

Photo-excited Toluidine Blue Disaggregates the Repeat Tau and Modulates End-binding Protein EB1, Cytoskeletal Structure in Neuronal Cells

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Research

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Abstract

Background

Alzheimer's disease is a progressive neurological disorder characterized by the intracellular accumulation of Tau protein aggregates. Inhibition of protein aggregation by photo-excited dyes is emerging as a novel strategy for the treatment of certain diseases. Toluidine Blue is a basic phenothiazine dye having the potency of photo-excitation by irradiation with a red light at 630 ± 20 nm. In the present work, we studied the effect of Toluidine Blue and photo-excited Toluidine Blue on the aggregation of repeat Tau using *in-vitro* assays.

Results

Results showed that Toluidine Blue efficiently inhibited the formation of higher-order aggregates which was evidenced by Thioflavin S fluorescence assay, SDS-PAGE and electron microscopy. Moreover, the photo-excited Toluidine Blue led to disaggregation of the mature repeat Tau fibrils, which were irradiated in the dark chamber customized in our lab. Further, studies on the effect of Toluidine blue on cell viability in Neuro2a cells using MTT assay showed that Toluidine Blue was not toxic to neuronal cells at lower concentrations but at high concentrations ($> 5 \mu\text{M}$) both Toluidine Blue and photo-excited Toluidine Blue induced significant toxicity. Immunofluorescence studies on the cytoskeleton of Neuro2a cells show that Toluidine Blue and photo-excited Toluidine Blue treatment at a non-toxic concentration of $0.5 \mu\text{M}$ stimulated the formation of actin-rich lamellipodia and filopodia structures. Tubulin networks were also differentially modulated after the treatment of Toluidine Blue and photo-excited Toluidine Blue. End-binding protein 1 (EB1) levels were observed to increase after Toluidine Blue and photo-excited Toluidine Blue treatment indicating the accelerated microtubule polymerization. The overall study suggested that Toluidine Blue inhibited the aggregation of soluble Tau and photo-excited Toluidine Blue disaggregated the pre-formed Tau filaments.

Conclusions

In our study, TB and PE-TB were observed to be potent against Tau aggregation. We observed a distinctive modulation of actin, tubulin networks and EB1 levels after TB and PE-TB treatment, which clearly suggested that TB and PE-TB have potency against the cytoskeleton deformities. Thus, from the present work, we are stating that TB could be a potent molecule against Tauopathy.

Background

Alzheimer's disease (AD) is characterized by accumulation of neurofibrillary tangles (NFTs), which are aggregates of intracellular Tau [1, 2]. The aggregated Tau has been reported to be involved in several other neurodegenerative diseases including Pick's disease, corticobasal degeneration, progressive supranuclear palsy, post-encephalitic parkinsonism *etc.*, which are termed as Tauopathies [3, 4]. Tau is a natively unfolded cytoskeleton-associated protein having the physiological role of stabilizing

microtubules and cytoskeleton structures [5]. Neurodegenerative diseases have been reported to be closely associated with cytoskeleton abnormalities in neuronal cells involving the actin and tubulin deformities [6, 7]. Actin and tubulin are abundant cytoskeleton proteins. (Bray & Gilbert 1981). Tubulin is known to involve in the formation of microtubules whereas, actin majorly assists the cells in substratum adhesion, synapse formation and cell motility by the formation of structures as lamellipodia, filopodia, podosomes *etc* [8, 9]. End-binding protein 1 (EB1) is another class of cytoskeletal associated protein, which has been reported to be located at the growing end of microtubules [10]. Among all these cytoskeleton proteins microtubule-associated protein Tau and its aggregates are majorly focused in the context of generation of neuronal abnormalities. Tau protein has a domain organization comprising of a projection domain and microtubule-binding domain. Microtubule-binding domain of Tau has 4 repeat region, which is considered as the aggregation prone region of Tau (Gustke *et al.* 1994)The hexapeptide VQIINK and VQIVYK present in the 2nd and 3rd repeat of Tau respectively, are reported to be involved in Tau aggregation [11, 12]. To study the aggregation inhibition propensity of certain molecules against Tau, the repeat region of Tau has been targeted [13, 14]. Several compounds of natural and synthetic origin have been studied against Tau aggregation [15, 16]. These compounds can be categorized in two groups based on their mode of action, firstly disaggregation of the mature Tau fibrils and the other by inhibition of the aggregation of Tau as reported for anthraquinones and EGCG respectively [17]. Several dyes such as methylene blue, Toluidine blue (TB) and rose bengal have been reported to modulate Amyloid- β peptides aggregation which is the other hallmark protein of AD [17-19]. Phthalocyanine dye has been reported to be effective against pathological prion protein (PrPC) [20]. Moreover, photo-active chlorin 6 dye was also found to reduce the aggregation by modulating the histidine residues in which protein (Amyloid- β)? [21]. Methylene blue derived Leuco-methylthionium Bis (Hydromehanesulphonate) (LMTM) entered phase-3 clinical trial for the treatment of mild AD [22] however, LMTM in further studies was found to be ineffective [23]. TB is a phenothiazine dye having structural similarity with methylene blue. The basic dye TB has been reported for its application in histology. TB has an affinity for nucleic acid and it majorly binds to tissues rich in DNA and RNA. TB has been widely applied for vital staining which facilitates the detection of early malignant lesions [24]. Several studies have revealed the anti-bacterial and anti-biofilm properties of TB but, the role of TB in neurodegeneration has not been explored extensively. In the present study, we evaluated the potency of TB against repeat Tau aggregation. Furthermore, the effect of TB in the presence of irradiation was also studied in disaggregating the matured fibrillary aggregates of repeat Tau. In the present study, the potency of TB and PE-TB against Tauopathy was studied in various aspects.

Results

TB inhibit the Tau aggregation *in-vitro*

The four repeat region in Tau is considered as aggregation-prone, VQIINK and VQIVYK hexapeptides present in second and third repeat majorly contribute to the aggregation propensity of Tau. The repeat Tau has a basic charge with an isoelectric pH of 9.6 (Fig. 1A). TB is a basic dye belonging to the

phenothiazine group of compounds (Fig. 1B). The aggregation inhibition property of TB was studied against heparin-induced Tau aggregation. The fluorescence studies suggested that TB efficiently inhibited Tau aggregation in a concentration-dependent manner. The inhibition was observed at a concentration of 2 μM and the rate of inhibition increased proportionally with the concentration of TB (Fig. 1C). 40 μM of TB found to inhibit 80% of aggregation ($p \geq 0.001$), suggesting TB as a potent molecule against Tau aggregation (Fig. 1D). The electron micrograph of TB treated Tau showed broken and fragile fragment unlikely of the untreated sample, comprising of long and thick filamentous Tau aggregates (Fig. 1E-F).

Photo-excited TB dissolved the matured Tau filaments

TB has an absorption maxima of 630 nm, the irradiation of TB at 630 ± 20 nm leads to the conversion of TB to photo-excited form, which generates singlet oxygen species (Fig. 2A). In our study, we exposed TB under a red LED light source for 180 minutes for photo-excitation with an irradiance of 9.9×10^6 Watt/m². The Tau treated with PE-TB showed no higher-ordered aggregates, which was evidenced by SDS-PAGE (Fig. 2B). The electron microscopy images support the observation that PE-TB efficiently disaggregated the mature repeat Tau fibrils (Fig. 2C-D). Furthermore, ThS assay was carried out for evaluating the disaggregation potency of PE-TB. These results suggested that in comparison to untreated aggregates fluorescence intensity was decreased in PE-TB treated aggregates samples (Fig 2 E). In SDS-PAGE we did not observe any protein bands in PE-TB treated samples however, we observe decreased fluorescence in PE-TB treated samples which may be due to the small peptides which were not visible on SDS-PAGE. Additionally, in our experiments, negligible heat changes were observed after 180 minutes of irradiation. These *in-vitro* experiments indicated that PE-TB to be an effective molecule against repeat Tau aggregates.

The cytotoxicity of higher concentration of TB

The current experiment aimed to study the toxicity for TB and PE-TB against mouse neuroblastoma cells, Neuro2a. The cells were treated with varying concentrations of TB (1-100 μM) for 24 hours. Additionally, TB incubated cells were irradiated with 630 ± 20 nm red light for 10 min with an irradiance of 5.5×10^5 Watt/m². These results suggested that TB-induced low levels of toxicity than PE-TB. TB found to have minimal toxicity at a concentration of 40 μM whereas a concentration of 120 μM induced-toxicity. However, PE-TB was found to be toxic at a concentration of 20 μM (Fig. 3A-B). We speculate that the singlet oxygen produced by PE-TB might lead to the generation of toxicity. Furthermore at a concentration of 80 μM and above TB was observed to be internalized in neuronal cells (Fig. 3C). Here we speculate that TB was able to cross the cell membrane easily and thus the accumulation of higher concentration of TB leads to toxicity. The overall results suggested that TB has low toxicity even at higher concentrations.

Modulation of cytoskeleton by PE-TB

Tubulin is a basic unit of cytoskeleton, which polymerizes leading to the formation of microtubules. The effect of PE-TB on cytoskeleton was observed by immunofluorescence studies. Neuro2a cells were

incubated for 24 hours with varying concentrations of PE-TB (0.5 and 50 μM). The immunofluorescence studies suggested that as compared to untreated cell control, cells treated with 0.5 μM PE-TB have high fluorescence intensity of tubulin, whereas no differential results were observed in cells treated with 50 μM of PE-TB treatment (Fig. 4A-B). The quantification of fluorescence images suggested a minimal increase in tubulin intensity. Tubulin is the basic unit of microtubules thus, the modulation of tubulin intensity suggested modification of cytoskeleton after PE-TB treatment (Fig. 4C). Although the morphological changes in the cell after PE-TB treatment was not appreciably significant minimal changes in elongated dendritic extensions were observed in treated cells (Fig. 4D). Moreover, to analyse the effect of TB and PE-TB on cytoskeleton we analysed the actin modulation (Fig. 5A). Our studies indicated that TB treatment induces actin structure in cells, as the cells were observed to have actin-rich “Lamellipodia” structures after TB exposure. We also observed the elevated numbers of fine hair-like protrusions or “Filopodia” in cells. While the cells treated with PE-TB observed to have lamellipodia structures, on contrary filopodia structures were not apparent after PE-TB treatment (Fig. 5B). Furthermore, we studied the End-binding protein 1 (EB1) expression in neurons. These results suggested that TB and PE-TB treatment enhance the levels of EB1, which suggest that TB and PE-TB treatment might accelerate the microtubule polymerisation in cells (Fig. 6A-B). The elevated levels of EB1 indicated that there could be an increased rate of microtubule polymerization after the TB and PE-TB treatment.

Discussion

The pathological state of Tau leads to the formation of Tau aggregates, several factors including post-translational modification, reactive oxygen species and mutations result in the generation of Tauopathies [3, 29]. Several studies have been done for screening of the compounds against Tauopathy [30]. Various classes of dyes were tested for their potency against neurodegeneration. The aggregation inhibition potency of methylene blue for Tau has been already reported illustratively. Xanthene dye such as erythrosine B was reported to reduce the Amyloid- β -mediated toxicity in cells. Photo-excited rose bengal was found potent in inhibiting the Amyloid- β aggregation in liver cells. Furthermore, the sulfonated dye Congo red was reported to attenuate the Amyloid- β aggregation. Phenothiazine class of dyes are reported to have therapeutic potency against numerous diseases [31]. Methylene blue and its derivatives have been reported to be neuroprotective molecules [32, 33]. TB is a known basic dye used in histological staining but the medicinal properties of TB have not been studied illustratively [34]. Here we have studied the efficiency of TB against Tau aggregation and our observations suggested that as its parent compound methylene blue, TB was also efficient in inhibiting the aggregation of repeat Tau. Recently researches have investigated that irradiation plays a crucial role in the treatment of AD. The intranasal red light probe, which irradiates red light on brain areas found to show significant recovery in a mouse model of AD [35]. Similarly, light-based intracranial implants have been patented for the treatment of Parkinson's disease patients [36]. Moreover, EGCG irradiated with near infra-red light found to reduce the Amyloid- β plaques deposition in neuronal cells [37]. TB is a well-reported photosensitizer, which was found efficient as an antibacterial molecule [38]. In our studies, we irradiated TB with 630 nm red light to study its effect on Tau aggregates. TB also reduced the Amyloid- β aggregation but the effect of TB was found ineffective

against Tau hyperphosphorylation [39]. We found that PE-TB potentially disaggregated the pre-formed Tau filaments. The PE-TB led to the generation of singlet oxygen species, thus we speculate that singlet oxygen contributes to the disaggregation potency of TB. The SDS-PAGE and electron microscopic studies clearly indicated the disaggregation of Tau filaments by PE-TB. ThS dye binds specifically to protein aggregates thus by tracing the ThS fluorescence, we could observe the extent of protein aggregation. In our results, the PE-TB treated aggregates showed low ThS fluorescence as compared to untreated aggregates, which indicated that PE-TB disaggregated the repeat Tau filaments. *In-vitro* studies including SDS-PAGE, electron microscopy and ThS fluorescence clearly advocated that PE-TB treatment dissolved the mature Tau filaments. The phenothiazine dye-induced low levels of toxicity in cells, but on photo-irradiation the dyes induced toxicity in cells due to the generation of singlet oxygen species [40]. In our work, we found that TB was not significantly toxic to cells even at a high concentration of 40 μ M but the PE-TB reduced the viability of cells at a concentration of 20 μ M. These results suggested that TB at lower concentrations could be considered biocompatible. The potency of photo-irradiation in modulating the cytoskeleton has been reported in human hepatoma cells [41]. The reports suggested that irradiation inhibited the cell growth by modulating the cytoskeleton. In our results, we observed that PE-TB treatment minimally increases the tubulin intensity in neuronal cells, which indicated that PE-TB could modulate the cytoskeleton structure. The actin cytoskeleton plays a crucial role in cell motility and cell synapse formation [42]. The lamellipodia are the actin-rich structures and the modulation in lamellipodia led to change in cell motility and adhesion [9]. Similarly, the modulation in filopodia structure alters the cell synapse formation and motility [43]. Thus, the alterations in actin network after TB and PE-TB treatment led us to the conclusion that the dye has the potency to modulate cell cytoskeleton networks. Hence, the overall results suggest that PE-TB could have a therapeutic potency against Tauopathy (Fig. 7).

Materials And Methods

Materials

MES (M3671), BSA (82516), BES (14853), BCA (B9643), CuSO_4 (C2284) ThS (T1892), Toluidine Blue (T3260), MTT (M2128) were purchased from Sigma. IPTG (420322) and DTT (3870) were purchased from Calbiochem. Other chemicals such as Ampicillin (2007081), NaCl (194848), KCl (194844), Na_2HPO_4 (191437), KH_2PO_4 (19142), EGTA (194823), MgCl_2 (191421), PMSF (195381), Ammonium acetate (191404), Heparin (904108) and DMSO were from purchased from MP Biomedicals and protease inhibitor cocktail was from Roche. Copper coated carbon grids were purchased from Ted Pella (01814F, carbon type-B, 400 mesh, Cu). DMEM advanced F12 media (12634010), Fetal bovine serum (16000044), Pensterc cocktail (04693159001), Anti-anti (15240062) were purchased from Gibco.

Purification of recombinant Repeat Tau

The recombinant Tau expressed in *E.coli* BL 21* cells were purified by the method suggested in earlier literature [15]. Briefly, the *E.coli* was grown in Luria-Bertani broth at 37°C, at 180 rpm in an orbital shaker (INFORS HT) till culture obtained OD_{600} of 0.5. In the log phase, the culture was induced with 0.5 mM

isopropyl β -D-1-thiogalactopyranoside (IPTG) and incubated for further 4 hours at 37 °C in an orbital shaker at 180 rpm. Further, 4 hours of post-induction incubation time was followed according to the standardized protocol[17]. The culture was pelleted down by centrifuging at 4000 rpm for 10 minutes in Avanti JXN 26. The pellets were further suspended in lysis buffer (50 mM MES, 1 mM EGTA, 2 mM $MgCl_2$, 5 mM DTT, 1 mM PMSF and 50 mM NaCl), the lysis was carried out under high pressure. The culture suspension was lysed at 15000 psi of pressure in a homogenizer (Constant cell disruption system) with a continuous lysis cycle. After 2 cycles of lysis the lysate was collected and stored on ice for reducing the protein degradation. The subsequent step of lysis is the lysate heating at 90°C for 20 min in a water bath (Benchmark) without any agitation. After heating the lysate was allowed to cool and then centrifuged at 164,700 \times g (Optima XPM ultracentrifuge, Beckman Coulter) for 45 min at 4°C. The supernatant was collected and dialyzed against buffer A. Tau is positively charged protein, hence the purification was done by cation-exchange chromatography as described in previous studies[25]. The repeat Tau was eluted by giving a linear gradient of 1 M NaCl, the fraction under the elution peak was collected and concentrated. The desired molecular weight of our protein was 13.4 kDa, hence the quality of protein was observed on 17% sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE). The concentration of protein was estimated by bicinchoninic acid assay (BCA assay) [26]. Briefly, bovine serum albumin (BSA) was diluted for making the standard graph. Tau protein was diluted in ratios of (1:100, 1:200, and 1:400). BCA reagent was prepared freshly by mixing bicinchoninic acid and $CuSO_4$ in the ratio of 4:1. After the addition of BCA to protein, the mixture was incubated at 37°C for 60 minutes in dark. The absorbance was measured at 562 nm in Tecan M 200 multimode plate reader and concentration was estimated referring to standard graph.

Preparation of repeat Tau-fibrils

The soluble repeat Tau was induced to form aggregates by incubating with heparin as suggested in the published literature[17, 27]. 100 μ M of soluble repeat Tau was incubated with 25 μ M heparin. The assembly was carried out in 20 mM BES buffer supplemented with 1 mM DTT, 25 mM NaCl, and protease inhibitor cocktail. The assembly mixture was kept at 37°C for 3 days and the aggregates were confirmed by SDS-PAGE and electron microscopy. The heterogeneous Tau aggregates were observed on SDS-PAGE as bands of higher molecular weight (25-150 kDa) [28]. Moreover, the electron microscopy images suggested the presence of long tangled Tau filaments, which supported the presence of aggregates in our sample.

ThS fluorescence assay

Thioflavin S (ThS) assay was used to monitor the assembly of Tau fibrils. The procedure was followed according to earlier published work [17]. The Tau protein was measured at a concentration of 2 μ M incubated with 8 μ M ThS dissolved in ammonium acetate. The mixture was incubated at room temperature in dark for 10 min and the fluorescence was measured at an excitation wavelength of 480 nm and emission was measured at 520 nm in Tecan M 200 multimode plate reader.

Electron microscopy

The morphological analysis of Tau fibrils was done by scanning under transmission electron microscopy. Tau was incubated at a concentration of 2 μM on 400 mesh copper-coated carbon grid for 90 seconds. After 2 subsequent washes with milliQ, the grid was incubated with 2% uranyl acetate for 120 seconds. The grids were blot dried and kept at room temperature. The scanning was done using Tecnai T20 electron microscope.

Photo-irradiation of TB

TB has an absorption maxima of 630 nm, thus it was photo-excited by a red light. For photo-excitation, a dark chamber was designed, which comprises of a commercially procured red LED light source (3.5 watts). The setup was equipped with a thermometer to measure heat changes during the irradiation. The repeat Tau aggregates were mixed with various concentrations of TB (2-40 μM). The mixture was transferred to 96 well black well plate. The plate was exposed to red light in the dark chamber for the photo-excitation of TB. After 180 minutes of irradiation, the samples were taken out from the plates and analyzed by various biochemical and biophysical assays including SDS-PAGE, ThS fluorescence assay and electron microscopy. The irradiation dose or irradiance was calculated by the formula

$$E = \frac{\text{Power}}{\text{Area}} * \text{Time of irradiation}$$

Where E is irradiance, time was calculated in terms of seconds.

Cell biology studies

Mouse neuroblastoma cells Neuro2a (ATCC: CCL-131), were cultured in advanced DMEM F12 media. 10^4 cells/well were seeded in 96 well plate for the viability assay. The cells were treated with various concentrations of TB viz, 1, 2.5, 5, 10, 20, 40, 80 and 120 μM for 24 hours. After the incubation, 10 μl of 5 mg/ml MTT solution was added in each well. The plate was further incubated at 37°C for 4 hours. The formazan crystals were dissolved in 100% DMSO and the absorbance was measured at 570 nm.

Immunofluorescence analysis

Neuro2a cells (50,000/well) were seeded on a glass coverslip and allowed to incubate for 24 hours at 37°C. The cells were treated with various concentrations of TB (0.5 and 50 μM) and irradiated for 10 minutes with a red light. After 24 hours of incubation with PE-TB, the cells were processed for immunostaining. The treated and untreated cells were fixed with absolute methanol for 20 minutes at -20°C. 0.2% Triton X-100 was used for cell permeabilization. For avoiding the non-specific binding of the antibody, the cells were incubated with 5% horse serum for 1 hour. The cells were incubated with anti-tubulin (Thermo PA1-41331; dilution 1:250) and K9JA (Dako A0024; dilution 1:500) antibody. After overnight incubation, the cells were incubated secondary antibody tagged with Alexa Fluor 488 (Thermo,

A11034) (anti-rabbit; dilution 1:1000) and Alexa Fluor 555 (Thermo, A32727) (Anti-mouse; dilution 1:500). The nucleus was stained with DAPI. After the incubation coverslips were mounted using mounting media (70% glycerol) and were sealed on a glass slide. These slides were allowed to air dry at room temperature before the scanning. These samples were scanned on Zeiss Axio observer inverted microscope using 63X magnification in oil emersion and at 40% light intensity.

Disaggregation of PE-TB

TB treated repeat Tau aggregates were irradiated with red light for 180 minutes. The PE-TB treated aggregates and untreated samples were incubated with 8 μ M of ThS dye (diluted in 50 mM ammonium acetate) for 10 minutes in dark. The samples were transferred in 384 black well plate and the fluorescence measurement was recorded on excitation of 480 and emission of 521 nm. For further analysis the PE-TB treated aggregates and untreated control aggregates were loaded on 10% SDS-PAGE. The SDS-PAGE was stained with 1% coomassie brilliant blue solution for 10 minutes, after destaining the gel was analysed for the presence of higher-order aggregated in treated and untreated samples.

Study of effect of PE-TB on cytoskeleton

The effect of TB and PE-TB on cytoskeleton was observed in Neuro2a cells. The cells were treated with 0.5 μ M TB and were irradiated for 10 minutes. The treated cells were incubated at 37°C for 24 hours and the effect on cytoskeleton modulation were monitored by immunofluorescence and western blot analysis.

Statistical Analysis

The statistical data for the fluorescence measurement were plot by using triplicate reading and the viability assay was plotted using duplicate readings. Untransformed (raw) data were analyzed and plotted by SigmaPlot 10.0 software. The data was analyzed for the significance by unpaired Student's t-test.

Abbreviations

AD: *Alzheimer's disease*

BCA: *Bicinchoninic acid*

BSA: *Bovine serum albumin*

DMSO: *Dimethyl sulfoxide*

MB: *Methylene blue*

MTT: *Methylthiazolyldiphenyl-tetrazolium bromide*

NFTs: *Neurofibrillary tangles*

PHFs: Paired helical filaments

PDT: Photodynamic therapy

PE-TB: Photo-excited TB

SEC: Size exclusion chromatography

SDS-PAGE: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

TEM: Transmission electron microscopy

TB: Toluidine blue

ThS: Thioflavin S

Declarations

Ethical approval

Not applicable. There were no animal experiments have been carried out in the current manuscript.

Consent for publication

Not applicable

Availability of data and material

All the data generated during various experiments are available from the authors on reasonable request.

Competing interests

The authors declare that they have no competing interest.

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Author Contributions

S.C designed the experiments. T.D and N.V carried out the experiments. T.D and S.C analyzed the data and wrote the manuscript. S.C. conceived the idea of the project, provided resources, supervised and wrote the manuscript. All authors contributed to the discussions and manuscript review.

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Figures

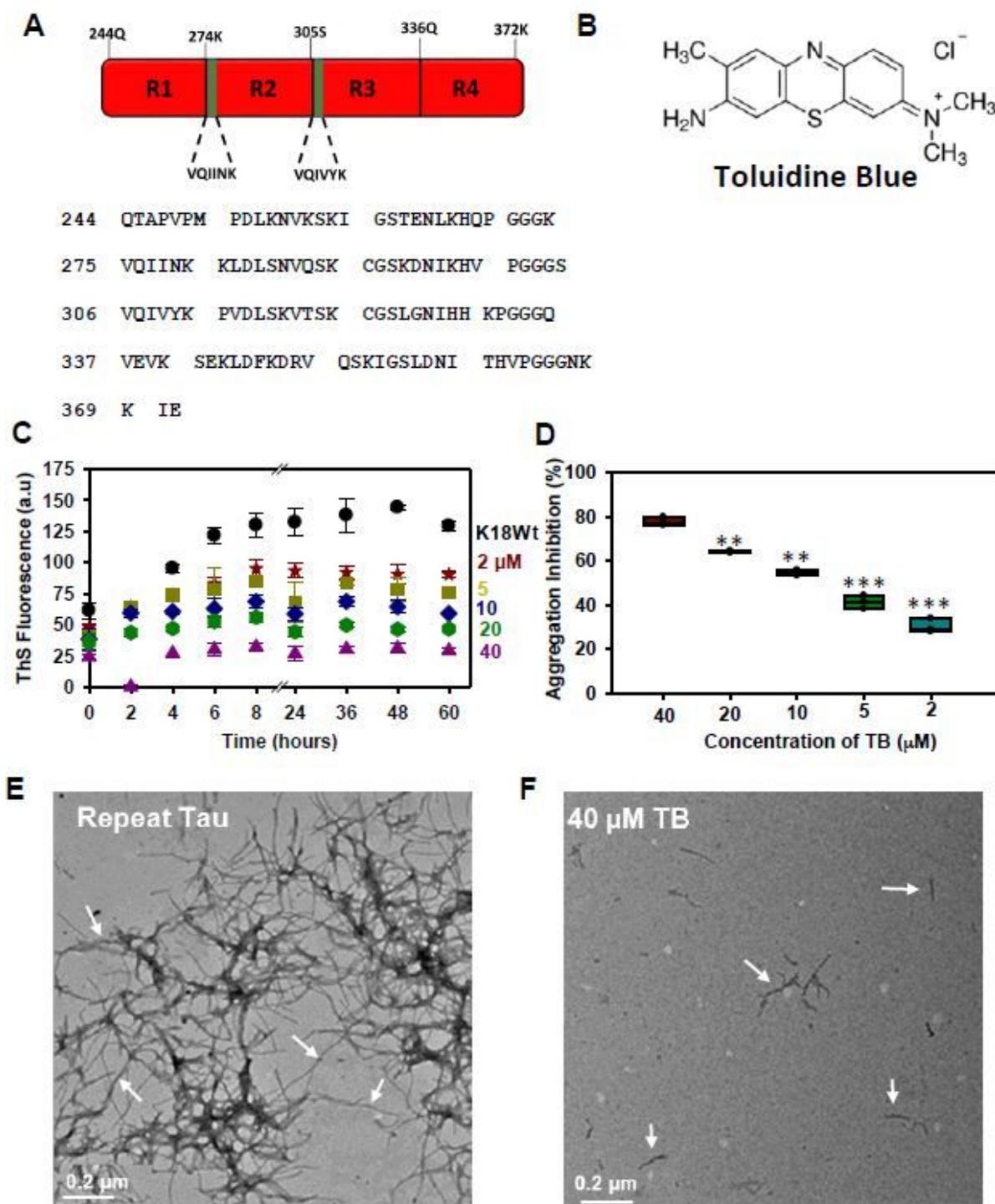


Figure 1

The aggregation propensity of repeat Tau with Toluidine Blue. A) The four repeat region of Tau is prone to aggregation. The TB is a phenothiazine dye having a basic charge. B) Aggregation of repeat Tau in presence of TB was probed by ThS fluorescence. The result suggested the aggregation inhibition of Tau in a concentration-dependent manner with respect to TB. C) The endpoint fluorescence analysis to quantify the rate of aggregation inhibition by TB in a concentration-dependent manner. E, F) The electron

microscopic analysis of aggregated Tau and TB treated Tau. The significance was calculated using unpaired Student's t-test in SigmaPlot 10.0 software. * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$, the statistical difference between control and treated groups.

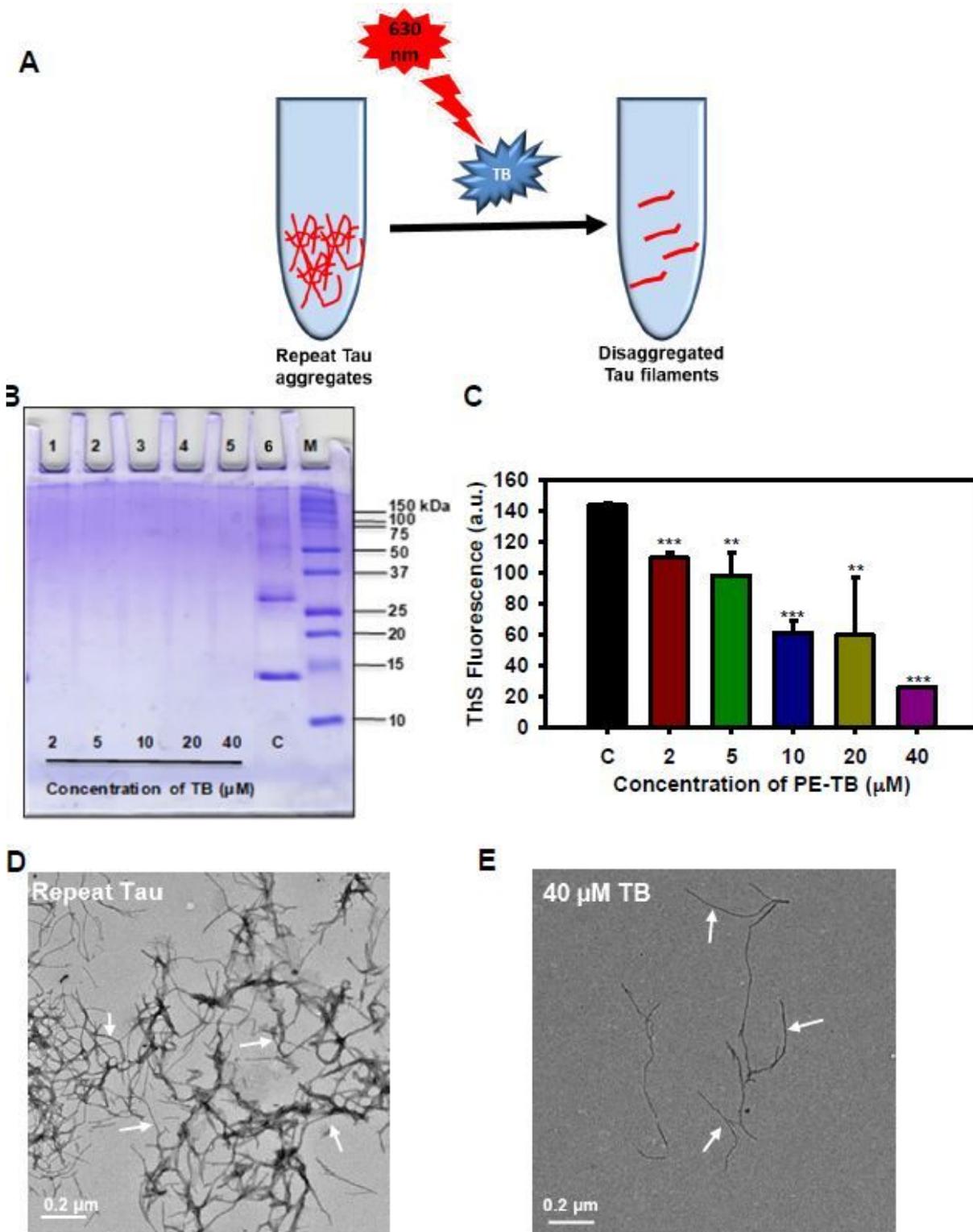


Figure 2

Effect of PDT on repeat Tau aggregates at various concentrations of photosensitizer. A) The repeat Tau aggregates were incubated with TB, which was further irradiated with 630 nm of red light. B) PE-TB (2-40

μM) on repeat Tau aggregates. The 17% SDS-PAGE showing the effect of PE-TB on higher molecular weight aggregates of repeat Tau. C) The ThS fluorescence showing the modulation in fluorescence intensity for PE-TB treated samples and untreated control. D, E) The electron microscopic images showing the morphologic changes in PE-TB treated aggregates vs. untreated control. The significance was calculated using Student's t-test in SigmaPlot 10.0 software. *p<0.05, **p<0.001, ***p<0.0001, the statistical difference between control and treated groups.

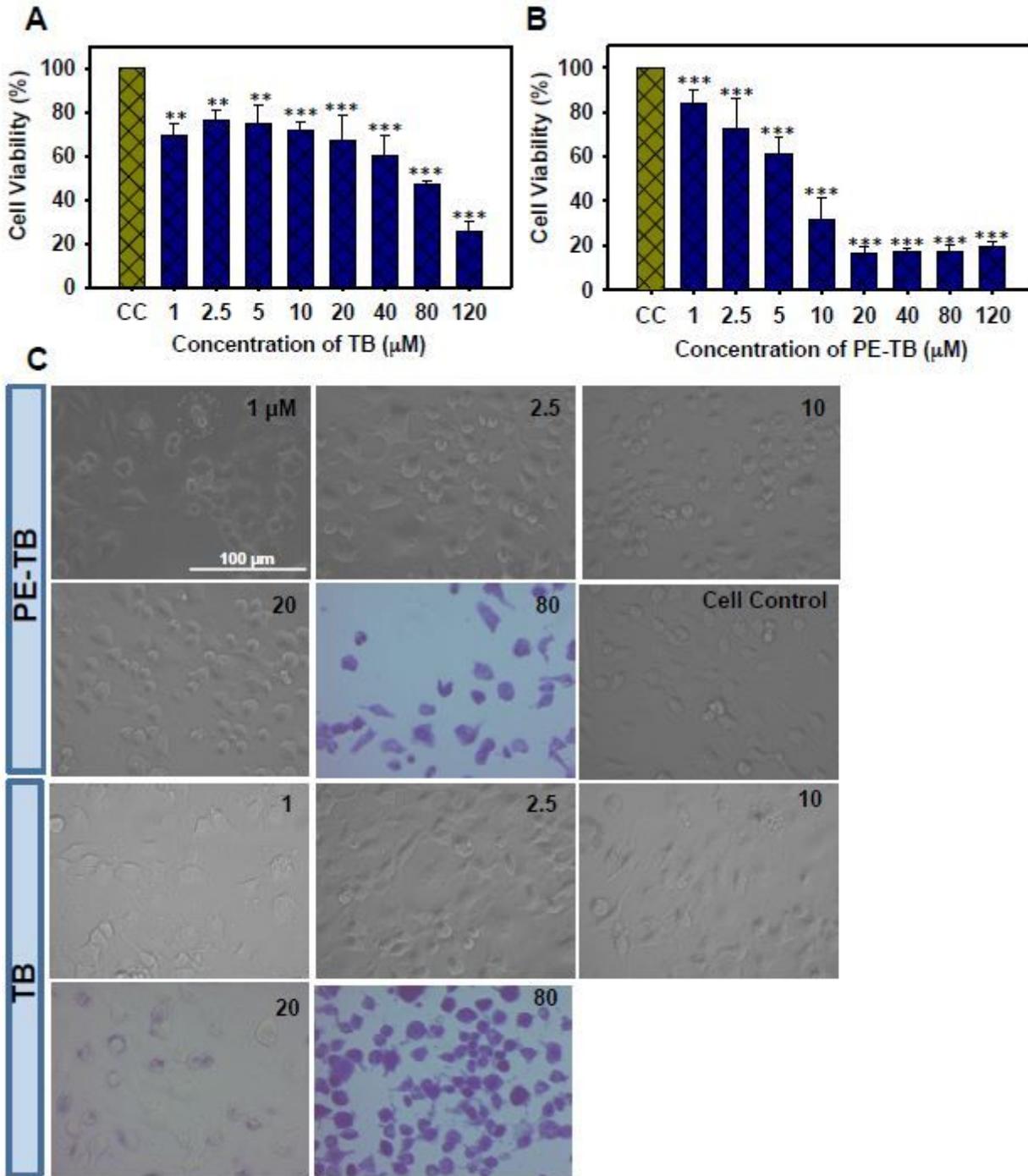


Figure 3

The effect of a higher concentration of TB was observed on Neuro2A cell viability. A) The effect of non-photo-excited TB was observed on Neuro2A cells. B) Photo-excited TB was found to be toxic at higher concentrations. C) Morphological analysis of TB treated cells. The significance was calculated using the Student's t-test in SigmaPlot 10.0 software. * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$, the statistical difference between control and treated groups.

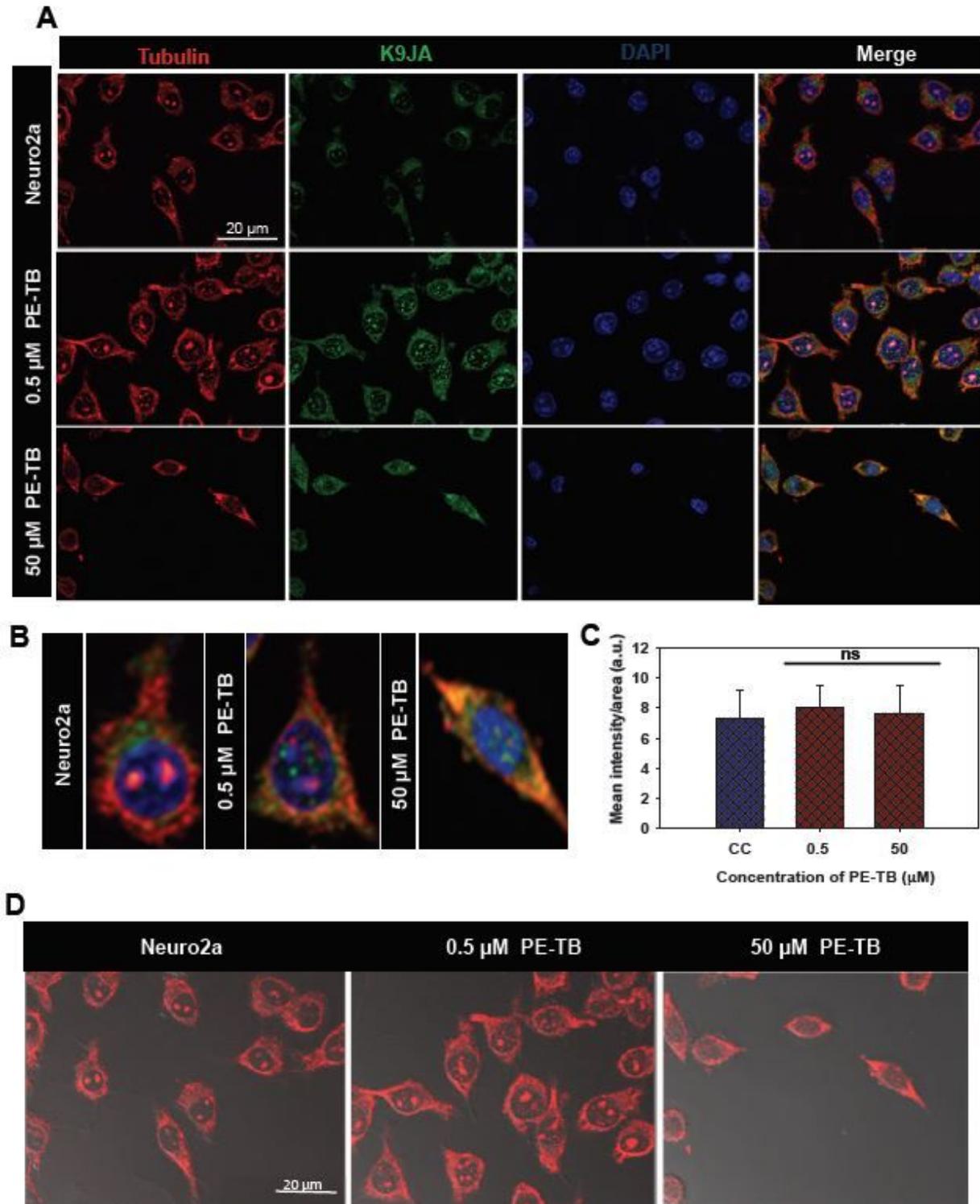


Figure 4

The effect of PE-TB on the cytoskeleton. A) The effect of PE-TB on the cytoskeleton was studied by immunofluorescence. The cells treated with various concentrations of PE-TB (0.5 μ M and 50 μ M) showed modulation in tubulin intensity. B) The single-cell images showing the differential level of tubulin in PE-TB treated cells. C) The quantification of immunofluorescence images suggested that the intensity of tubulin increased after PE-TB treatment to cells. D) Images including DIC showing the morphological changes after PE-TB treatment of Neuro2A cells.

Figure 5

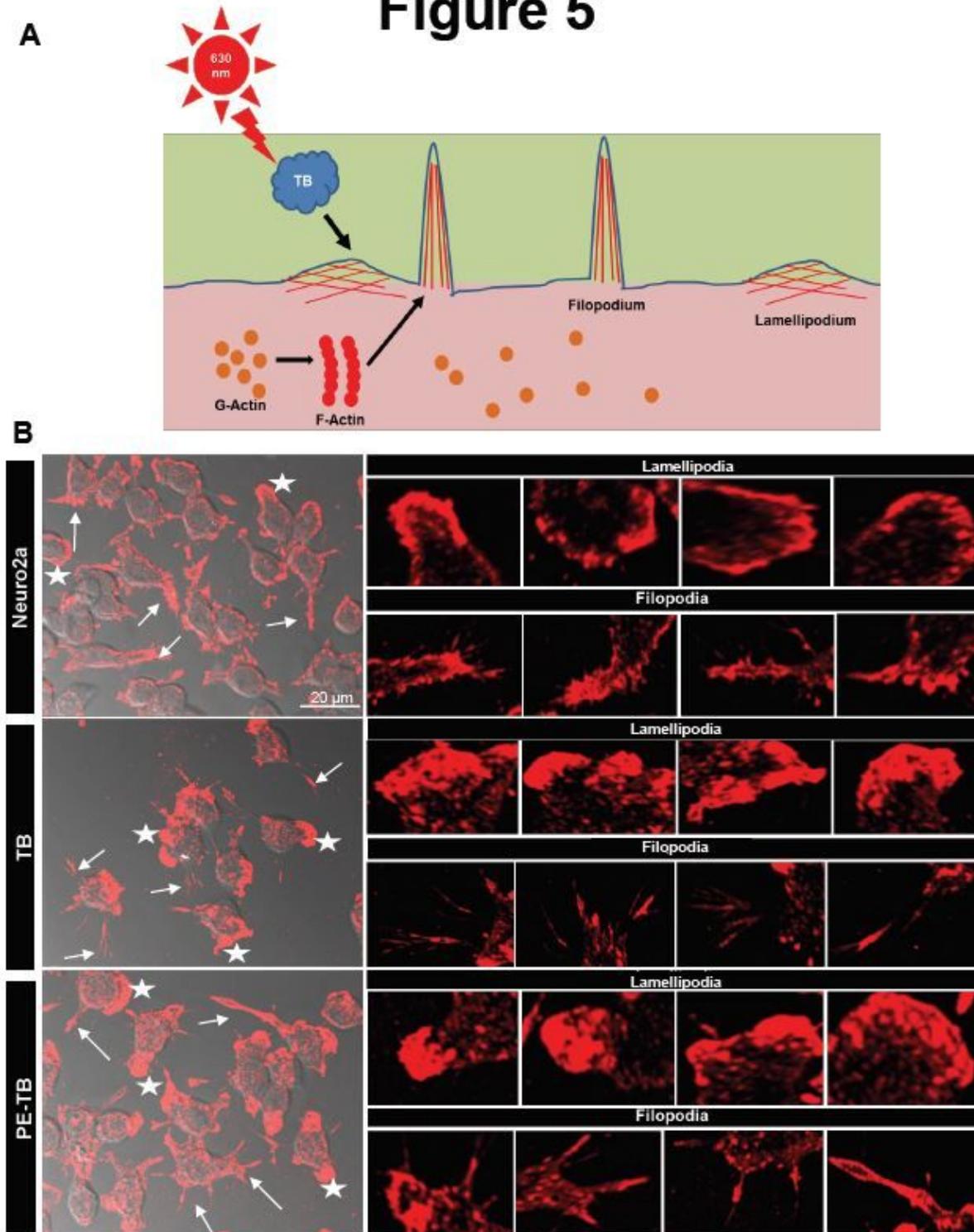


Figure 5

TB induces modulation in the cytoskeleton. A) The schematic diagram for the alteration of actin cytoskeleton after TB and PE-TB treatment. B) The immunofluorescence images showing the changes in the actin cytoskeleton, the filopodia (arrow marked) and lamellipodia (star marked) structures after TB and PE-TB treatment.

Figure 6

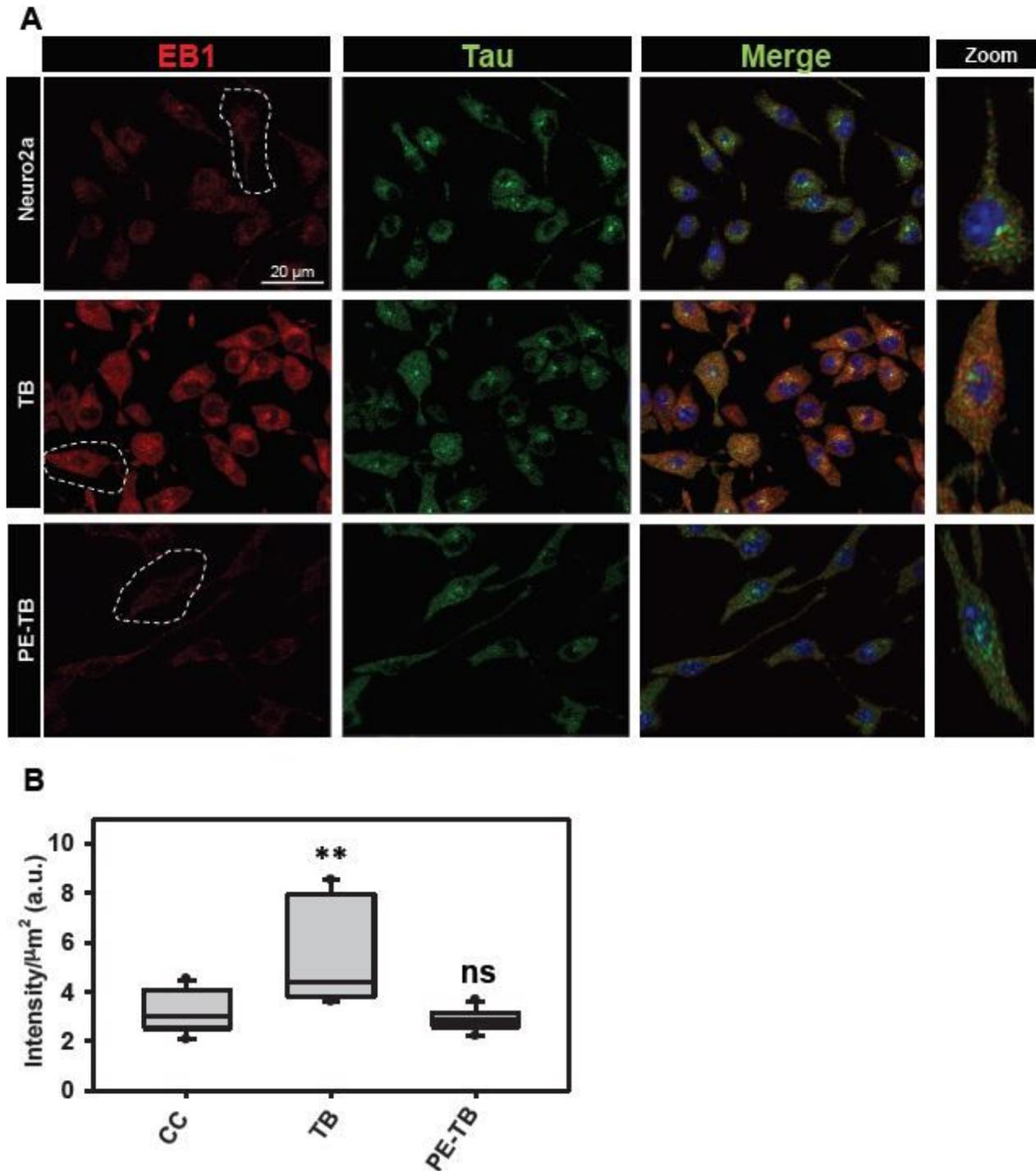


Figure 6

TB and PE-TB induce changes in EB1 expression. A) The immunofluorescence images showing the changes in EB1 levels after TB treatment. B) The quantification of immunofluorescence images suggested that TB increases the EB1 levels in cells. The quantification was done by using Zen 2.0 blue software.

Figure 7

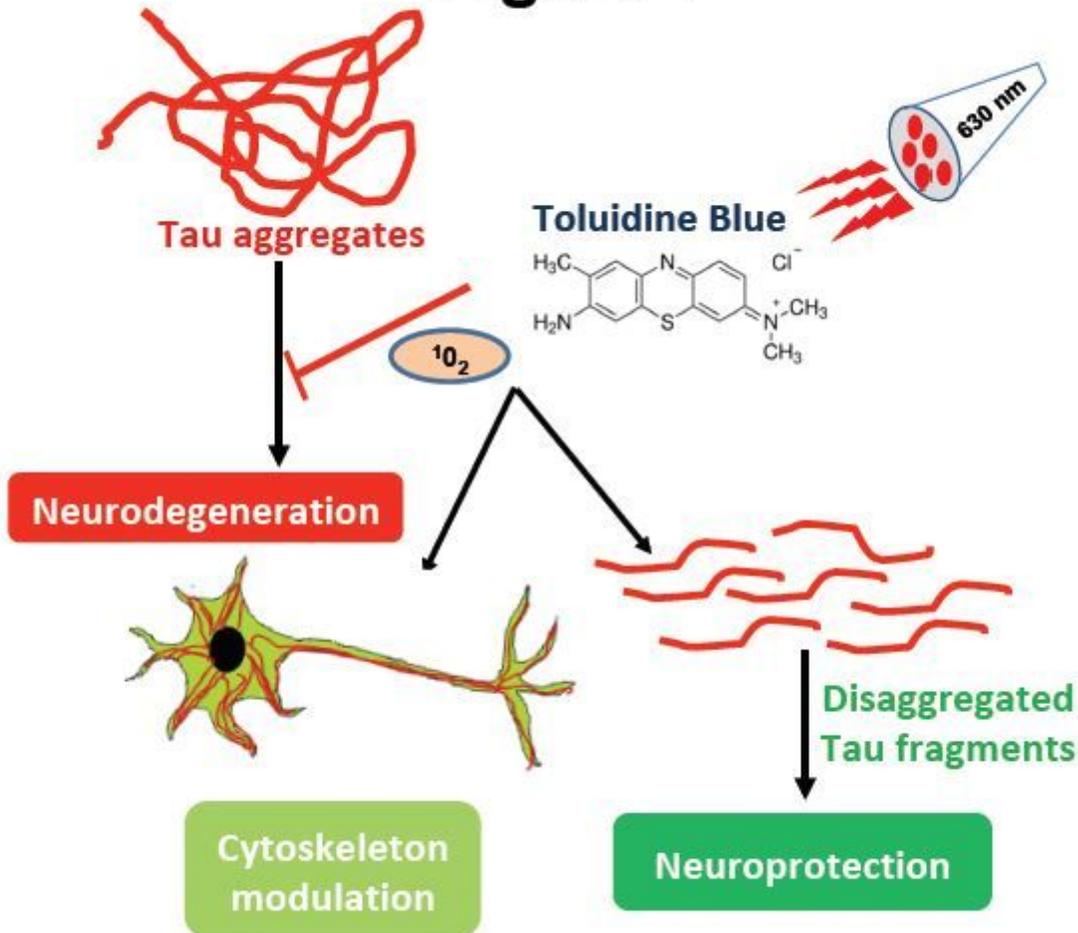


Figure 7

The TB and PE-TB dissolve the Tau fibrils. Tau aggregates are one of the hallmarks of AD. Accumulation of Tau aggregates leads to the generation of neurodegenerative diseases. TB on irradiation with 630 nm red light gets converted to PE-TB. PE-TB disaggregates the pre-formed Tau and modulates the cytoskeleton. Thus, PE-TB could be a potent molecule against Tauopathy.