

A Brief Overview of Pathological Tau and Inhibitors and Modifiers of Tau Aggregation for Treatment of Tauopathies

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ABSTRACT

Tauopathies make up a significant portion of neurodegenerative diseases, affecting tens of millions of people. Tauopathies are defined by abnormal tau protein aggregation. Current approaches to tauopathy inhibition and therapeutic development require an in-depth understanding of post-translational modifications and propagation of pathological tau. Research thus far has produced a variety of inhibiting and mitigating compounds to alleviate tau aggregation. These compounds employ distinct mechanisms, including: kinase-, enzyme-, and secretion-inhibition, direct aggregation inhibition, and upregulation of cellular degradation systems. Overall, the current progress and ongoing research in the development of tauopathy therapeutics has potential, while also highlighting some crucial unknowns in the field that warrant further investigation.

Introduction

Of the vast number of people burdened with a neurological disease, a staggering more than 30 million are victims of tauopathies, in which an abnormal tau protein is the defining characteristic [1]. These tauopathies encompass many types of disorders, including neurofibrillary tangle dementia, chronic traumatic encephalopathy, and aging-related tau astrogliopathy [2]. Abnormal tau deposits are sometimes observed in Parkinson's disease and Alzheimer's disease [3]. While tau aggregation is the pathological hallmark of tauopathies [4], there is no established cure or treatment. Inhibition or mitigation of tau aggregates and its overall abnormal protein complexes would address a vast number of these neurodegenerative diseases. This review introduces tau structure, post-translational modifications of pathological tau, methods of pathological tau propagation, and a brief overview of a diverse set of tau aggregation inhibitors and degradation-inducers.

Overview on Pathological Tau

Tau Structure

First discovered by co-purification of microtubule-associated proteins from porcine brain lysate, human tau protein is usually found in the cytosol of neurons and glial cells, where it plays a crucial role in microtubule dynamics and structure [1], [5]. The protein is composed of four regions: an N-terminus, a proline-rich domain, a microtubule-binding domain (MBD), and a C-terminus. Tau stabilizes microtubules by binding to the microtubule surface through its microtubule binding domain. Human tau has six main isoforms, generated by alternate splicing of the N-terminus and the MBD. There are two main categories of cytoplasmic tau, those composed of 3 microtubule-binding potential repeat domains (MTBDs) (3R) or 4 MTBDs (4R) [3], [5]. In non-disease states, tau exists in an equal ratio of 3R to 4R tau, however this ratio is often imbalanced in neurodegenerative diseases [5].

Post Translational Modifications of Pathological Tau

Tau undergoes multiple post-translational modifications (PTMs). While the exact mechanism by which tau proteins become dysfunctional and assemble into pathological tau is still unclear, there are several hypotheses regarding irregular or misplaced PTMs [3]. Pathological tau may arise through unfolding and/or misfolding due to certain PTMs, which then trigger aggregation [6]. Consequently, tau's protein-protein interactions, such as its binding to the microtubules, are either lost or altered, leading to impaired axon functions [7]. Hyperphosphorylation of tau is one of the most studied PTM. Phosphorylation is a PTM where a phosphoryl group is added to a protein, typically by a kinase, a type of enzyme that catalyzes the transfer of a phosphoryl group from adenosine triphosphate (ATP) to the protein of interest. Tau can be phosphorylated by a variety of kinases, some of which are downstream of signaling pathways related to glial cells, such as microglia and astrocytes. Glial contributions to tau pathology is an area of active research [8]. Hyperphosphorylation disengages tau from microtubules and missorts tau to synapses and somatodendritic compounds, causing aggregates followed by toxicity and synaptic dysfunction [1], [9]. Other PTMs that promote tau aggregates include acetylation, nitration, glycation, and truncation of the C-terminus [3].

Pathological Tau Propagation

Propagation of pathological tau is an area with immense research potential. It is generally agreed that tau inclusions propagate along neuroanatomically connected brain regions, similar to prion diseases [10], [11], [12]. Abnormally assembled protein can cause the same abnormal configuration in another of the same kind of protein, setting off a self-amplification that causes otherwise healthy cells to obtain pathological deposits. Tau propagation also allows for seeding of the protein in a templated misfolding process, where hyperphosphorylated tau causes other, not necessarily phosphorylated, tau proteins to become misfolded [11], [13]. Thus, understanding how this misfolding can be prevented while paying special attention to those un-phosphorylated tau with the potential to become misfolded, could serve as key information to finding new therapeutics. While cell-to-cell propagation of tau is commonly understood to occur through vesicles, tiny fluid-filled compartments enclosed by a lipid bilayer (similar to the lipid bilayer comprising the cellular membrane), emerging research supports vesicle-free secretion of free form tau in the extracellular space.

One method of secretion is related to exosomal secretion. This mechanism depends on various intracellular structures, such as endolysosomes. Tau is secreted in small exosomes or as unbound tau from the microtubule due to the fusion of the endolysosomes to the plasma membrane. Numerous studies reveal the presence of tau in exosomes ranging from those from neuroblastoma cells to the ones found in cerebrospinal fluid [11], [14], [15], [16]. Internalization of tau can occur through the traditional endo-lysosomal pathway involving dynamin-dependent endocytosis, or by micropinocytosis [17], [18]. Other methods of tau secretion include microvesicle shedding, in which the vesicles with tau are directly synthesized through outward budding of the plasma membrane, bypassing the Golgi apparatus where proteins are normally synthesized. The misfolding-associated protein secretion pathway (MAPS) is yet another method through which tau can be translated into endolysosomes by overloading of the ubiquitin-proteasome clearing system. Abnormal proteins and aggregates may also be delivered to endo-lysosomes through macroautophagy, the process in which cellular contents are degraded and recycled by the lysosomes, though it is still uncertain whether they deliver to endo-lysosomes or fuse directly to the plasma membrane [11].

Additionally, tau seems to interact with various cell surface receptors and blockage of such receptors decreases tauopathy *in vitro* [17]. Finally, while it's still ambiguous how templated misfolding and seeding occurs from a molecular perspective, both cell surface receptor interactions and the specifics of templated misfolding and seeding are opportune areas of research for novel therapy development [17], [18].

Tau therapeutic development is a wide range of study, with varying approaches for mitigating and/or inhibiting pathological tau deposits. Here, several studies will be highlighted to provide an insight into the current stage of therapeutics and potential next steps.

Inhibition and Mitigation

R-(-)-apomorphine & raloxifene

In a study demonstrating the feasibility and efficacy of using hyperphosphorylated tau-based assays to develop therapeutics and identify risk factors, conclusions were made that raloxifene and R-(-)-apomorphine are p-tau aggregation inhibitors. Both of these compounds were chosen because they were shown to reduce p-tau toxicity without thioflavin S (ThS), fibril dye, or inducers [19]. Additionally, both compounds can pass the blood brain barrier. Raloxifene, a selective estrogen receptor modulator used in the treatment of osteoporosis and breast cancer, showed a typical inhibition pattern — it prevented the increase of tau aggregation and kept aggregation levels in check, with a gradual decline. R-(-)-apomorphine is already conventionally used in treating Parkinson's. However, this study was able to demonstrate the specificity that hyperphosphorylated tau-based assays have by utilizing R-(-)-apomorphine, highlighting its potential in the further development of tau therapeutics [19]. In the case of R-(-)-apomorphine, 1N4R tau (p-tau) aggregation increased at a normal rate and then either stabilized or started to slowly decline— this was measured on account of reactivity with ThS. After the aggregation stabilized or started its gradual descent, there was a sharp drop in concentration starting at the 600 minute point. The distinction between the effects of both compounds on p-tau suggests that they each act on different parts of the tau fibrillogenesis [19].

A notable point when conducting such studies is that unmodified tau fibrils are susceptible to the oxidation of two cysteine tau residues, C291 and C322. However, p-tau aggregation is not affected when the residues are switched to alanine or serine [20]. Because of the apparent inhibitory and mitigating effects of R-(-)-apomorphine and raloxifene, it was also concluded that both compounds worked unrelated to the cysteine residues. Conducting further examination, SH-SY5Y neuroblastoma cells were treated with tau and then the compounds and analyzed to determine more specific effects. It was seen that when co-incubating p-tau with gradually rising amounts of R-(-)-apomorphine or raloxifene, the LD₅₀ values—the concentration of p-tau causing 50% of cell death— of p-tau also increased, showing cytotoxicity attenuation [19]. Both raloxifene and R-(-)-apomorphine are concentration-dependent. The cells were again treated with 0.8 μM and then with different amounts of the inhibitors. The EC₅₀—the concentration of a compound needed to reduce p-tau cytotoxicity by 50%— was 2.9 and 3.4 μM for R-(-)-apomorphine, and 4.8 μM for raloxifene, highlighting the increased potency of R-(-)-apomorphine [19]. Overall, the exact molecular explanation for the effects remains to be examined, but both compounds inhibit aggregation (suggested methods include better cellular tolerance to p-tau aggregates and decreased fibril toxicity in the presence of the compounds) [19].

EHT 5372

Another potential therapeutic focuses on kinase inhibition: EHT 5372 (methyl 9-(2,4-dichlorophenyl amino) thiazolo[5,4-f]quinazoline-2-carbimidate) is a novel and highly potent inhibitor of the human dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A). DYRK1A kinase is affiliated with neurofibrillary tangles in Down Syndrome, along with sporadic Alzheimer's [21]. Over-expression of DYRK1A has been implicated in tauopathy generation, based on studies done in cell cultures and transgenic mice [22]. DYRK1A also plays a role in inducing pathological hallmarks of tauopathies—high-throughput siRNA screening established DYRK1A's role in phosphorylating tau on a variety of amino acids (S202, T212, T231, S396, S404, AT8 (pS202 + T205), 12E8 (pS262 + pS356))—along with priming Tau for phosphorylation by GSK3 at other sites [23], [24]. Thus DYRK1A is a highly promising kinase target for potential tauopathy therapeutics.

EHT 5372 is the lead inhibitor for the DYRK family: the compound inhibited DYRK1A activity with a 0.22 nM IC₅₀ (the concentration at which a drug is able to inhibit a particular biological process by 50%), DYRK1A with a 0.28 nM IC₅₀, DYRK2 with a 10.8 nM IC₅₀, and DYRK3 with a 93.2 nM IC₅₀. However, there was no inhibition recorded for DYRK4. EHT 5372 also demonstrated notable selectivity over 339 tested kinases, along with having almost 30-fold more potency than other known tools— harmine, EGCG (epigallocatechin-gallate), L41 and TG003 [21]. In addition, EHT 5372 inhibits the direct phosphorylation of Tau; utilizing an *in vitro* phosphorylation assay with recombinant human DYRK1A and Tau, it was confirmed that DYRK1A directly phosphorylated Tau protein at Serine 396.

HEK293 cells, that express DYRK1A but are devoid of endogenous Tau, were co-transfected with DYRK1A and Tau expression vectors and monitored, with special attention at S396 [21]. When specifically DYRK1A was co-transfected to maximize DYRK1A effect, Tau phosphorylation more than doubled, confirming DYRK1A's phosphorylation-inducing effect. This phosphorylation, which only occurred in the presence of Tau, DYRK1A, and ATP, was dose-dependently inhibited when EHT 5372 was added; after 24h incubation, the cells were treated with varying concentrations of the compound. EHT 5372 dose-dependently reduced pS396 levels (IC_{50} of 1.7 μ M) and cell viability stayed over 87%. Again, the potency of EHT 5372 was emphasized when compared to other compounds, which did not exhibit as much activity as EHT 5372 [21]. Then, rat cortical and SH-SY5Y-Tau441 cells were also used to determine that EHT 5372 inhibition of DYRK1A reduces cellular Tau phosphorylation at multiple phosphoepitopes [21]. Another interesting point is that A β 42 also stimulates DYRK1A expression [22]; Consequently, A β 42 stimulated Tau phosphorylation at pS396, but also AT8 epitopes, in the cortical neurons and SH-SY5Y-Tau441 cells. This was then dose-dependently inhibited by EHT 5372 in both cell types. Phosphorylated Tau levels were also reverted back to the control situation. Although, a noteworthy point is that neither A β 42 or EHT 5372 showed any significant effect on total Tau levels [21]. Despite this, kinase inhibition still proves to be a promising aspect of aggregation inhibition.

GSK1482160

Tau pathology may be propagated through secretion of tau-containing exosomes, as discussed above. Although the exact molecular target is still unidentified, microglia do play a role in the process. P2X purinoceptor 7 (P2RX7) is an ATP-gated cation channel enriched in microglia, and it triggers exosome secretion. A study was conducted to examine the therapeutic effects of a CNS penetrant P2RX7 inhibitor, GSK1482160, on the early disease stage of P301S tau transgenic mice [25]. GSK1482160 was previously entered into clinical trials as a treatment for inflammatory pain, however, no results have been published [26]. GSK1482160 seemed to have no effect on P301S tau transgene expression, but it did significantly reduce pS199 tau levels in the hippocampal—but not cortical—regions of the mice. pT181 and pT231 tau levels were unchanged. Oral GSK1482160 treatment also significantly reduced Alz50⁺ misfolded tau, along with MC1⁺ misfolded tau, in pyramidal cell bodies and neuropils in neuronal cell body and dendrites of hippocampal and EC regions [25]. Tsg101 is a specific marker of ESCRT-I that is involved in exosome synthesis. GSK1482160 reduction of Alz50⁺ and MC1⁺ misfolded tau was coupled with decreased Tsg101 accumulation in the CA1, GCL and EC layer II neurons in P301S mice. This concomitant reduction highlighted the implied role of exosomes in tau propagation— GSK1482160-induced suppression suggests that exosomes are the method by which P2RX7 regulates pathological tau propagation to hippocampal neurons [25]. It was also observed that extracellular vesicle (EV) secretion can be stimulated from neurons and astrocytes through ATP, but EV secretion is not affected by P2RX7 inhibition. GSK1482160, however, significantly and dose-dependently suppressed exosome secretion from h-Tay-phagocytosed microglia. A striking observation confirming GSK1482160's effects was that the inhibitor-treated mice displayed improved working and contextual memory through Y-maze and fear conditioning tests [25].

PE859

Another orally administered compound, tested on JNPL3 P301L-mutated human tau transgenic mice, is PE859. PE859 (3-[(1E)-2-(1H-indol-6-yl)ethenyl]-5-[(1E)-2-[2-methoxy-4-(2-pyridylmethoxy)phenyl]ethenyl]-1H-pyrazole) is a novel compound developed to inhibit tau aggregation. PE859 inhibited heparin-induced tau aggregation of 3RMBD (includes only 3 repeat domain, excludes N-terminal, second microtubule-binding domain, and C-terminal), along with full-length tau in a dose-dependent manner [27]. For the 3RMBD, the IC_{50} value was 0.81 μ M and 2.33 μ M for the full-length tau. After 1h incubation, ThT fluorescence intensity drastically decreased and was inhibited, reaching a plateau after 10h. PE859 inhibits aggregation through beta-sheet structure formation [27]. It also was determined that PE859 can cross through the blood-brain barrier and can be distributed into the tissue of the CNS (this was determined by calculating the time-dependent change of PE859 concentration in the blood and the brain). The mice were subject to the rotarod test, along with consistent hind-leg checkups. The observations indicated that the compound

was able to delay motor dysfunction in the JNPL3 mice [27]. Both sarkosyl-insoluble tau (sarkosyl is an ionic detergent that solubilize protein in brain tissue that are in the native foldings [28]) and sarkosyl-soluble tau saw a significant decrease in amount between the vehicle and the PE859 group; the insoluble tau being the most obvious of the two. However, there was no notable difference for tris-soluble tau. Thus, it was concluded that PE859 delayed motor dysfunction through accumulation inhibition of sarkosyl-insoluble tau [27].

Degradation

Ubiquitin-Proteasome System

A promising degradation mechanism of pathological tau is related to the UPS— ubiquitin-proteasome system. The UPS is the pathway in which damaged and misfolded proteins in the nucleus and cytoplasm are removed. The first principal step is ubiquitylation, in which ubiquitin is covalently attached to a substrate protein. The second step is proteasomal degradation where the proteasome unfolds substrates and inserts polypeptide chains through the inner channel, where they cleave into short peptides. After release, the cellular aminopeptidases process the peptides into amino acids and then recycle them [29]. Reportedly, the UPS is downregulated in the cases of neurodegenerative disorders [30]. It is implicated in such diseases due to the existence of deposits comprising ubiquitinated proteins in affected neurons. Aggregation-prone proteins associated with neurodegenerative disorders causing UPS downregulation delay the degradation of proteasome substrates. Due to such delay, cell signaling, cell-cycle progression, apoptosis, etc., all are compromised. Thus, the malfunctioning UPS system may be the cause of cellular demise in neurodegenerative disorders [29]. Another strong factor related to the UPS to consider is the proteasome-associated deubiquitinating enzyme (DUB) USP14, which works to regulate the UPS. USP14 disassembles the ubiquitin (Ub) tag on the substrate, causing a delay or inhibition of degradation because of the weakened interaction between the Ub proteasome receptors and polyUb substrate chains. Substrate deubiquitination by USP14 can be a very fast process, and without the Ub tag, the substrate can be released prematurely, preventing the sometimes essential degradation of misfolded and abnormal proteins [30], [31]. Additionally, there seems to be some evidence, although not completely clear, that USP14 suppresses autophagy along with the UPS [32].

USP-1, USP14-2, USP14-3

Three specific RNA aptamers, USP14-1, USP14-2, and USP14-3, were identified that have inhibitory effects on USP14, causing the degradation of tau. Aptamers are single-stranded nucleic acid molecules sequenced through systematic evolution of ligands by exponential enrichment (SELEX) [30]. The aptamers can bind to multiple different targets with high specificity, along with repressing enzymatic activity of proteins. The study's results suggested that the aptamers' GGAGG and G-rich sequences are significant for USP14 interactions. USP14 RNA aptamers showed significant inhibition of proteasomal DUBs, with USP14-3 being the most potent but also most selective to USP14. With an *in vitro* study, 70% of Ub-Sic1 proteins were degraded in the control reaction [30]. With the addition of the aptamers, almost all of the proteins were degraded by proteasomes, with similar kinetics for such degradation as USP14(C114A), a catalytically inactive mutant version of USP14. The effect of the aptamers on tau degradation was tested on HEK293-derived cell line expressing the htau40 isoform. The cells also expressed SDS-resistant tau. When USP14-3 was transfected into the cells, tau level decreased in a dose-dependent manner, with up to a 25% decrease. It also reduces cytotoxicity— the USP14 aptamers exhibited no significant cytotoxicity at concentrations up to 100 µg/mL— and fairly preserved cell viability. However, the exact mechanism of aptamer inhibition is still unknown [30].

IU1-47

Another already existing small-molecule inhibitor of USP14 is IU1, and it can increase degradation rates of substrates. A novel compound, IU1-47 was synthesized after examining various IU1 analogs, and IU1-47 was found to be 10-fold more potent than IU1 [31]. Increasing potency seemed to have a relation with increased lipophilicity from fluorine

to chlorine, along with piperidine having more potency than pyrrolidine. *In vitro*, IU1-47 was able to block USP14's rapid deubiquitination of Sic1 (which almost reached an endpoint at 2 minutes, without the presence of IU1-47). IU1-47 treatment in wild-type mouse embryonic fibroblasts (MEFs) showed that the compound can stimulate tau degradation [31]. Notably, IU1-47 did not have any effect on USP14-lacking MEFs, showing that USP14 inhibition is the key to IU1-47-induced degradation. Cortical primary neuronal cultures were transfected with wild-type human tau, and *in vitro*, the cells were treated with IU1-47 and/or MG132, a proteasome inhibitor. IU1-47 cells depicted reduced tau levels, whereas the effect was switched with the presence of MG132, again clarifying that IU1-47 degradation occurs through the UPS [31]. Hippocampal primary neurons, along with cortical neurons, were transfected with various tau forms. IU1-47 was shown to increase wild-type tau, mutants P301L and P301S tau, and the A152T tau variant rates of degradation, highlighting its ability to degrade phosphorylated tau. The compound worked dose-dependently, decreased tau levels almost by 80% in some cases. Additionally, cell viability with IU1-47 was measured in an MTT assay, and the study concluded that IU1-47 was well-tolerated in MEFs [31]. This is an especially noteworthy due to the previous study mentioned seeing results that indicate IU1 (not IU1-47) being significantly cytotoxic for cells starting at 50 µg/mL [30].

Autophagy-Lysosomal Pathway

Similar to the UPS, another pathological hallmark of tauopathy is autophagy-lysosomal pathway (ALP) malfunction. The ALP is a mechanism that, like the UPS, is responsible for the degradation of intracellular macromolecules [33]. mTOR is a serine/threonine kinase that has a vital role in regulating cell autophagy. mTOR inhibits autophagy by phosphorylation-dependent suppression of ULK1/2 and the VPS34 complex, along with utilizing TFEB phosphorylation to intercept expression of lysosomal and autophagy genes [34].

Rapamycin

Rapamycin is an FDA approved drug which inhibits mTORC1, thus allowing the formation of autophagosomes and the stimulation of the ALP. A study was conducted using P301S tau transgenic mice in order to confirm the effects of rapamycin on tau aggregation. The study consisted of a group of long-term rapamycin treated mice and a group of vehicle treated mice [35]. Rapamycin was detected in the brain after administration, confirming that it is able to pass the blood-brain barrier. Along with that, there was an increase in LC3II by 229% in the rapamycin treated P301S mice, suggesting the increased accumulation of autophagosomes as related to the ALP. There was a large reduction in cortical tangles in the rapamycin treated group compared with the vehicle group, along with Gallyas tangle count reduction and cortical tau hyperphosphorylation reduction [35]. Short-term treatment also had a significant decrease in cortical tangles and in tau hyperphosphorylation at the AT8 epitope. There was also noteworthy minimization of sarkosyl insoluble tau in the forebrain of both long and short term mice treatment groups. Finally, hyperphosphorylated tau at the AT8 and AT100 epitopes also noticeably decreased. However, there was no detectable decrease of forebrain soluble tau in the mice along with no decrease in soluble tau protein generation [35].

Rapamycin is currently in clinical trials for reducing measures of mild cognitive impairment and Alzheimer's [36]. However, as it is ongoing, no results are published.

OSI-027, AZD2014, AZD8055

Another recent study has identified three mTOR inhibitor compounds suggested to be more potent than rapamycin—OSI-027, AZD2014 and AZD8055. The inhibitors exhibited increased autophagy activation strengths through increased vesicle numbers compared to rapamycin [37]. FTD patient-derived neurons that expressed tau-A152T or P301L were used to effect tau burden. In the A152T cells, the compounds reduced tau concentrations more than rapamycin, with ~80% downregulation of tau and p-tau for OSI-027 and AZD2014, and ~60% for AZD8055, contrasting rapamycin which achieved only ~40%. The EC₅₀ concentration was 100nM for AZD2014 and AZD8055, and the EC₅₀ was 1 µM for OSI-027. 24h treatment of the neurons did not induce any decrease in cell viability besides for a ~20% decrease with AZD8055 at 30 µM, while rapamycin became toxic at conditions equal to or greater than 5 µM.

For the P301L neurons, the compounds reached maximum tau reduction at 5 μ M, accompanied with 60-70% tau reduction [37]. Rapamycin once again proved to be less potent by only reducing tau by 50%. As for P301L neuronal viability, the compounds had no effect, with the exception of rapamycin at greater than or equal to 5 μ M causing a 40% viability decrease. The three inhibitor compounds also were shown to be 60-90% more effective in downregulating insoluble tau compared to soluble tau [37]. The compounds also generated an increase in LC3-II by threefold, ATG12/ATG5 by twofold, and lysosomal LAMP1 by fourfold. After the mTOR inhibitor treatment, in A152T cells, lysosomes and phagolysosomes were found enriched with tau and P-tau S396, relative to the cytosol [37]. In cell media, exosome presence was verified through observing exosome membrane proteins CD9 and CD81. In the p301L cells, with AZD2014 or OSI-027 treatment, there was a major presence of tau in phagolysosomes. The increase of autophagy components corroborates the implication that the inhibitors reduce tau through the ALP [37]. Finally, the mTOR inhibitors induced tau degradation at 24h and the effect continued for 12-16 days, with the first 4 days post-treatment suggesting an increase in neuronal viability and no decrease in viability for the days after [37].

Methylthioninium Chloride

Methylthioninium Chloride, also known as Methylene Blue (MB), is one of the more well-founded and promising therapeutic out of the existing pool. MB was found to induce autophagy as its main mechanism in tau aggregation inhibition, through brain slice cultures from mouse model FTD and a transgenic mouse model with tauopathy, all treated with MB [38]. In the slice cultures, MB managed to decrease sarkosyl insoluble tau to $87.2 \pm 0.94\%$ of control values, and phosphorylated tau to a range between 76.9 and 85.6% of ser199, ser262, ser422, and ser396/404. Treatment of a transgenic mouse model yielded significant decrease in overall tau, however, the effects on sarkosyl insoluble tau levels were insignificant [38]. MB also managed to decrease levels of p62 ($79 \pm 1.5\%$ of control), an autophagy marker whose degradation indicates that autophagy has been stimulated. Cathepsin D ($122 \pm 5.8\%$ of control), BECN1 ($131.9 \pm 10\%$ of control), and LC3-II ($139.41 \pm 10.9\%$ of control) are all markers where MB incited an increase of their presence [38]. p62 is involved with directing ubiquitinated proteins to autophagic vacuoles, part of the previously mentioned ubiquitin-proteasome system, while Cathepsin D is a prime lysosomal protease in charge of protein degradation, part of the autophagy-lysosomal pathway mentioned above [39], [40]. BECN1 is involved in autophagic vesicle formation, and LC3-II can be found in the autophagosome membrane [38]. Thus, the increase of all these markers signified autophagy induction and hinted at implications that MB acts on upregulation of not only the UPS, but also the ALP. Additionally, MB was shown to promote vacuole formation through observance of increased LC3 (a central protein in autophagosome biogenesis) positive puncta, and blocking autophagy rescinded MB effects on tau, confirming its role in aggregation inhibition through autophagy induction [38]. Another study describes how MB decreased hyperphosphorylated tau and astrogliosis through upregulating nrf2 genes and reducing oxidative stress, along with improving behavioral deficits in P301S mice [41]. The effects of MB were recapitulated in one more MB study done on full-length pro-aggregant human Tau transgenic mice, which suggested that applying MB in a preventative way, before functional deficits begin, can preserve the cognition of the transgenic mice [42].

MB has various compounds and analogs derived from it, and some of them were even tested for clinical use, such as TRx0237. TRx0237 managed to reach Phase III trials, however it did not show any efficacy at the Phase III level [43]. A recent study conducted in response to the Phase III clinical trial results suggests a different characteristic of MB—while reducing the number of tau fibrils, MB also seems to increase the presence of granular tau oligomers [44]. With supporting data that oligomer formation is essential for neuronal death [45], the study concluded that the increase of oligomers negated the effect of tau fibril reduction. [44]. So, another key point of study may be ensuring that compounds focus more on oligomer prevention or degradation along with, or rather than, fibril reduction.

Discussion

The current research on therapeutics for tauopathies has made notable progress and has immense potential. Further development of therapies would benefit from a deeper understanding of the exact mechanisms leading to pathological

tau, tau propagation, and the role of extracellular tau. Finding parallels to other proteins with unconventional secretions that are better understood, compounds that mask binding sites on tau, and cell surface receptors that interact and interfere with internalization of pathological tau are all potential targets for therapy development.

As tauopathy itself is a multidimensional disease topic, therapeutic efforts also take on a variety of approaches. Such approaches include focuses on kinase inhibition, enzyme inhibition, and direct aggregation inhibition (i.e., R-(-)-apomorphine). Certain drugs originally used for treating other illnesses, such as raloxifene, can also be found to apply to tauopathies, based on studies conducted through hyperphosphorylated tau-based assays. However, even though such drugs are confirmed to reduce aggregation, not much is understood on the specifics of how the compounds function on a molecular scale. These specifics are a key point of information that can open the doors to creating more potent and effective analogs to be developed as therapeutics.

Inhibition of kinases implicated in tauopathy generation seems to be a promising approach for therapeutics. Inhibition of complexes triggering exosome secretion have been reported to be effective, however, the effects of exosome secretion inhibition on the rest of the cell and cell viability has to be thoroughly studied and the inhibitors must be able to have precise specificity so as to not interfere with other cellular functions. Aggregation, kinase, and exosome secretion inhibition are all perspectives that exhibit potential for tauopathy therapeutics.

The other approach is upregulating protein degradation systems that are normally compromised or malfunctioning in tauopathies, the UPS and ALP. Out of all mentioned anti-aggregation compounds, those related to preserving and enhancing protein degradation system function seem to be the most promising because the presented compounds are all shown to preserve cell viability and seem to be more understood in terms of mechanisms of pathological tau downregulation. That is, the compounds have a clear target, and by focusing on degradation rather than mitigation of proliferation, there may be a stronger therapeutic effect on tauopathies. Additionally, the compound MB, which functions through upregulation of degradation, has even reached the point of Phase III clinical trials. However, the stipulation on MB's derivatives and similarly functioning compounds is that further research should focus on tau oligomers along with tau fibrils to ensure that there is a reduction or inhibition of both facets—rather than an increase of one and decrease of the other, causing the overall negation of the compound's inhibitory effects. Out of all the compounds and inhibitors presented, OSI-027, AZD2014 and AZD8055 appear to have the highest potential. These compounds are most attractive due to their ability to considerably degrade tau while preserving and even increasing cell viability, along with the ability to preserve the effect for multiple days, clearly showing solid potential for therapeutic development. Plus, specific and reasonable evidence is set forth to validate the methods by which the mTOR inhibitors reduce tau in two different neuronal cell cultures by confirming action by observing increased amounts of ALP components.

Overall, continued research on the molecular processes such as tau propagation, post-translational modifications causing pathological tau, method of inhibition itself, etc., could allow for development of favorable therapeutics and further insight into various areas of neurology and beyond. Despite there being much left to understand and uncover, considerable progress has been made in regards to therapeutics for tauopathies, reinforcing the potential to alleviate this issue that burdens millions of people.

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