

Carboplatin alleviates astrocytic TDP-43 neurotoxicity by inhibiting NF- κ B activation

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a rare and progressive motor neuron disease; however, its exact pathogenic mechanisms remain unclear. Currently, no effective treatments are available for this disease. Therefore, in this study, we investigated the anti-inflammatory effects of the anti-cancer agent, carboplatin, on neuronal cells and its potential therapeutic effects against ALS. Carboplatin inhibited NF- κ B phosphorylation in the transactive response DNA-binding protein (TDP)-43-transfected astrocytes, reducing pro-inflammatory cytokine levels, without affecting the TDP-43 protein levels. In neuron-astrocyte co-culture models, carboplatin effectively alleviated TDP-43-induced toxicity by restoring mitochondrial integrity, specifically rescuing basal respiration, ATP production, and maximal respiratory capacity. *In vivo*, carboplatin rescued the locomotor deficits in glial-specific TDP-43-expressing *Drosophila*, without altering TDP-43 protein levels and subcellular localization. These findings suggest that TDP-43-induced astrocytic damage compromises mitochondrial functions in adjacent neurons, and that carboplatin-mediated restoration of TDP-43-mediated astrocyte damage is critical for neuronal survival and functions. Therefore, carboplatin, a chemotherapeutic agent, represents as a potential therapeutic candidate for TDP-43-associated proteinopathies.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the progressive and fatal loss of the upper and lower motor neurons, leading to muscle weakness, paralysis, and death within 3–5 years of symptom onset (Pasinelli and Brown, 2006). ALS is of two major types: sporadic ALS, which lacks a well-defined pathogenesis, and familial ALS, which is inherited. Transactive response DNA-binding protein (TDP)-43 is a key factor in the development of both forms of ALS (Neumann et al., 2006). TDP-43 is a highly conserved nuclear RNA/DNA-binding protein involved in the regulation of gene expression, alternative splicing, and RNA processing (De Conti et al., 2015; Hallegger et al., 2021; Tollervey et al., 2011). It is a key component of cytoplasmic inclusions observed in various neurodegenerative diseases, including ALS, frontotemporal dementia, Alzheimer's disease (AD), and Parkinson's disease (PD) (Meneses et al., 2021; Steinacker et al., 2019; Yamashita et al., 2022). The overexpression of wild-type or mutant

human TDP-43 causes progressive motor neuron degeneration in mice (Mitchell et al., 2015). Similarly, the loss of function of the *Drosophila* ortholog *TBPH* or the zebrafish ortholog *tardbp* leads to locomotor deficits and motor neuron axon abnormalities (Diaper et al., 2013; Hewa-madduma et al., 2013). However, the specific pathogenic mechanisms associated with abnormal TDP-43 expression and formation of cytoplasmic inclusions remain unclear. Previous studies have suggested a link between neuroinflammation, immune-mediated mechanisms, and neurodegeneration in ALS.

TDP-43 interacts with nuclear factor-kappa B (NF- κ B), a critical inflammatory response mediator, and activates NF- κ B signaling in microglia. This activation induces the production of pro-inflammatory cytokines, including interleukin (IL)-1 β and tumor necrosis factor- α (TNF- α) (Correia et al., 2015; Zhao et al., 2015). Mitochondrial dysfunction is a prominent pathological feature of TDP-43-associated proteinopathies in both neuronal and glial cells, contributing to the induction of neuroinflammation. TDP-43 overexpression in astrocytes

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leads to mitochondrial dysfunction in neuronal cells (Lee et al., 2020). TDP-43 accumulation in the mitochondria disrupts several key functions, including fission/fusion dynamics, ATP production, and Ca^{2+} homeostasis in neurons (Gao et al., 2019; Huang et al., 2020). Activated glial cells release inflammatory factors that regulate mitochondrial function (Lin et al., 2022). In neuroinflammatory diseases, including ALS, astrocytes are driven into a neurotoxic state by cytokines secreted by microglia, which release various pro-inflammatory cytokines that exacerbate cellular damage and mitochondrial injury in neighboring cells including neurons (Singh, 2022; Staats et al., 2022). Recent studies have consolidated the NF- κ B pathway as a primary driver of TDP-43-induced neurotoxicity. Furthermore, emerging evidence has demonstrated that TDP-43 directly regulates p65-mediated apoptotic pathways (Yu et al., 2025), particularly by triggering neuroimmune signaling in astrocytes, leading to nucleocytoplasmic disruption (Zhou et al., 2025). The therapeutic potential of targeting this signaling pathway is supported by findings that inhibiting NF- κ B signaling effectively restores motor performance in *superoxide dismutase 1* and *fused in sarcoma* (FUS)-linked ALS models (Cao et al., 2024; Pelaez et al., 2023).

Carboplatin is a second-generation, platinum-based chemotherapeutic agent widely used to treat various cancers, including ovarian, lung, and bladder cancers (Zhang et al., 2022). Its cytotoxic effects are primarily mediated by the formation of DNA crosslinks that prevent DNA replication and transcription, leading to cell death (Cruet-Hennequart et al., 2009). Notably, carboplatin exhibits a markedly lower neurotoxic profile compared with other platinum-based agents, such as cisplatin. Recent reviews suggest that carboplatin neurotoxicity is dose-dependent and negligible at lower concentrations, partly because of its reduced propensity to generate reactive oxygen species (ROS) or to cause significant DNA damage compared with first-generation analogs such as cisplatin (Kanat et al., 2017). Although its cytotoxic effects on tumor cells are well-documented, its potential neuroprotective roles in alleviating neuroinflammation and neurodegenerative diseases remain unclear. Furthermore, although carboplatin is primarily used in oncology, growing evidence suggests that it also exerts neuroprotective effects on non-cancerous tissues and organs, including the central nervous system (CNS). Carboplatin also modulates the inflammatory responses in neurons (Lv et al., 2023). Recently, it has attracted attention as a potential therapeutic agent for neurodegenerative diseases because of its ability to alleviate inflammation and restore mitochondrial function (Boonmee et al., 2021; Cha et al., 2022). However, the specific effects of carboplatin on TDP-43-induced neuroinflammation and mitochondrial dysfunction remain unclear. Leveraging its wide therapeutic window, we propose that sub-therapeutic doses of carboplatin may be repurposed to modulate key pathological signaling pathways central to the pathogenesis of ALS.

This study aimed to bridge this knowledge gap by examining the effects of carboplatin on TDP-43-induced toxicity using *in vitro* and *in vivo* models. Using molecular and functional assays, we investigated the mechanisms by which carboplatin modulates NF- κ B signaling, inflammatory cytokine production, and mitochondrial function in TDP-43-associated neurodegenerative diseases. We assessed the neuroprotective effect of carboplatin on TDP-43-mediated neurodegeneration using various experimental models, including cell culture models of astrocytes and neurons, and *Drosophila* models expressing TDP-43 in glial cells. Carboplatin inhibited TDP-43-induced NF- κ B activation, reduced pro-inflammatory cytokine levels in astrocytes, restored mitochondrial function in neurons, and alleviated locomotor deficits in *Drosophila* models. By targeting both neuroinflammation and mitochondrial dysfunction, carboplatin emerges as a promising therapeutic agent for TDP-43-associated proteinopathies including ALS. This study highlights the potential of carboplatin as a novel therapeutic agent for neurodegenerative diseases characterized by TDP-43 accumulation and toxicity.

2. Materials and methods

2.1. Reagents and antibodies

The following reagents were used in this study: carboplatin (Cat# C2538; Sigma Aldrich), lipopolysaccharide (LPS; Cat# L4391; Sigma Aldrich), and recombinant interferon- γ (IFNG/IFN- γ ; Cat# 585-IF; R&D Systems). The following antibodies were used for immunoblotting: rabbit anti-TDP-43 (Cat# 10782-2-AP; Proteintech Group), rabbit anti-NF- κ B p65 Ser536 (Cat# 3033; Cell Signaling Technology), rabbit anti-NF- κ B (Cat# 8242; Cell Signaling Technology), rabbit anti-histone H3 (Cat# 9715; Cell Signaling Technology), and horseradish peroxidase (HRP)-conjugated anti- α -tubulin (Cat# 9099; Cell Signaling Technology), and HRP-conjugated rabbit IgG (Cat# sc-2004; Santa Cruz Biotechnology) antibodies.

2.2. Primary cell cultures

Primary astrocytes were cultured as described previously (Lee et al., 2020). Postnatal day 1–2 mouse pups were sacrificed via decapitation, and their brains were rapidly removed and placed in a culture dish containing Hank's balanced salt solution (HBSS; Gibco). Whole brains were homogenized and filtered through a 70- μ m strainer, and the cells were seeded in T75 culture flasks. The cells were grown at 37 °C in a humidified atmosphere containing 5% CO_2 . The culture medium was initially changed after five days and every two days thereafter. Cells were used after 14–21 days of culture. Secondary pure astrocyte cultures were obtained by shaking mixed glial cultures at 250 rpm for 4 h, after which the culture medium was discarded. Astrocytes were dissociated using trypsin-ethylenediaminetetraacetic acid (Life Technologies) and centrifuged at 800 $\times$ g for 30 min. The isolated astrocytes were then seeded in plates with Dulbecco's modified Eagle's medium (Life Technologies) supplemented with 10% heat-inactivated fetal bovine serum (Gibco) and 50 μ g/mL penicillin-streptomycin.

Primary cerebral cortical neurons were cultured as described previously (Lee et al., 2020). Embryonic day 17 mouse embryos were sacrificed and their brains were rapidly removed and placed in a culture dish containing HBSS (Gibco). The cortices were isolated, transferred into conical tubes, and washed twice with HBSS. The cortical tissues were enzymatically digested with prewarmed papain (20 U/mL) (Worthington Biochemical Corporation) and DNase I (0.005%) for 30 min at 37 °C. Following digestion, the tissues were mechanically dissociated using 1000- and 200 μ L pipette tips to achieve complete homogenization. The dissociated cells were further centrifuged at 130 $\times$ g for 10 min at room temperature, and the resulting cells were seeded in poly-D-lysine (Sigma Aldrich)-coated plates with neurobasal media supplemented with 2 mM glutamine (Gibco), N2 (Gibco), B27 (Gibco), and 50 μ g/mL penicillin-streptomycin (Gibco). The culture medium was initially changed after five days and every three days thereafter. Cells were used after 14 days of culture. The animals used in this study were obtained and cared for in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the Korea Brain Research Institute (IACUC-24-00017) and were performed in accordance with the approved guidelines and regulations of the Korea Brain Research Institute.

2.3. Transfection

Primary astrocytes were transfected using the Neon electroporation device (Thermo Fisher Scientific). A total of 100×10^4 cells were electroporated with 5 μ g of either green fluorescent protein (GFP) (pCMV6-AC-GFP; Cat# PS100010; OriGene Technologies), wild-type human TDP-43 (pCMV6-AC-TDP-43-GFP; Cat# RG210639; OriGene Technologies), or TDP-43 Q331K mutant plasmids. The Q331K mutant construct

was generated from the wild-type TDP-43 vector using site-directed mutagenesis and the sequence was verified prior to use. Electroporation was performed under the following conditions: 1300 V, 20 ms, and two pulses. Transfection efficiency was assessed by flow cytometry 24 h post-transfection. Analysis was performed using a MoFlo Astrios cell sorter (Beckman Coulter), with non-transfected astrocytes serving as negative controls to quantify the GFP-positive populations. The percentages of GFP-positive cells were 54% for the GFP control, 38% for TDP-43-GFP, and 36% for TDP-43 M337V-GFP (Supplementary Fig. S1). These transfection rates were consistent across multiple independent experiments. Transfected cells were plated in 6-well culture dishes and treated with carboplatin (500 nM) or phosphate-buffered saline (PBS) for 24 h. All assays were conducted 2 days post-transfection to minimize nonspecific toxicity caused by the transfection procedure.

2.4. Cytotoxicity assay

Primary astrocytes (8×10^4 cells/mL) were cultured in 96-well plates and treated with carboplatin for 24 h, with PBS serving as a negative control. Cytotoxicity was assessed using the Cell Counting Kit-8 (CCK-8; Cat# ALX-850-039; Enzo Life Science) according to the manufacturer's instructions. Briefly, 10 μ L of CCK-8 reagent was added to each well, and the plate was incubated at 37 °C for 2 h. Absorbance was then measured at 450 nm using a microplate reader (Tecan), and cell viability was reported as a percentage relative to the control. All experiments were conducted in triplicate.

2.5. Nitrite measurement

Primary astrocytes (8×10^4 cells/well) were treated with carboplatin or LPS/IFN- γ in 96-well plates. Nitrite concentrations in the cell culture media were analyzed using the Griess Reagent System (Cat# G2930; Promega) as described previously (Lee et al., 2020). Briefly, a 50 μ L aliquot of the culture medium was mixed with 50 μ L of Griess reagent, and optical density at 550 nm was measured using the microplate reader (Tecan). All experiments were conducted in triplicate.

2.6. Immunoblotting

Primary astrocytes were lysed using radioimmunoprecipitation assay (RIPA) buffer (Cell Signaling Technology) supplemented with a protease and phosphatase inhibitor cocktail (Roche). The protein concentration in the lysate was measured using the Pierce BCA Protein Assay Kit (Cat #78440; Thermo Fisher Scientific). Subsequently, 10 μ g protein was mixed with 4 $\times$ NuPAGE LDS Sample Buffer (Invitrogen) and 10 $\times$ NuPAGE Sample Reducing Agent (Invitrogen) and boiled at 95 °C for 5 min. Proteins were separated using NuPAGE 4–12% Bis-Tris gels (Novex) and transferred to polyvinylidene difluoride (Novex) membranes according to the manufacturer's instructions. The membranes were blocked with 5% skim milk and sequentially incubated with primary and HRP-conjugated secondary (anti-rabbit IgG) antibodies. Detection was performed using the ECL Prime Kit (Amersham Biosciences). Data from three independent experiments were analyzed and relative protein expression levels were quantified using Fusion-FX software (Vilber Lourmat).

2.7. Nuclear extraction

Nuclear fractions were prepared using NE-PER Nuclear Extraction Reagent (Cat# 78833; Thermo Fisher Scientific) according to the manufacturer's instructions. Briefly, primary astrocytes were harvested, washed with ice-cold PBS, and resuspended in Cytoplasmic Extraction Reagent I. Following incubation on ice, Cytoplasmic Extraction Reagent II was added, and the lysates were centrifuged at 16,000 $\times$ g for 5 min at 4 °C. The resulting supernatants representing the cytoplasmic fractions were collected. The remaining pellets were resuspended in ice-cold

Nuclear Extraction Reagent, vortexed intermittently for 40 min on ice, and centrifuged at 16,000 $\times$ g for 10 min at 4 °C to obtain the nuclear fractions. Nuclear extracts were mixed with 4 $\times$ Bolt LDS Sample Buffer and 10 $\times$ Bolt Sample Reducing Agent, boiled at 95 °C for 5 min, and subjected to immunoblot analysis. The purity of the nuclear fractions was confirmed by immunoblotting for histone H3.

2.8. Quantitative reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from cultured cells and the heads of 20 adult flies using TRIzol® reagent (Life Technologies) and followed by purification with the RNeasy Mini Kit (QIAGEN) according to the manufacturer's instructions. For cDNA synthesis, 100 ng of RNA was reverse transcribed at 37 °C for 120 min using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Quantitative PCR was performed using the One-Step SYBR® PrimeScript™ RT-PCR Kit (Perfect Real Time; Takara Bio Inc.) on an Applied Biosystems 7500 Real-Time PCR System. 18S rRNA was used as the internal reference gene, and relative mRNA expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method. PCR reactions were initially incubated at 95 °C for 3 min for polymerase activation and DNA denaturation. After the pretreatment, reactions were subjected to the following thermal cycling conditions: 40 cycles of denaturation at 95 °C for 5 s and annealing/extension at 60 °C for 30 s. Primer sequences used in the PCR assays are listed in Supplementary Table S1.

2.9. Enzyme-linked immunosorbent assay (ELISA)

The secretion of pro-inflammatory cytokines (IL-1 β , IL-6, and TNF- α) from TDP-43-transfected astrocytes (1.5×10^5 cells/well) was quantified using ELISA Kits specific for each cytokine (R&D Systems). Briefly, 96-well plates were coated with the capture antibodies, blocked, and incubated with culture supernatants or recombinant standards. Following incubation with biotin-conjugated detection antibodies, streptavidin-HRP and substrate solution were applied according to the manufacturer's instructions. Absorbance was measured at 450 nm using a microplate reader (Tecan) with a reference wavelength of 540 nm.

2.10. Astrocyte-conditioned media (ACM)

For the preparation of ACM, primary astrocytes transfected with GFP or TDP43-GFP were treated with carboplatin (500 nM) or PBS for 24 h. Following treatment, the astrocytes were washed three times with PBS to remove any residual carboplatin and subsequently incubated in fresh growth medium for an additional 24 h in the absence of the drug. The ACM was then collected, centrifuged at 200 $\times$ g for 10 min to eliminate cellular debris, and stored at –80 °C until further use. The rigorous washing procedure ensured that the neurons were not directly exposed to carboplatin, thereby confirming that the observed neuroprotective effects were mediated non-cell-autonomously by astrocyte-secreted factors.

2.11. ACM-treated neuron culture

Primary cortical neurons (10×10^4 cells/well) in 96-well plates were stimulated for five days with ACM from GFP-transfected, GFP-transfected carboplatin-treated, TDP-43-transfected, and TDP-43-transfected carboplatin-treated cells (referred to as GFP ACM, GFP + carboplatin ACM, TDP-43 ACM, and TDP-43 + carboplatin ACM, respectively). Following stimulation, cells were subjected to a CCK-8 assay.

2.12. Mitochondrial activity

Primary cortical neurons cultured in XF24-well plates (10×10^4 cells/well; Seahorse Bioscience) were stimulated with GFP ACM, GFP +

carboplatin ACM, TDP-43 ACM, or TDP-43 + carboplatin ACM for five days. Because TDP-43 ACM induces neuronal toxicity and reduces cell numbers, normalization strategies were applied to minimize the potential confounding effects of cell loss on oxygen consumption rate (OCR) measurements. Then, the cells were washed twice with the XF Base Medium supplemented with 2 mM L-glutamine, 10 mM D-glucose, and 1 mM sodium pyruvate (pH 7.4), and incubated at 37 °C in a non-CO₂ incubator for 1 h. Mitochondrial dysfunction was assessed using the XF Cell Mito Stress Test Kit (Seahorse Bioscience), according to the manufacturer's guidelines, using the XF24 Extracellular Flux Analyzer (Seahorse Bioscience). The 24-well utility plate was hydrated, treated with 2 μ M oligomycin, 2 μ M carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone, and 0.5 μ M antimycin A + rotenone, and calibrated by the analyzer. Basal OCR, ATP production, maximum reserve, and respiratory capacity were measured as described above, with averages obtained from five wells per condition in each experiment. The OCR was normalized to the total protein concentration (OD) to compensate for differences in cell numbers resulting from ACM-induced neuronal toxicity, ensuring that the observed OCR changes reflected mitochondrial function rather than variations in cell numbers. After Seahorse analysis, the plate was centrifuged at 280 $\times$ g for 5 min, the medium was aspirated, and the cells were washed twice with PBS before lysis with RIPA buffer. Finally, protein concentrations in the cell lysates were determined using a BCA Assay Kit (Pierce).

2.13. Chloromethylfluorescein diacetate (CMFDA) staining assay

To evaluate neuronal viability, primary cortical neurons were incubated with 5 μ M CellTracker™ Green CMFDA (Invitrogen) for 30 min at 37 °C. After removing the dye solution, the cells were maintained in fresh medium without the dye for an additional 45 min to allow for complete esterification. The labelled neurons were then mounted and visualized under a fluorescence microscope. Images were acquired from three randomly selected fields per well, and CMFDA-positive cells were quantified. Data are expressed as the percentage of labelled cells relative to the untreated controls.

2.14. *Drosophila* stocks

For the experiments involving *Drosophila melanogaster*, formal ethical approval from the IACUC was not required in accordance with institutional and national guidelines, as these involved only invertebrate models. Nevertheless, all procedures were conducted in compliance with established ethical standards for laboratory animal care. All stock flies were maintained at 25 °C under normal humidity (60%) on standard food. Crosses between flies were performed according to the standard procedure, and progeny were raised at a normal temperature or 25 °C. The *UAS-TDP-43* line was kindly provided by Nancy M. Bonini (University of Pennsylvania, Philadelphia, PA, USA). The pan-galial driver *Repo-Gal4* and *UAS-TDP-43^{Q331K}* lines were obtained from the Bloomington *Drosophila* Stock Center. All transgenic lines were backcrossed with *W¹¹¹⁸* strain for ten generations prior to use. Based on their genetic background, *W¹¹¹⁸* flies were used as controls.

2.15. Drug feeding assay

The standard fly food was mixed with various concentrations of carboplatin (LKT Laboratories). An equal volume of distilled water was used as the vehicle control for carboplatin. The parent flies were provided with food supplemented with carboplatin. The 3rd instar larvae were selected for use in subsequent experiments.

2.16. Locomotive activity assay

For the larval crawling assay, 3rd instar larvae were washed with PBS to remove residual food, dried on clean filter paper, and placed on a

2% grape juice-agar Petri plate. Larvae from each genetic line were allowed to crawl for 90 s. To quantify the crawling behavior of the larvae, lines were drawn to track larval movement, and the distance traveled was measured using ImageJ software. The results from at least 15 larvae were averaged for each transgenic line.

2.17. Immunoblotting assay for flies

Proteins were extracted by homogenizing the heads of the flies in LDS Sample Buffer (Cat# NP0007; Invitrogen). Total protein extracts (10 μ g) were separated on 4–12% gradient sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene fluoride membranes (Cat# IPVH00010; Millipore). The membranes were blocked with 4% non-fat dry milk in Tris-buffered saline containing 0.1% Tween-20 for 1 h and incubated with the following primary antibodies overnight: rabbit anti-TDP-43 (1:1000; Cat# 10782-2-AP; Proteintech Group), rabbit anti- β -actin (1:5000; Cat# 4967 L; Cell Signaling Technology), mouse anti-*Drosophila* lamin C (1:1000; Cat# LC28.26; DSHB), and rabbit anti- α -tubulin (1:1000; Cat# 9099; Cell Signaling Technology) antibodies. After washing with TBST, the membranes were incubated with the following HRP-conjugated secondary antibodies: HRP-conjugated goat anti-rabbit IgG (1:2000; Cat# AP307P; Millipore), and goat anti-mouse IgG HRP conjugate (1:2000; Cat# AP308P; Millipore) antibodies. Protein bands were detected using an ECL Plus Kit (Cat# RPN2106; Amersham).

2.18. Cellular fractionation assay

For subcellular fractionation, the heads of 20 male flies were lysed using the reagent included in the Nuclear Extraction Kit (Cat# RPN2106; Active Motif) according to the manufacturer's instructions. Protein extracts from different subcellular fractions were mixed with SDS Loading Buffer, boiled, and subjected to SDS-PAGE for Western blot analysis.

2.19. External eye microscopy

The heads of 5-day-old male flies were mounted on glass slides for imaging. Eye images were captured using a Leica MZ10 F stereomicroscope equipped with a Leica DFC450 digital camera. Images were converted to grayscale using Adobe Photoshop 7.0. For quantitative analysis of pigment loss, a comparable region of the eye was selected for each genotype, and grayscale thresholds were adjusted to define the depigmented areas. The proportion of white (depigmented) regions was quantified using the ImageJ software.

2.20. Statistical analyses

Data were analyzed using Student's *t*-test (Vassar Stats; www.vassarstats.net) or one-way analysis of variance, followed by Tukey's multiple-comparison test, as appropriate (GraphPad Prism Software). Statistical significance was set at $p < 0.05$.

3. Results

3.1. Carboplatin alleviates TDP-43-induced inflammation in astrocytes by inhibiting NF- κ B phosphorylation

Astrocytes are crucial cells that initiate inflammatory responses in adjacent neurons by secreting various pro-inflammatory molecules, including cytokines, which induce or exacerbate neuroinflammation (Cekanaviciute and Buckwalter, 2016). In addition, astrocytes generate ROS such as nitric oxide (NO), which induce neurotoxicity. During chronic inflammation, these molecules promote neuronal damage and have been implicated in the pathogenesis of various neurodegenerative diseases (Adamu et al., 2024). The primary mechanism underlying the

antitumor activity of carboplatin involves the induction of cross-linking between and within DNA strands, resulting in DNA damage, inhibition of DNA unwinding and DNA synthesis, and ultimately cytotoxicity (Stankovic et al., 2020). However, few studies have investigated the role of carboplatin in resulting neuroinflammation in neurodegenerative diseases. Furthermore, it remains unclear whether carboplatin exerts therapeutic effects against neuroinflammation in patients with ALS.

To determine the optimal treatment concentration, cell viability was assessed using a CCK-8 assay following treatment with various concentrations of carboplatin. Significant toxicity to astrocytes was observed at concentrations ≥ 10 nM, with approximately a 20% reduction in the number of viable cells compared to the control (Supplementary Fig. S2). However, no further reduction in cell viability was observed at concentrations ≥ 500 nM; therefore, 500 nM was selected as the working concentration of carboplatin. To validate this optimal working concentration, astrocytes were stimulated with LPS and IFN- γ in the presence of various doses of carboplatin and NO production was evaluated as a marker of inflammatory activation. Carboplatin reduced NO production in a dose-dependent manner, reaching maximal efficacy at a sub-cytotoxic concentration of 500 nM (Supplementary Fig. S2 and S3). Notably, this 500 nM (50 μ M) dose is also clinically relevant; considering the reported cerebrospinal fluid (CSF)/plasma ratio of $\sim 3\%$ and clinical plasma peaks of 50–100 μ M (Jacobs et al., 2005), a CNS concentration of 500 nM is readily achievable in human patients. Consequently, 500 nM was selected for all subsequent experiments, as it significantly attenuated the LPS/IFN- γ -induced inflammatory response (Fig. 1A) without compromising cell viability.

TDP-43 contributes to excessive neuroinflammation in astrocytes and activates microglia via the NF- κ B signaling pathway (Zhao et al., 2015). Therefore, we hypothesized that the NF- κ B signaling pathway plays a pivotal role in TDP-43-mediated neuroinflammation and may be modulated by carboplatin in astrocytes. To verify this, we extracted proteins from astrocytes transfected with GFP or GFP-tagged TDP-43 and assessed the phosphorylation levels of NF- κ B subunit p65 using a phospho-specific antibody targeting Ser536. The phosphorylation of p65 at Ser536 is critical for the nuclear translocation of NF- κ B, which drives the expression of inflammatory genes. TDP-43-transfected cells

showed significantly higher NF- κ B p65 (Ser536) phosphorylation than GFP-transfected cells (Fig. 1B). Notably, TDP-43-induced phosphorylation of NF- κ B p65 (Ser536) was significantly inhibited by carboplatin; however, carboplatin did not alter the total NF- κ B protein levels in both the GFP- and GFP-tagged TDP-43-transfected cells (Fig. 1B). Consistent with these findings, nuclear fractionation revealed that TDP-43 overexpression markedly increased the nuclear accumulation of NF- κ B p65 compared to GFP controls. However, carboplatin treatment significantly inhibited TDP-43-induced nuclear accumulation of NF- κ B p65 (Fig. 1C). These results indicate that carboplatin effectively inhibits the nuclear translocation of active NF- κ B, thereby modulating the canonical NF- κ B signaling pathway at the level of subcellular trafficking. To further clarify the effect of carboplatin on TDP-43, we examined TDP-43 protein levels by immunoblotting. Notably, carboplatin did not alter the elevated TDP-43 protein levels in TDP-43-overexpressing cells (Fig. 1D). These results suggest that carboplatin protects astrocytes against TDP-43-induced toxicity without directly affecting TDP-43 protein levels. Specifically, carboplatin suppresses NF- κ B activation by inhibiting p65 phosphorylation at Ser536 and its subsequent nuclear translocation. Notably, this protective effect occurs independently of changes in TDP-43 protein levels, thereby establishing a specific mechanistic link between the modulation of NF- κ B signaling and the attenuation of astrocytic neuroinflammation.

3.2. Carboplatin restores the pro-inflammatory cytokine levels in astrocytes

The inflammatory activation of astrocytes is a well-established feature of ALS, including superoxide dismutase-1-linked ALS (Guttenplan et al., 2020). TDP-43 contributes to the pathological production of microglial pro-inflammatory cytokines by activating the NF- κ B pathway and inflammasomes in ALS (Deora et al., 2020; Zhao et al., 2015). We previously showed that TDP-43 overexpression in astrocytes induces neuroinflammation through a protein tyrosine phosphatase 1B-dependent inflammatory response, characterized by the increased levels of inflammatory cytokines, such as *IL-1 β* , *Tumor necrosis factor- α* , *IL-6*, *lipocalin 2*, and inducible nitric oxide synthase (Lee et al.,

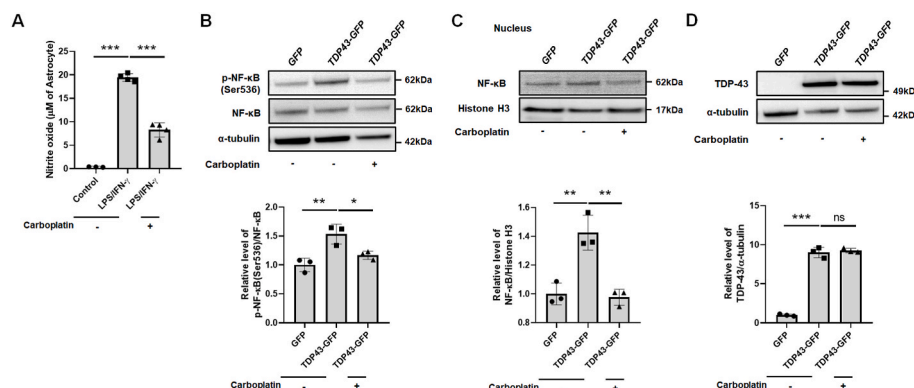


Fig. 1. Carboplatin alleviates inflammation and inhibits transactive response DNA-binding protein 43 (TDP-43)-induced nuclear factor- κ B (NF- κ B) activation in astrocytes. (A) Primary astrocytes were pretreated with carboplatin (500 nM) for 30 min and subsequently treated with lipopolysaccharide (LPS)/interferon- γ (IFN- γ) (100 ng/mL + 50 U/mL) for 24 h. Nitrite level in the culture medium was then measured. Carboplatin significantly reduced LPS/IFN- γ -induced nitric oxide (NO) production in primary astrocytes. Data are presented as the mean $\pm$ standard deviation (SD) of three independent experiments. *** p < 0.001 (one-way ANOVA, followed by Tukey's multiple-comparison test). (B-D) Primary astrocytes transfected with GFP or TDP-43-GFP were treated with carboplatin (500 nM) for 24 h and subjected to immunoblot analysis. (B) Immunoblot analysis of GFP- or TDP-43-GFP-transfected cells showing protein levels of phosphorylated NF- κ B (Ser536) and total NF- κ B following 24 h carboplatin treatment. Carboplatin significantly reduced TDP-43-induced phosphorylated NF- κ B (Ser536) levels in primary astrocytes. Tubulin was used as a loading control for normalization. Data are presented as the mean $\pm$ SD of triplicate samples. * p < 0.05 and ** p < 0.005 (one-way ANOVA, followed by Tukey's multiple-comparison test). (C) Nuclear fractionation analysis of NF- κ B p65 in GFP- or TDP-43-GFP-transfected cells treated with carboplatin (500 nM) for 24 h. TDP-43 overexpression markedly increased nuclear accumulation of NF- κ B p65, which was significantly reduced following carboplatin treatment. Histone H3 was used as a nuclear loading control. Data are presented as the mean $\pm$ SD of triplicate samples. ** p < 0.005 (one-way ANOVA, followed by Tukey's multiple-comparison test). (D) Immunoblotting analysis of TDP-43 protein levels in GFP- and TDP-43-GFP-transfected cells 24 h after carboplatin (500 nM) treatment. Carboplatin did not alter TDP-43 levels in TDP-43-GFP-transfected astrocytes. Tubulin was used as a loading control for normalization. Data are presented as the mean $\pm$ SD of triplicate samples. *** p < 0.001; ns, not significant (one-way ANOVA, followed by Tukey's multiple-comparison test).

2020). To elucidate the role of carboplatin in regulating the secretion of inflammatory cytokines, we measured the mRNA expression of inflammatory cytokines and chemokines in astrocytes expressing GFP, GFP-tagged TDP-43, and carboplatin-treated GFP-tagged TDP-43 using

RT-PCR. TDP-43 mRNA expression, similar to its protein level, was unaffected by carboplatin (Fig. 2). Notably, TDP-43 levels in astrocytes significantly increased the mRNA expression of *IL-6*, *nitric oxide synthase 2*, *lipocalin2*, and *IL-1 β* compared with GFP controls. However, the

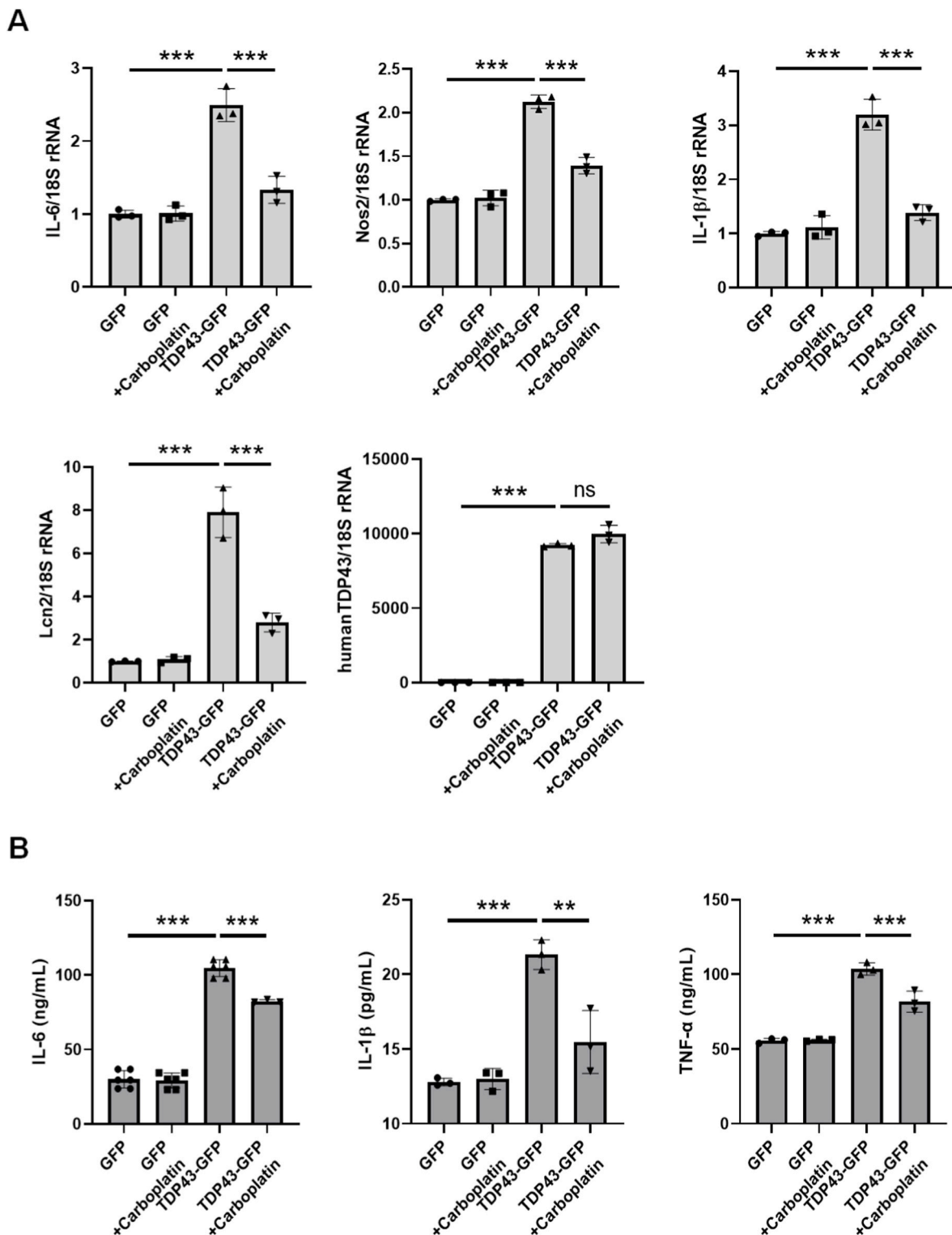


Fig. 2. Carboplatin attenuates TDP-43-induced pro-inflammatory responses in primary astrocytes. Primary astrocytes were transfected with TDP-43-GFP or GFP control plasmids and subsequently treated with carboplatin (500 nM) for 24 h. (A) Relative mRNA expression of pro-inflammatory genes was quantified by qRT-PCR. Transcript levels were normalized to 18S rRNA and are presented as the mean $\pm$ SD. *** p < 0.001; ns, not significant (one-way ANOVA, followed by Tukey's multiple-comparison test). (B) Conditioned media from the same cultures were collected, and secreted cytokine levels (IL-1 β , IL-6, and TNF- α) were determined by ELISA. ** p < 0.005 and *** p < 0.001 (one-way ANOVA, followed by Tukey's multiple-comparison test).

increased cytokine levels were reduced following carboplatin treatment. Notably, carboplatin alone did not induce any significant changes in the cytokine levels in the GFP control group (Fig. 2A). To assess whether TDP-43 expression in astrocytes promotes cytokine release, we measured the levels of inflammatory cytokines in the culture medium from astrocytes expressing GFP-tagged TDP-43 using ELISA. Secretion of inflammatory cytokines, including IL-6, IL-1 β , and TNF- α , was elevated in the medium, and this increase was attenuated following carboplatin treatment (Fig. 2B). To investigate whether carboplatin modulates the inflammatory responses induced by ALS-linked Q331K mutant TDP-43

expression, we quantified the mRNA expression of inflammatory cytokines in astrocytes expressing GFP alone, GFP-tagged TDP-43 Q331K, or GFP-tagged TDP-43 Q331K following carboplatin treatment. Notably, TDP-43 Q331K expression in astrocytes significantly increased the mRNA expression of *IL-6*, *IL-1 β* , *Nos2*, and *Lcn2* compared with GFP controls. Carboplatin treatment markedly attenuated these elevated cytokine levels compared to the control, whereas TDP-43 Q331K mRNA expression, remained unaffected (Supplementary Fig. S4A). Furthermore, survival was reduced by approximately 60% in the TDP-43 Q331K ACM-treated neurons. CCK-8 assay revealed that carboplatin

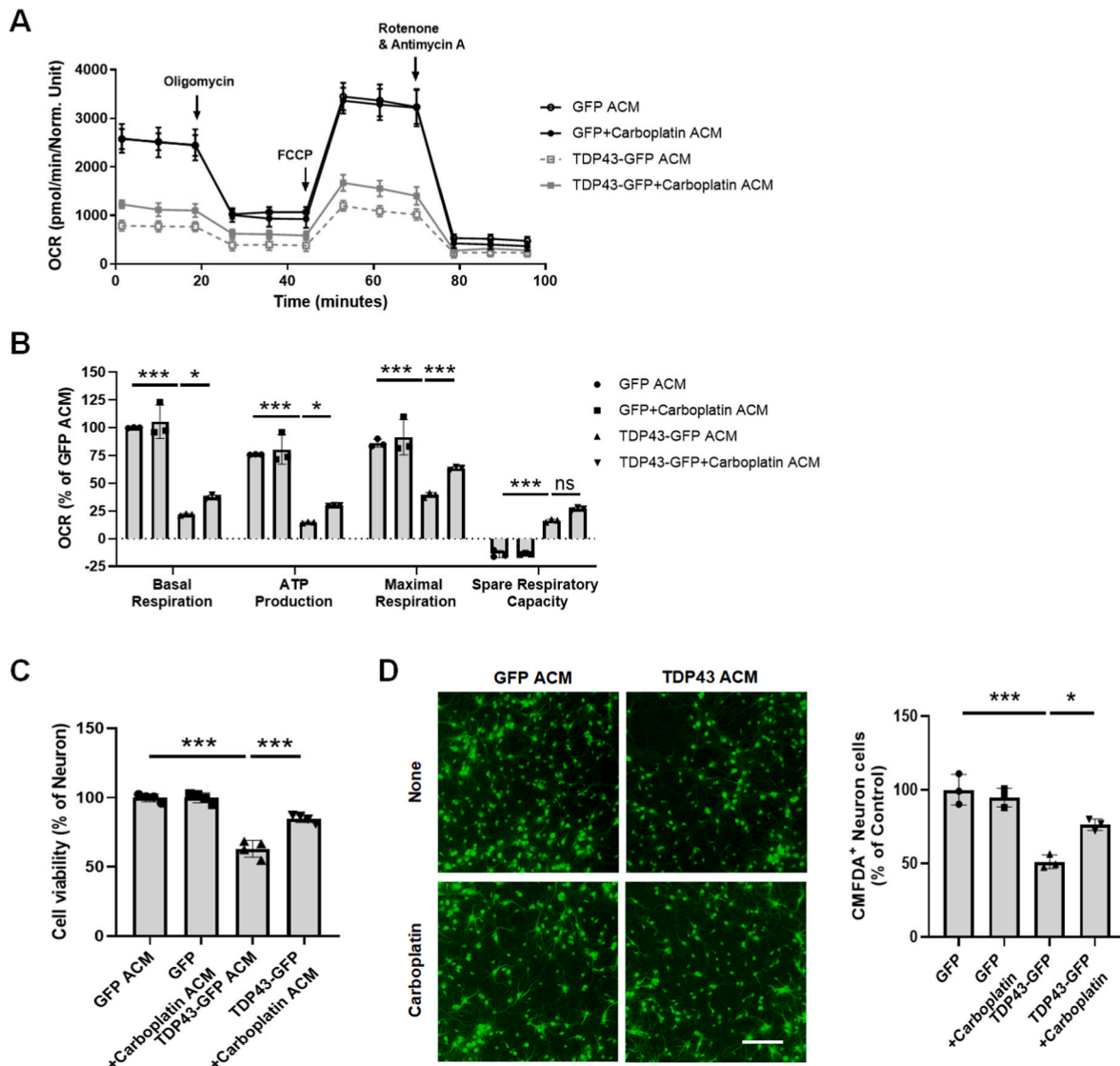


Fig. 3. Carboplatin alleviates mitochondrial dysfunction and neuronal toxicity in neurons exposed to TDP-43-expressing astrocytes. (A–C) Astrocytes transfected with GFP and TDP-43-GFP were treated with carboplatin (500 nM) for 24 h, and astrocyte-conditioned media (ACM) was collected. (A) Primary cortical neurons in XF24-well culture plates were treated with GFP ACM, GFP + carboplatin ACM, TDP-43 ACM, or TDP-43 + carboplatin ACM for five days. Mitochondrial dysfunction in ACM-treated cells was analyzed using the Seahorse XF analyzer to measure the basal oxygen consumption rate (OCR), ATP production, maximum reserve, and respiratory capacity. OCR values were normalized to the total protein concentration (OD). (B) Quantification of OCR, ATP production, maximum reserve, and respiratory capacity, expressed as a percentage of the basal values. Data are presented as the mean $\pm$ standard deviation (SD). * p < 0.05 and *** p < 0.001; ns, not significant (one-way ANOVA, followed by Tukey's multiple-comparison test). (C) Neuronal viability after ACM treatment was measured using the Cell Counting Kit-8 (CCK-8) assay. Data are presented as the mean $\pm$ SD. *** p < 0.001 (one-way ANOVA, followed by Tukey's multiple-comparison test). (D) In parallel experiments, primary cortical neurons were stained with chloromethylfluorescein diacetate (CMFDA) to evaluate neuronal survival. Representative CMFDA staining images and quantification of CMFDA-positive neuronal cells following treatment with conditioned media from GFP- or TDP43-GFP-expressing cells in the absence or presence of carboplatin. Images were acquired from three independent biological replicates for each experimental condition. For each biological replicate, 1–2 fields were captured and used for quantification. When two fields were acquired from the same biological replicate, field-level measurements were averaged to obtain one biological replicate value. Statistical analysis was performed using biological replicate values, and individual fields were not treated as independent biological replicates. Scale bars, 100 μ m. Data are presented as the mean $\pm$ SD. * p < 0.05 and *** p < 0.001; ns, not significant (one-way ANOVA, following by Tukey's multiple-comparison test).

significantly restored neuronal survival (Supplementary Fig. S4B). These results suggest that carboplatin alleviates neuroinflammation by inhibiting TDP-43-induced production of inflammatory cytokines in astrocytes.

3.3. Carboplatin rescues astrocytic TDP-43-induced mitochondrial dysfunction in neurons

Mitochondrial dysfunction is a key factor in several neurodegenerative diseases, including AD, PD, and ALS. TDP-43 is associated with mitochondrial dysfunction and disruption of mitochondrial dynamics in neurons (Gao et al., 2019; Wang et al., 2013). Moreover, TDP-43 expression in astrocytes increases the levels of inflammatory factor in the culture medium, thereby enhancing mitochondrial dysfunction and neuronal toxicity (Lee et al., 2020). Therefore, TDP-43-induced mitochondrial abnormalities in neurons and astrocytes may play central roles in the pathogenesis of these diseases.

To investigate the effect of carboplatin on TDP-43-induced neuronal toxicity in astrocytes, we used neuron-astrocyte transwell co-culture systems as well as neuronal cultures treated with ACM (Lee et al., 2020). To examine whether TDP-43 overexpression in astrocytes induces mitochondrial dysfunction in neurons, we measured the cellular OCR in primary cortical neurons treated with ACM using a Seahorse XF24 Extracellular Flux Analyzer and a Mitochondrial Stress Test Kit (Seahorse Bioscience). OCR serves as an indicator of mitochondrial respiration. Using electron transport chain modulators, such as oligomycin, carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone, and rotenone/antimycin A, we analyzed various mitochondrial respiratory parameters, including basal respiration, ATP production, maximal respiration, and spare respiratory capacity. Basal mitochondrial respiration, ATP production, and maximal respiration were significantly reduced in the TDP-43 ACM-treated primary cortical neurons (Fig. 3A). The TDP-43-induced neuronal mitochondrial dysfunction was substantially alleviated following carboplatin treatment (Fig. 3A and B). Moreover, survival was reduced by approximately 40% in the TDP-43 ACM-treated neurons. Consistent with the restoration of mitochondrial function, CCK-8 assay showed that carboplatin significantly restored neuronal survival (Fig. 3C). We also confirmed the CCK-8 assay data by staining with the fluorescent live-cell tracker CMFDA. Consistently, the number of CMFDA-positive neurons markedly decreased among TDP-43 ACM-treated primary cortical neurons, and carboplatin treatment significantly attenuated TDP-43 ACM-induced neuronal toxicity (Fig. 3D). Taken together, these findings suggest that TDP-43-induced damage to astrocytes compromises mitochondrial function in adjacent neurons and that restoration of TDP-43-mediated astrocyte damage by carboplatin is critical for neuronal survival and function.

3.4. Carboplatin mitigates locomotive dysfunction in glial-specific TDP-43-expressing flies

NF- κ B pathway is a key regulator of inflammation in *Drosophila*, where NF- κ B activation leads to antimicrobial peptide production (Panayidou and Apidianakis, 2013). Furthermore, glial cell-specific expression of human TDP-43 increases inflammation and neurotoxicity in *Drosophila* (Krupp et al., 2023; Lee et al., 2020).

To investigate the effect of carboplatin on TDP-43-induced toxicity *in vivo*, we treated flies expressing TDP-43 with carboplatin. *Drosophila* larvae expressing TDP-43 in glial cells were raised on food supplemented with carboplatin throughout their larval stages. Flies with glial cell-specific TDP-43 expression driven by *Repo-Gal4* exhibited significant locomotor deficits (Fig. 4A). However, carboplatin significantly mitigated TDP-43-induced locomotor dysfunction in the larvae (2 μ g/mL) (Fig. 4A). Immunoblotting assays of brain extracts from TDP-43-expressing adult flies revealed that carboplatin did not affect TDP-43 protein levels (Fig. 4B). To determine whether carboplatin affects the localization of TDP-43 in neuronal cells, we performed subcellular

fractionation assays to measure the TDP-43 levels in the cytoplasm and nucleus. Notably, carboplatin did not affect TDP-43 levels in either the cytoplasmic or nuclear fractions (Fig. 4C). These results suggest that carboplatin restores TDP-43-induced locomotor defects independently of TDP-43 protein. Collectively, our results showed that carboplatin effectively protects glial cells against TDP-43-induced toxicity, highlighting its potential as a therapeutic agent for TDP-43-associated proteinopathies.

3.5. Carboplatin attenuates neuroinflammation induced by glial TDP-43 expression in *Drosophila*

We next investigated whether glial cell-specific expression of TDP-43 in flies elicits an innate immune response accompanied by neuroinflammatory activation, and whether carboplatin treatment can suppress this response. In *Drosophila*, the Toll and immune deficiency (Imd) signaling pathways are key components of the innate immune system, mediating proinflammatory responses by regulating the production of antimicrobial peptides (AMPs) (De Gregorio et al., 2002; Ferrandon et al., 2007). Activation of these pathways requires induction of transcription factors Dorsal and Relish, the *Drosophila* homolog of mammalian NF- κ B (Kleino and Silverman, 2014). These transcription factors regulate the expression of most inflammatory genes, including AMPs such as *Diptericin*, *Cecropin*, and *Attacin*, which function analogously to proinflammatory cytokines during inflammation (Ferrandon et al., 2007). Overexpression of TDP-43 in glial cells significantly increased the mRNA expression of *Dorsal* and *iNOS*. Carboplatin treatment attenuated the expression of TDP-43-induced inflammatory genes without altering TDP-43 expression levels (Fig. 4D). As elevated AMP expression is a hallmark of innate immune and inflammatory activity, we quantified the relative mRNA expression of AMPs in TDP-43-expressing flies. Compared to the control, glial-specific expression of TDP-43 led to a significant upregulation of *Diptericin B* and *Attacin C* mRNA in the brain. Importantly, carboplatin treatment markedly suppressed the upregulation of *Diptericin B* and *Attacin C* in the brains of TDP-43-expressing flies (Fig. 4D). Consistent with the protein level of TDP-43, the expression of TDP-43 mRNA was not affected by carboplatin (Fig. 4D).

Next, we assessed the effects of carboplatin in an *in vivo* *Drosophila* model expressing ALS-associated TDP-43 Q331K mutant. Targeted expression of mutant TDP-43 in the eye was driven by the *GMR-Gal4* system. Consistent with its pathogenic role, overexpression of TDP-43 Q331K resulted in a rough-eye phenotype characterized by disrupted ommatidial organization and pigmentation loss. Notably, carboplatin treatment significantly ameliorated both the rough-eye phenotype and pigmentation defects in TDP-43 Q331K-expressing flies (Supplementary Fig. S4C). Collectively, these results indicate that carboplatin effectively suppresses the neuroinflammatory response associated with glial TDP-43 expression *in vivo*.

4. Discussion

Neuroinflammation is a hallmark of many neurodegenerative diseases in which glial cells, particularly microglia and astrocytes, are activated in response to neuronal injury or stress (Adamu et al., 2024; Cekanaviciute and Buckwalter, 2016). Chronic neuroinflammation contributes to the progression of various neurodegenerative diseases, including AD, PD, and ALS. Glial cells are involved in the pathogenesis of these diseases by exacerbating inflammatory responses and promoting neuronal damage (Adamu et al., 2024). The activation of astrocytes and microglia by toxic proteins such as TDP-43 and FUS leads to the release of various pro-inflammatory molecules that perpetuate neuronal injury (Ajmone-Cat et al., 2019; Correia et al., 2015). The NF- κ B pathway is a critical regulator of neuroinflammation, and its activation leads to the transcription of several inflammatory genes involved in the immune responses, including cytokines. TDP-43 activates the NF- κ B

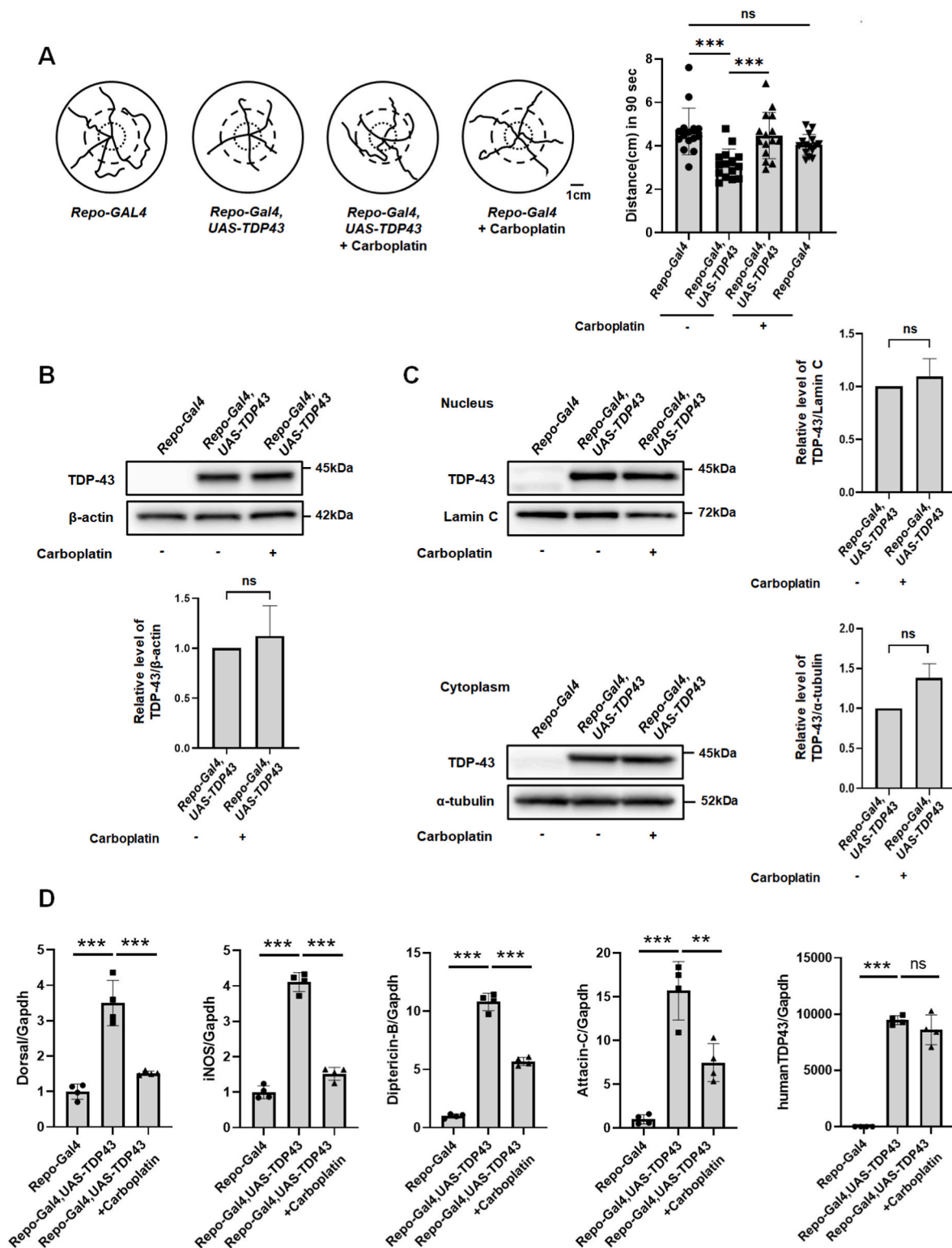


Fig. 4. Carboplatin alleviates TDP-43-induced toxicity in glial cells of *Drosophila*. (A) Larvae expressing TDP-43 in glial cells were treated with different concentrations of carboplatin. Movement of the 3rd instar larvae was monitored for 90 s, and average distance of the larval trail was calculated ($n > 15$ each). Data are presented as the mean $\pm$ standard deviation (SD). *** $p < 0.001$; ns, not significant (one-way ANOVA, followed by Tukey's multiple-comparison test). (B) Immunoblots showing TDP-43 protein levels in head extracts from adult flies. β -actin was used as a loading control. Data are presented as the mean $\pm$ SD of triplicate samples. ns., not significant (Student's t -test). (C) Immunoblot analysis of nuclear and cytoplasmic TDP-43 protein levels in the adult fly head extracts. Lamin C and α -tubulin were used as loading controls for the nuclear and cytoplasmic fractions, respectively. Data are presented as the mean $\pm$ SD of triplicate samples. ns., not significant (Student's t -test). (D) *Repo-Gal4* and *Repo-Gal4/UAS-TDP-43* transgenic flies were treated with carboplatin (2 μ g/mL) for 5 d, after which total RNA was isolated from fly heads and subjected to qRT-PCR analysis. mRNA levels of *Dorsal*, *iNOS*, *Dipteracin B*, and *Attacin C* were quantified. Carboplatin did not affect *TDP-43* mRNA expression. *Gapdh* was used as an internal control for normalization. Data are presented as the mean $\pm$ SD. ** $p < 0.005$ and *** $p < 0.001$; ns, not significant (one-way ANOVA, followed by Tukey's multiple-comparison test).

signaling pathway, resulting in the production of pro-inflammatory cytokines that exacerbate inflammatory responses and neuronal damage (Lee et al., 2020). The overproduction of ROS, including NO, is another critical aspect of TDP-43-induced neuroinflammation. Astrocytes are particularly susceptible to these inflammatory mediators, which further exacerbate the pathological progression of neurodegenerative diseases (Vandenbark et al., 2021). Considering the central role of inflammation in the pathogenesis of TDP-43-associated diseases, targeting inflammatory pathways is a potential therapeutic strategy for these diseases.

In this study, we investigated whether neuroinflammation induced by TDP-43 expression in astrocytes is alleviated by carboplatin. Carboplatin is a second-generation, platinum-based anti-cancer drug approved for the treatment of several cancer types (Zhang et al., 2022). Although carboplatin is primarily used in oncology, it also exerts neuroprotective effects on non-cancerous tissues and organs, including the CNS (Potenza et al., 2024). We recently demonstrated that carboplatin reduces FUS aggregation and apoptotic cell death of neurons in the brain tissue of a *Drosophila* ALS model (Cha et al., 2022). Carboplatin increases FUS solubility in neurons of FUS-expressing fly brains (Cha et al., 2022). These reports suggest possible therapeutic effects of carboplatin against ALS. Despite limited direct evidence of its anti-inflammatory effects, carboplatin effectively alleviated LPS-induced inflammation in mouse astrocytes in this study, possibly by suppressing inflammatory responses. Additionally, carboplatin reduced NF- κ B phosphorylation and nuclear translocation induced by TDP-43 in astrocytes, thereby inhibiting inflammatory pathway activation (Fig. 1B and C). Furthermore, carboplatin reduced TDP-43-induced synthesis and secretion of pro-inflammatory cytokines from astrocytes (Fig. 2). Increased glial cell-mediated neuroinflammation is a key pathological process in neurodegenerative diseases that leads to the elevated secretion of pro-inflammatory cytokines that contributes to neuronal damage (Vandenbark et al., 2021). To investigate the effects of carboplatin in this process, we treated mouse neurons with culture media containing various pro-inflammatory cytokines secreted by TDP-43-expressing astrocytes and assessed neuronal survival. Carboplatin significantly improved neuronal survival and restored mitochondrial function in neurons induced by astrocytic TDP-43 (Fig. 3). We also induced TDP-43 overexpression in astrocytes and examined mitochondrial dysfunction in neurons treated with culture media containing the cytokines secreted by these astrocytes. Carboplatin alleviated TDP-43-associated phenotypic alterations, including the reduction in the mitochondrial respiration rate and ATP synthesis in neurons, thereby mitigating inflammation-induced mitochondrial dysfunction (Fig. 3). Notably, reduced cellular respiration and ATP production are hallmarks of ALS. Mitochondrial dysfunction promotes neuroinflammation by increasing ROS production, creating a vicious cycle that accelerates disease progression (Lin et al., 2022). Furthermore, TDP-43 pathology is associated with the dysregulation of the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway, the master regulator of antioxidant defenses (Lastres-Becker et al., 2022). Whether this involves a direct suppression or an insufficient compensatory increase in Nrf2 activity, the resulting disruption in redox homeostasis further exacerbates the oxidative burden. In this context, the restoration of mitochondrial function by carboplatin likely helps alleviate this ROS accumulation, thereby compensating for the compromised antioxidant defenses and breaking the pathological cycle. Our findings indicate that TDP-43 expression significantly impairs mitochondrial oxygen consumption in neurons, and that carboplatin restores both ATP production and maximal respiratory capacity in these cells. Collectively, our results suggest that carboplatin exerts protective effects by inhibiting NF- κ B phosphorylation in astrocytes, thereby alleviating inflammation and mitochondrial dysfunction in the surrounding neurons caused by pro-inflammatory cytokine secretion. Furthermore, in a *Drosophila* disease model, carboplatin significantly restored the locomotor activity impaired by glial-specific TDP-43 expression. These results suggest that carboplatin exerts similar effects in both *in vivo* *Drosophila* and *in vitro* cellular models. However, in both mouse glial

cells and *Drosophila* models, carboplatin did not affect the cytoplasmic mis-localization of TDP-43. Therefore, although carboplatin does not directly modulate the pathophysiology of TDP-43 aggregation and mis-localization, it mitigates TDP-43-induced astrocyte-mediated neurotoxicity by improving mitochondrial function and reducing oxidative stress. Furthermore, carboplatin treatment mitigated the aberrant cytokine release and neurotoxic effects induced by ALS-associated TDP-43 Q331K mutation in primary astrocytes. Notably, a similar protective effect against the neurotoxicity induced by mutant TDP-43 was observed in the *Drosophila* disease model. Overall, our findings suggest that carboplatin, a chemotherapeutic agent, is a promising therapeutic target for TDP-43-associated proteinopathies such as ALS. Although our study focused primarily on ALS, cytoplasmic TDP-43 aggregation is a common pathological hallmark of frontotemporal dementia and limbic-predominant age-related TDP-43 encephalopathy. Therefore, a therapeutic strategy that targets the astrocytic TDP-43-mediated inflammatory axis may have broader implications for these related TDP-43 proteinopathies.

While our previous studies demonstrated that protein tyrosine phosphatase 1B (PTP1B) inhibition mitigates TDP-43-induced neuroinflammation by modulating specific phosphatase-related signaling (Lee et al., 2020), the current study identifies carboplatin as a distinct and potent modulator of the NF- κ B-mediated astrocytic response. Unlike PTP1B inhibitors, which are largely in the preclinical stage, carboplatin is an FDA-approved agent that offers a more rapid path for clinical translation through drug repurposing. These findings suggest that carboplatin may provide a broader or complementary therapeutic window for the treatment of TDP-43-associated neurodegeneration.

Drug repurposing offers a cost-effective and time-efficient strategy for identifying novel therapeutics, particularly for incurable neurodegenerative diseases, such as ALS, where the development of new chemical entities often fails in late-stage clinical trials (Carroll et al., 2025). Several repurposed drugs, including edaravone, masitinib, and lithium, which primarily target oxidative stress, neuroinflammation, or protein aggregation have been evaluated for the treatment of ALS (Cha and Kim, 2022; Xu et al., 2021). In contrast to these approaches, the present study identifies carboplatin, a clinically approved anticancer agent, as a novel repurposing candidate that mitigates TDP-43-induced astrocyte-mediated neurotoxicity through NF- κ B pathway inhibition and restoration of mitochondrial function. To the best of our knowledge, this is the first report demonstrating such effects of carboplatin in ALS-relevant models, thus expanding the therapeutic scope of drug repurposing strategies for TDP-43 proteinopathies.

Despite the promising effects of carboplatin observed *in vitro* and *Drosophila* models, its poor blood-brain barrier (BBB) permeability and known systemic toxicity limit its direct clinical applicability for neurodegenerative diseases (Drean et al., 2016; Jacobs et al., 2005). The concentrations used in this study may not be readily achievable in the CNS through standard systemic administration without causing adverse effects. Therefore, carboplatin should be considered primarily as a tool compound to demonstrate that the pharmacological inhibition of NF- κ B in astrocytes can mitigate TDP-43-induced neurotoxicity. To address these pharmacokinetic limitations and facilitate clinical translation, future research should focus on advanced delivery strategies designed to enhance CNS bioavailability, while minimizing systemic exposure. For instance, the development of lipophilic platinum (IV) prodrugs that undergo intracellular reduction to their active platinum (II) forms (Jimenez-Macias et al., 2022), or the use of nanoparticle-based carriers functionalized with ligands to exploit receptor-mediated transcytosis, could significantly improve BBB penetration (Wiley et al., 2013). Furthermore, integrating non-invasive techniques such as magnetic resonance-guided focused ultrasound to transiently permeabilize the BBB offers a localized approach to increase carboplatin concentrations in affected brain regions (Hynynen et al., 2001; Lipsman et al., 2018). These approaches may facilitate the translation of the mechanistic insights gained through this study into clinically viable therapeutic

interventions for TDP-43-associated proteinopathies.

Consent for publication

All authors have read the manuscript and agree for publication.

Ethics approval and consent to participation

Not applicable.

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CRedit authorship contribution statement

Jihye Song: Data curation, Formal analysis, Investigation, Methodology. **Shinrye Lee:** Data curation, Formal analysis, Investigation, Methodology. **Jiyoung Lee:** Data curation, Investigation. **Yaeeun Gil:** Data curation, Investigation. **Taehyun Kim:** Data curation, Investigation. **Seung-Yoon Kim:** Data curation, Investigation. **Sun Joo Cha:** Data curation, Investigation. **Hyung-Jun Kim:** Supervision, Writing – review & editing. **Kiyoung Kim:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ejphar.2026.178968>.

Data availability

Data will be made available on request.

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