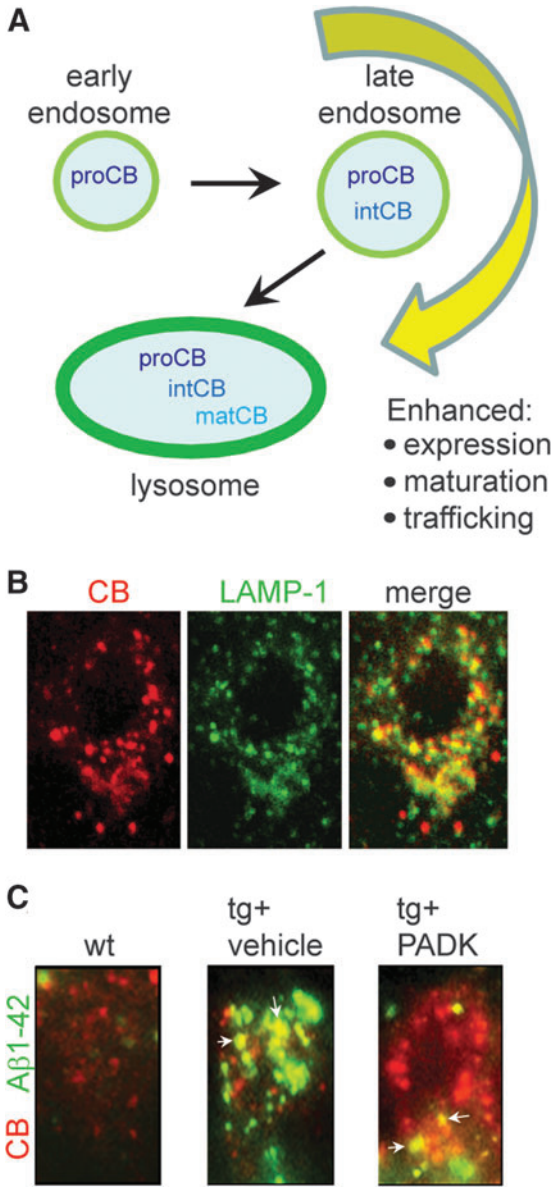

FIG. 1. (**A**) Lysosomal modulation increases the cathepsin B content of lysosomes and reduces intracellular amyloid beta 1–42 ($A\beta_{1-42}$) in APPswe/PS1δE9 hippocampus. The lysosomal modulator Z-Phe-Ala-diazomethylketone (PADK) has been shown to increase expression of procathepsin B (proCB), as well as produce molecular changes consistent with enhanced cathepsin maturation and trafficking.[8,23,29] The latter steps may involve processing/trafficking of active intermediate (intCB) and mature forms (matCB) of cathepsin B from early endosomes and late endosomes, and subsequently to lysosomes to promote protein degradation. (**B**) To confirm localization, hippocampal tissue from PADK-treated mice (intraperitoneally, 20 mg/kg per day × 9 days) exhibiting increased mature cathepsin B levels was stained with anti-cathepsin B (red) and anti- lysosomal-associated membrane protein 1 (LAMP1) (green). The merged image shows that the PADK-modulated hydrolase highly co-localizes with LAMP1-positive lysosomes in pyramidal neurons. (**C**) The increase in organellar cathepsin B (red) in PADK-treated APPswe/PS1δE9 transgenic mice (tg) was associated with a decrease in intracellular $A\beta_{1-42}$ in CA1 pyramidal neurons. Arrows denote co-localization in organelles, the image capturing intact $A\beta_{1-42}$ before being digested by cathepsin B. View-field widths, $\approx$12 μm. wt, Vehicle-treated wild-type mice.

included the loss of pre- and postsynaptic proteins. Gradual loss of transport occurred as protein deposits and microtubule deterioration became evident. A microtubule-stabilizing agent was used to show that, indeed, disruption of microtubule integrity accounted for the chloroquine-induced synaptic decline.[51] Upon removal of the lysosomal disruptor, the protein accumulation events continued to persist, especially in pyramidal neurons. In contrast, when chloroquine was replaced by the positive lysosomal modulator PADK, the three- to eight-fold enhancement of lysosomal enzymes was associated with the clearance of protein oligomers and aggregates.[8,23] The PADK treatment generated pronounced increases in cathepsin isoforms as compared to the modestly elevated levels found in response to the chloroquine-mediated lysosomal stress, the result being enhanced lysosomal function with the capacity to clear pre-existing protein deposits.

In addition to clearance of PHF-tau and amyloidogenic fragments, lysosomal enhancement also led to indications of microtubule stabilization, further establishing a link between protein accumulation and the loss of microtubule integrity.[7,23] In the slice model, chloroquine-induced protein accumulation was found to be associated with reduced microtubule integrity by assessing tubulin acetylation, a specific posttranslational modification that indicates stable microtubules. Disruption of microtubules and transport capability is thought to be a key contributor to Alzheimer disease. Reduced levels of acetylated tubulin as well as increased tubulin fragmentation occurred in close association in the protein accumulation model. These changes also significantly correlated with transport failure and the reduction in excitatory postsynaptic potential (EPSP) size when measuring synaptic responses in the CA1 region.[7,30] The cellular content of acetylated tubulin was gradually restored by PADK-mediated lysosomal enhancement to levels similar to those expressed by control slices.[23] Importantly, the positive influence on microtubule integrity by lysosomal enhancement was further indicated by the restoration of microtubule-based transport through distal dendritic branches, spine structures, and long axons.

Positive lysosomal modulation elicited protection against the pre-existing protein accumulation events in the slice model, thus indicating that the protein deposition–microtubule destabilization–transport failure–synaptic decay cascade is able to be reversed. In addition to the recovery of microtubule stability markers, transport function, and tubulin-binding proteins in hippocampal slices treated with low-dose PADK, synaptic proteins also exhibited concomitant recovery as well as improved maintenance. The lysosomal modulation elicited many indications of pre- and postsynaptic maintenance,[8,23,38] including protected levels of synaptic vesicle proteins, different α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subunits (GluR1, GluR2, GluR3), *N*-methyl D-aspartate (NMDA)-type glutamate receptors, and other neurotransmitter receptors. In addition, improved levels were found for stargazin, a member of the transmembrane AMPA receptor regulatory proteins (TARP) family of transmembrane AMPA receptor-associated proteins, and other proteins involved in the trafficking and clustering of transmitter receptors in central neurons.[38] Together, these findings indicate that, at early-stage protein accumulation pathology before overt neuronal degeneration, pharmacologically-controlled modulation of

the lysosomal system provides: (1) Enhanced clearance of protein deposits, (2) restoration of microtubule integrity, (3) re-establishment of vital transport mechanisms, and (4) synaptic and functional recovery.

Following the *in vitro* proof-of-principle studies, recent work tested the effect of positive lysosomal modulation in transgenic mouse models of Alzheimer disease.[29] As in the hippocampal slice model in which the novel approach reduced PHF-tau species, comparable effects on Aβ clearance and synaptic protection were produced by a lysosomal modulator in transgenic mice. The modulator PADK, when administered intraperitoneally into mice daily for 1–2 weeks, enhanced the cathepsin B levels in pyramidal neurons in the brain, with no effect on neuronal morphology or cell density. The PADK treatment influenced the lysosomal content of cathepsin B as determined in brain tissue double-stained for cathepsin B and LAMP1, the merged immunofluorescence image showing that the modulated cathepsin B co-localized with LAMP1-positive organelles in pyramidal neurons (Fig. 1B). While intracellular cathepsin B levels increased markedly, the number of cathepsin B-positive organelles per neuron and the level of LAMP1 staining were unchanged. Thus, as confirmed by measuring cathepsin B activity in isolated lysosomes,[29] lysosomal modulator treatment enhanced the content of active cathepsin B in lysosomes.

As a test of whether lysosomal proteolytic activity can be enhanced to reduce Aβ accumulation, the PADK injections in APP_{SwInd} and APPswe/PS1δE9 transgenic mice caused 3- to 10-fold increases in cathepsin B activity, whereas neprilysin and insulin-degrading enzyme were unchanged.[29] The up-regulated levels of cathepsin B corresponded with reduced levels of Aβ_{1-42}. Brain sections from the different APPswe/PS1δE9 treatment groups were double-stained for Aβ_{1-42} (green) and cathepsin B (red) to distinctly show the link between the positive modulation of cathepsin B in neurons and the clearance of intracellular Aβ_{1-42} (Fig. 1C). The immunofluorescence images revealed punctate Aβ_{1-42}–positive material within CA1 pyramidal neurons, and the intracellular accumulation was reduced by PADK in correspondence with enhanced labeling intensity of cathepsin B-positive organelles. The sparse number of organelles exhibiting co-localization of cathepsin B and intact Aβ_{1-42} (arrows) provides further evidence of the efficient degradation of Aβ_{1-42} peptide within positively modulated lysosomes.

These experiments, using two different transgenic mouse models, suggest that positive modulation of lysosomal enzymes starting at early or latter stages of Alzheimer disease can promote protective clearance of toxic material.[29] The enhanced clearance of intracellular Aβ also led to the reduction of extracellular deposits, thus suggesting new ideas regarding Aβ metabolism as well as equilibrium processes that contribute to the dynamics of extracellular protein deposition. Using an alternative approach, lentiviral delivery of cathepsin B was shown to significantly reduce Aβ accumulation in a transgenic mouse model, whereas the genetic inactivation of the cathepsin increased the abundance of Aβ_{1-42}.[20] Together, the findings further implicate cathepsin B as an Aβ-degrading enzyme, both studies indicating that cathepsin B plays an anti-amyloidogenic role by degrading Aβ_{1-42} into less pathogenic peptide species.

The evidence of intracellular Aβ clearance and concomitant reduction of extracellular deposits by the lysosomal modulator PADK were found to protect against further aspects of protein accumulation pathology.[29] The findings suggest that positive lysosomal modulation reduces Aβ_{1-42} through intracellular truncation that also influences extracellular deposition, with one or both effects explaining the elimination of behavioral and synaptic protein deficits in two transgenic mouse models. Figure 2 shows PADK-mediated reduction of Hematoxylin & Eosin–stained deposits in the hippocampal neuropil of APPswe/PS1δE9 transgenic mice. The protein clearance was associated with the preservation of neuronal integrity, as indicated by the recovered distribution of calbindin labeling. Learning deficits have been previously shown to correlate

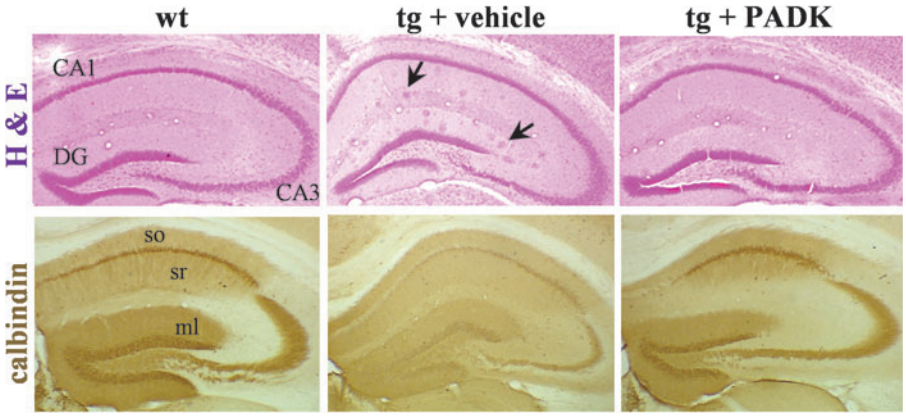

FIG. 2. The lysosomal modulator Z-Phe-Ala-diazomethylketone (PADK) reduces protein deposits and preserves neuronal integrity in hippocampus of APPswe/PS1δE9 transgenic mice. APPswe/PS1δE9 mice (tg) of 20–22 months were treated with PADK (intraperitoneally, 20 mg/kg per day) or vehicle for 11 days, and nontransgenic control mice (wt) received vehicle injections. Fixed sections were Hematoxylin & Eosin–stained (top) and assessed for calbindin immunolabeling (*bottom*). Typical extracellular deposits of the APPswe/PS1δE9 mice (arrows denote two of them) were dramatically reduced in number by lysosomal modulation in the stratum radiatum and other brain areas. Recovery of calbindin-positive structures and cellular expression was also produced, especially evident in the CA1 dendritic fields and the molecular layer of the dentate gyrus. View-field width, approximately 1.8 mm. H & E, Hematoxylin & Eosin; DG, dentate gyrus; ml, molecular layer; so, stratum oriens; sr, stratum radiatum. (Color image is available online at www.liebertpub.com/rej).

strongly with the depletion of the calcium-binding protein in hippocampus, and mice that had cathepsin B genetically inactivated had lower hippocampal calbindin staining, consistent with their increased Aβ_{1-42} levels.[20] In addition to the recovery of calbindin-positive dendrites and cellular expression, PADK-treated APP_{SwInd} and APPswe/PS1δE9 mice exhibited similar indications of pre- and postsynaptic protection.[29] The lysosomal modulation preserved measures of specific markers of synaptic components, and, in fact, improved levels of a neurotransmitter receptor subunit significantly correlated with the degree of cathepsin B enhancement in the brain. The PADK-mediated clearance and synaptic protection translated to behavioral protection as the treated mice exhibited reduced deficits in motor memory, exploratory inhibition, and episodic memory tasks.

Positive modulation of cathepsin expression and trafficking of the hydrolases may enhance the cooperation between autophagosomes and lysosomes to promote catabolic processes and control toxic material that builds up as the brain ages. Perhaps in PADs, as well as in LSDs, endogenous compensatory signaling occurs in response to the toxic buildup, although at insufficient levels to prevent accumulation pathology and associated neuronal compromise. Such endogenous responses may delay overt neurodegeneration and account for the gradual disease progression often exhibited by several types of protein accumulation pathology. Lysosomes are the primary site for removal of misfolded and accumulating proteins to maintain cellular homeostasis, and enhancing lysosomal function above that produced by the endogenous modulatory response can protect the brain against a distinct pathogenic cascade. Reducing accumulation events in neurons and other cells is essential for slowing the progression of PADs and LSDs. Small-molecule lysosomal modulators like PADK support the concept of pharmacologically controlled lysosomal enhancement to remove toxic deposits and offset cognitive decline. Members of the new class of compounds are being tested in aged animals and in models of frontotemporal dementia and LSDs. Development of efficacious lysosomal modulators will further the efforts to provide a therapeutic avenue that enhances protein clearance, promotes synaptic integrity, and slows the progression of proteinopathies.

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Author Disclosure Statement

Dr. Bahr is a founding scientist of Synaptic Dynamics, Inc., that is developing modulators for the enhancement of lysosomal capacity.

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