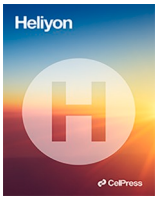
Heliyon 10 (2024) e31378

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Neuroprotective effects and mechanisms of action of artemisinin in retinal ganglion cells in a mouse model of traumatic optic neuropathy

Shirui Zhou [a], Wangzi Li [b], Ruohan Lv [c], MingChang Zhang [a,**], Wei Liu [a,*]

[a] Department of Ophthalmology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China
[b] Department of Cardiovascular Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China
[c] Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

ARTICLE INFO

Keywords:
Artemisinin
Optic nerve crush
Retinal ganglion cells
Tau protein
Traumatic optic neuropathy

ABSTRACT

Introduction: Traumatic optic neuropathy is known to be a critical condition that can cause blindness; however, the specific mechanism underlying optic nerve injury is unclear. Recent studies have reported that artemisinin, considered vital in malaria treatment, can also be used to treat neurodegenerative diseases; however, its precise role and mechanism of action remain unknown. Therefore, in this study, we aimed to investigate the impact and probable mechanism of action of artemisinin in retinal ganglion cells (RGCs) in a mouse model of traumatic optic neuropathy induced by optic nerve crush (ONC).

Methods: ONC was induced in the left eye of mice by short-term clamping of the optic nerve; oral artemisinin was administered daily. The neuroprotective effect of the drug was assessed using Tuj-1 staining in RGCs. In addition, the inflammatory response and the expression levels of phosphorylated tau protein and tau oligomers were observed using RT-qPCR, TUNEL assay, and fluorescence staining to investigate the underlying mechanisms.

Results: Artemisinin increased the survival rate of RGCs 14 days after ONC. Artemisinin significantly reduced the levels of inflammatory factors such as CXCL10, CXCR3, and IL-1β in the retina and decreased the apoptosis of RGCs. Moreover, downregulation of the phosphorylation of tau proteins and the expression of tau oligomers were observed after artemisinin treatment.

Conclusion: Our results suggest that artemisinin can increase the survival rate of RGCs after ONC and reduce their apoptosis. This effect may be achieved by inhibiting the inflammatory response it triggers and downregulating tau protein phosphorylation and tau oligomer expression. These findings suggest the potential application of artemisinin as a therapeutic agent for neuropathy.

1. Introduction

The retina and optic nerve are essential structures for visual transmission. The axons of retinal ganglion cells (RGCs) make up the majority of the optic nerve. The intracanalicular segment is the section of the optic nerve that passes through the small optic canal and

* Corresponding author.
** Corresponding author.
E-mail addresses: m202275984@hust.edu.cn (S. Zhou), noharakikoz@163.com (W. Li), flarie@foxmail.com (R. Lv), mingchangzhang@hotmail.com (M. Zhang), weiliu0113@hust.edu.cn (W. Liu).

https://doi.org/10.1016/j.heliyon.2024.e31378
Received 21 December 2023; Received in revised form 14 May 2024; Accepted 15 May 2024
Available online 21 May 2024

is particularly susceptible to crush from trauma [1]. Schwann cells do not exist in the optic nerve; hence, the optic nerve and RGCs barely regenerate after injury, leading to irreversible visual impairment [2]. Traumatic optic neuropathy (TON) is a major cause of vision loss, wherein the RGCs are substantially lost [3]. Optic nerve injury can be divided into direct and indirect types according to the anatomical damage and into primary and secondary types according to the mechanism of injury [4]. TON leads to the progressive death of RGCs, causing irreversible vision loss in patients. However, existing theories are unable to explain the specific mechanism underlying optic nerve injury and RGC death. Therefore, commonly used clinical treatments rarely aid vision [5].

Tau protein is the primary microtubule-associated protein in mature neurons and is essential for microtubule assembly. Hyperphosphorylation inhibits the biological activity of tau protein [6]. Hyperphosphorylated tau proteins tend to detach from microtubules and aggregate into tau oligomers that translocate from axons to dendrites and the cytosol, interfering with normal neuronal function [7]. In central neurodegenerative diseases, tau oligomers are neurotoxic; injecting exogenous tau proteins into neural tissues triggers neurodegenerative changes, suggesting that tau proteins are crucial in the pathogenesis of neurodegenerative diseases [7–9]. The tau protein has recently been found to play a role in glaucoma- and trauma-induced optic nerve damage [10,11].

Artemisinin is vital in malaria treatment, and artemisinin-based combination therapies are among the first-line, effective, and safe therapies for malaria [12]. However, recent studies have also revealed other properties of artemisinin, such as its anti-tumour and anti-inflammatory properties, as well as its protective role in autoimmune and neurodegenerative diseases such as Alzheimer (Deichelbohrer, 2017 #5)'s disease (AD) [13]. Artemisinin has been shown to protect RGC-5 cells in vitro, reduce cellular oxidative stress levels, and restore the physiological function of the rat retina following light exposure injury [14]. Furthermore, artemisinin and its derivative artemether can inhibit the phosphorylation of tau protein in a mouse model of AD [15,16].

Based on these findings, we hypothesised that artemisinin improves the survival of RGCs after optic nerve crush (ONC). To test this hypothesis, we established an ONC model using C67BL/6J mice and compared changes in RGC survival, inflammatory response, tau protein phosphorylation, and tau protein oligomer formation after artemisinin treatment.

2. Materials and methods

2.1. Animals

All *in vivo* animal experiments were approved by the Experimental Animal Ethics Committee of the Tongji Medical College, Huazhong University of Science and Tech-nology, China. A total of 52 8–10-week-old male C57BL/6J mice (weight 22–24 g) were procured from the Experimental Animal Centre of Tongji Medical College, Huazhong University of Science and Technology (Wuhan, China) and housed in SPF-class animal rooms with standard light and dark cycles (L:D = 12 h:12 h) at appropriate temperature and humidity. Food and water were promptly replenished and were freely available.

All animals were divided into operation (left eye) and sham operation (right eye) groups and further subdivided into the experimental group treated with artemisinin and the control group administered solvent (i.e., 10 % dimethyl sulfoxide (DMSO) + 90 % corn oil). Therefore, they were divided into the control-vehicle (Con-Veh) (26 in total) , optic nerve crush-vehicle (ONC-Veh) (26 in total) , control-artemisinin (Con-ART) (26 in total), and optic nerve crush-artemisinin (ONC-ART) (26 in total) groups. The animal experiments were conducted in compliance with the guidelines of the Association for Research in Vision and Ophthalmology for the Use of Animals in Ophthalmic and Vision Research.

2.2. Surgical procedures

We chose a well-established modelling approach successfully simulated in several experiments on multiple animals [17,18]. After anaesthesia with sodium pentobarbital (1 %, 40 mg/kg) and oxybutynin hydrochloride drops (0.4 %), each animal's left posterior bulbar optic nerve was exposed by temporal separation of the bulbar conjunctiva and sclera, and the optic nerve was clamped with microscopic toothless platform forceps 2 mm posterior to the bulb for 15 s. Carprofen (5 mg/kg) (Maclin, Shanghai, China) was injected subcutaneously once a day for analgesia after the procedure. Animals that underwent surgery in the left eye were included in the operation group. The right eye was used in a sham operation group (control group), with its optic nerve separated without clamping.

2.3. Drug application

The drug was administered by gavage at 200 mg/kg/d. The experimental group was administered 100 mg/kg of artemisinin solution each time, which was dissolved in 1 mL of DMSO and made into a 10 mg/L solution with 9 mL of corn oil before use. The control group was administered an equal amount of solvent.

Groups of mice whose specimens were collected at 9 h after surgery were administered drug or solvent by gavage from the day before surgery, i.e., 24 h pre-operatively, 12 h pre-operatively, 4 h pre-operatively, and 4 h post-operatively.

Those whose specimens were collected 3 days after surgery were administered drug or solvent by gavage from the day before surgery, i.e., 24 h before surgery, 12 h before surgery, 12 h after surgery, 24 h after surgery, 36 h after surgery, 48 h after surgery, 60 h after surgery, and 72 h after surgery. In addition, the mice whose specimens were collected 14 days after surgery were dosed twice daily from the day before surgery until day 14.

2.4. Counting of surviving RGCs

Artemisinin- or solvent-treated animals were sacrificed 14 days post-operatively using pentobarbital overdose anaesthesia. The retina was removing from the eye, fixed overnight with 4 % paraformaldehyde, and then the retina was peeled from the choroid and sclera and cut into a four-leaf clover shape. Subsequently, the retinal tissues were placed in 48-well cell culture plates, washed twice with phosphate-buffered saline (PBS) for 5 min each time, blocked with a 20:1 solution of 0.3 % Triton™ X-100 (Solarbio Life Sciences, China) to goat serum (Beyotime Biotechnology, China), and incubated overnight at 4 °C with primary antibody (see Table 1). Next, the specimen was washed twice with PBST, once with PBS, incubated with the secondary antibody for 4 h at 4 °C, and rewashed twice and once with PBST and PBS, respectively. The retinal ganglion cell layer (GCL) was placed upward at the centre of the slide and sealed. The Tuj-1-labelled surviving RGCs were counted using a laser-scanning confocal microscope (Leica Microsystems, IL, USA). Six to eight fields of view (1–2 fields per leaflet) with a relatively uniform distribution in the peripheral part of the tetrahymena retina were selected, and the number of RGCs in each field of view was counted and averaged. The researchers were blinded to the different mouse samples.

2.5. Assessment of inflammatory factor infiltration

Moreover, 12 h post-operatively, the artemisinin- or solvent-treated animals were sacrificed after pentobarbital overdose anaesthesia; retinal tissue was collected, and TRIzol (Nanjing Vazyme Biotech, China) was added to a volume of 1 mL. The TRIzol and tissues were mixed completely and 200 μL of chloroform was added. Subsequently, the cells were mixed and centrifuged at 15,000×*g* for 15 min at 4 °C in an ultracentrifuge (Xiangyi Centrifuge Instrument, China). The upper layer of the colourless and clear liquid was placed in 2 mL Eppendorf tubes, and isopropyl alcohol was added at 1:1 and mixed well. Next, the supernatant was removed; 1 mL of 75 % alcohol was added to each tube and the white sediment dissolved, then centrifuged at 15,000×*g* for 10 min. The procedure was repeated once, and the supernatant was removed and allowed to stand for 10 min. Furthermore, 50 μL of RNAase-free water (Nanjing Vazyme Biotech, China) was added to the supernatant to dissolve by shaking. The obtained RNA was reverse-transcribed into cDNA using an RNA extraction kit (DP431, Tiangen Biotech, Beijing, China). RT-qPCR was performed using 2× ChamQ SYBR qPCR Master Mix (Nanjing Vazyme Biotech, China) to detect the gene expression levels according to the manufacturer's instructions. Reaction volume is 10 μ L. Including 3 μ L cDNA,5 μ L 2× ChamQ SYBR qPCR Master Mix, 1.5 μ L ultra pure water and 0.5 μ L for each primer. Hprt served as an internal reference gene. Primer sequences are listed in Table 2.

To quantified the relative expression, we used the Con-Veh group as a control. First, Δt = target gene-internal reference gene. Then, $\Delta\Delta t = \Delta t$ for each group $-\Delta t$ for Con-Veh group. Finally, the relative expression was $2\text{-}\Delta\Delta t$.

2.6. Assessment of inflammatory cell recruitment

At 9 h after surgery, the mice were anaesthetised to expose the heart and isolate the right and left common carotid arteries. The thoracic cavity was flushed with pre-warmed heparinised PBS, and 0.2 mL sodium heparin working solution (SPH No. 1 Biochemical & Pharmaceutical Co., Ltd., China) was injected into the left ventricle. A perfusion hose was inserted from the apex of the heart to perfuse the pre-warmed Con A solution (see Table 1). Subsequently, both eyes were removed and fixed overnight, and the retinal pavement was made by avoiding light. Con A labelled blood vessels and leukocytes in the lining of blood vessels. CD45 antibodies (BD Pharmingen™, NJ, USA) (see Table 1) were used to stain the leukocytes. Adherent leukocytes means leukocytes inside the vassels and infiltrating leukocytes means leukocytes paravascular. The process of CD45 staining is the same as 2.4. Intravascular adhesion leukocytes and perivascular infiltrating leukocytes were counted using ImageJ software (National Institutes of Health, USA).

2.7. Evaluation of apoptosis in RGCs

Three days post-operatively, the artemisinin- or solvent-treated animals were sacrificed using pentobarbital overdose anaesthesia, and the eyes were removed and fixed in 4 % paraformaldehyde for 24 h. After gradient dehydration using alcohol and anhydrous

Table 1
Antibodies used in this study.

Antibody	Source	Identifier
Anti-TuJ-1 antibody	BioLegend	# 801,201
Anti-CD45 antibody	BD Pharmingen™	#550539
Rhodamine labeled Concanavalin(Con A)	Vector Laboratories	#RL-1002
Anti-Tau antibody	Millipore	# ABN454
Anti-p-Tau(Thr231) antibody	Thermo Scientific	#MN1040
Goat Anti-rat Alexa Fluor 488	Abcam	#ab150157
Goat Anti-rabbit Alexa Fluor 488	Thermo Fisher	#A11008
Goat Anti-mouse Alexa Fluor 488	Thermo Fisher	#A11001

Table 2
Sequences of the primers used for RT-qPCR.

Gene	Forward primers (5′–3′)	Reverse primers (5′–3′)
Cxcr3	TTGCCCTCCCAGATTTCATC	TGGCATTGAGGCGCTGAT
Cxcl10	CATCCCTGCGAGCCTATCC	CATCTCTGCTCATCATTCTTTTTCA
IL-1β	AGTTGACGGACCCCAAAAGA	GGACAGCCCAGGTCAAAGG
Hprt	GAAAGACTTGCTCGAGATGTCAT	CACACAGAGGGCCACAATGT

ethanol, the samples were treated with anhydrous ethanol and xylene mixed in equal proportions for 10 min and soaked twice in xylene for 8 min each. Ocular tissue blocks were immersed in paraffin wax for 1 h thrice. Placed the tissue in a paraffin embedded box. After the wax block cooled down, cut it into 4 μm wax slices using a rotary slicer (Leica Biosystems, IL, USA) and baked them in a 60 °C tissue spreading machine (Wuhan Junjie Electronics Co.,Ltd.,China) for 3 h. The sections were immersed in 0.1 % Triton X-100 for 15 min and washed with 1× PBS for 5 min. Subsequently, 50 μL of TUNEL reaction solution was added dropwise to the samples and incubated for 2 h at 37 °C in a wet box. The sections were washed twice with 1× PBST for 5 min each, followed by adding 100 μL DAPI staining solution (Beyotime Biotechnology, China), incubating for 20 min at 25 °C, and washing with PBS for 5 min. The slices were sealed with an anti-fluorescence quenching blocker (Wuhan antigene biotechnology Co. Ltd, China) and observed under a fluorescence microscope (Olympus, Tokyo, Japan). Finally, cells with positive Tunnel staining in the ganglion cell layer (GCL) of a single field of view were counted by Image J software (version 1.52a) (National Institutes of Health, USA).

2.8. Assessment of tau protein phosphorylation and oligomer formation

Sections were prepared using the specimens collected 3 days post-operatively. They were reheated, rinsed, blocked with goat serum diluted at 1:20 in PBS, and incubated with primary antibody(see Table 1) overnight at 4 °C. Subsequently, the sections were washed with PBS, incubated with the secondary antibody for 4 h at room temperature, and rewashed with PBS. Cell nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI) for 20 min, washed with PBS for 5 min, sealed with a fluorescent anti-bursting agent, and observed through microscopy. Three fields of view of the retinal limbal area were randomly selected for each section, observed using a laser confocal microscope, and photographed. Patterns of individual fields of view were recorded and the positive signal intensity of the GCL was analyzed with Image J software (version 1.52a) (National Institutes of Health, USA).

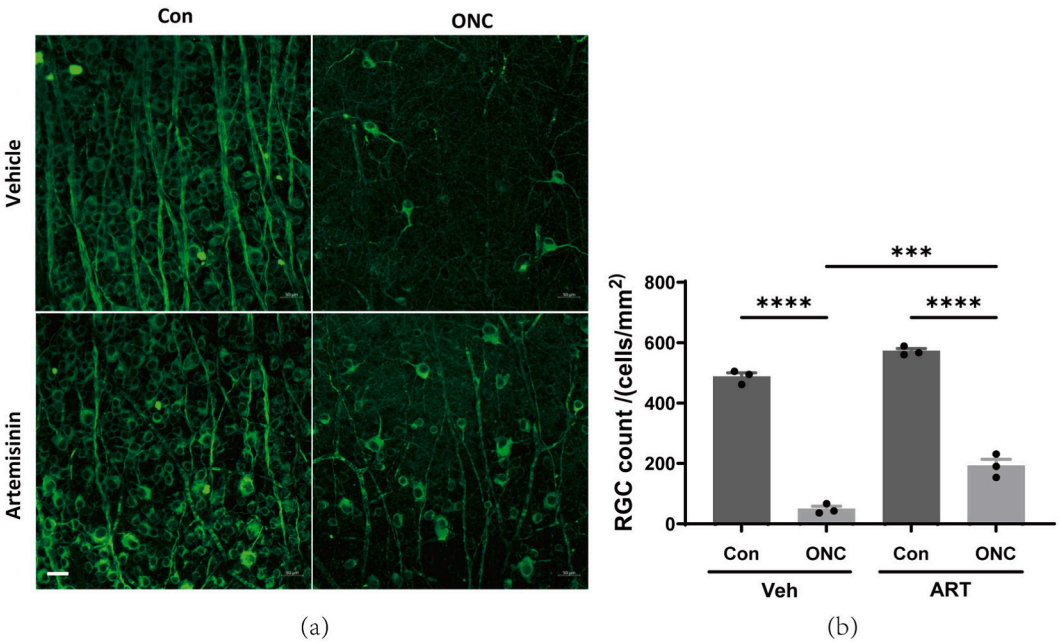


Fig. 1. Changes in the survival rate of RGCs after artemisinin treatment. (a) Representative plots of fluorescence staining of the peripheral part of the retina for each group; green (Tuj-1) indicates the RGC cytosol and axon, scale bar: 50 μm; (b) bar graphs indicate the mean number of RGCs per field of view (6–8 fields of view per specimen), n = 3 retinas, **: $p < 0.01$, ****: $p < 0.0001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.9. Statistical analyses

This study used GraphPad Prism 7.0 (GraphPad Software. Inc.) to statistically analyse and plot the obtained data. The results were expressed as the mean ± standard deviation (Mean ± SEM) of continuous variables. One-way Anova wasused to compare the means of the four groups. Statistics were judged significant at $P < 0.05$.

3. Results

3.1. Artemisinin has a protective effect on RGCs after ONC

To explore the role of artemisinin in optic nerve injury, we used a mouse ONC model and compared changes in RGCs in mice with and without artemisinin treatment. First, we demonstrated a significant reduction in RGCs after ONC by tuj-1 staining. Morphologically, the size and fluorescence intensity of normal RGCs are relatively uniform (Fig. 1a). Compared with the sham operation group, the cells in the operation group exhibited extensive cell death, shrinkage, light colouration, and morphological changes (Fig. 1a). Compared with mice in the Con-Veh group (487.15 ± 22.72 cells/mm^2), mice in the ONC-Veh group showed decreased survival of RGCs after 14 days post-operatively (48.88 ± 16.36 cells/mm^2) (Average reduction of 91.09 %) (Fig. 1b). Then, we gave mice artemisinin to explore its effects. For Con-ART and Con-Veh groups, there were no visible differences in cell morphology or staining (Fig. 1a), and no significant difference in the survival rate of RGCs (Fig. 1b). Compared with that in the ONC-Veh group, the survival rate of RGCs in the ONC-ART group increased 14 days post-operatively after treatment with artemisinin (191.6 ± 38,48 cells/mm^2) (Average increase of 291.98 %), and the difference was statistically significant ($p = 0.0005$, Fig. 1b). Indicating that artemisinin had a neuroprotective effect on damaged RGCs, and it had no apparent effect on normal RGCs.

3.2. Artemisinin can inhibit the inflammatory response

Inflammatory responses are crucial in post-traumatic retinal injury [19]. So we explored whether artemisinin can reduce the retinal inflammatory response after ONC. We first compared the expression levels of CXCR3, CXCL10, and IL-1β in each group. The results of RT-qPCR showed that, compared with those in the ONC-Veh group, the expression levels of inflammatory factors were significantly lower in the ONC-ART group (Fig. 2), with a percentage decrease of 45.5 %, 75.3 %, and 45.6 % in CXCR3, CXCL10, and IL-1β expression, respectively, indicating that the level of inflammation in the retina was suppressed after artemisinin treatment.

In addition to elevated levels of inflammatory factors, local retinal vascular validation is an important part in nerve injury, so we also compared the recruitment of leukocytes inside and outside the blood vessels in each group. The results of Con A and CD45 labelling of the retinal pavement showed almost no leukocyte adhesion in the retinal vessels in the sham operation group. However, after 9 h post-operatively, there was a significant increase in intravascular leukocyte adhesion and perivascular leukocyte infiltration in the ONC-Veh group (265.45 ± 118.011 cells/mm^2) ($p < 0.0001$, Fig. 3a and b) (1086.77 ± 483.61 cells/mm^2) ($p < 0.0001$, Fig. 3c and. d). In contrast, the leukocyte adhesion status was reduced in the ONC-ART group compared with that in the ONC-Veh group (22.15 ± 18.08 cells/mm^2) ($p = 0.0003$, Fig. 3a and. b) (339.08 ± 183.16 cells/mm^2) ($p < 0.0001$, Fig. 3c and. d). These results indicate that artemisinin treatment reduced retinal inflammatory cell recruitment.

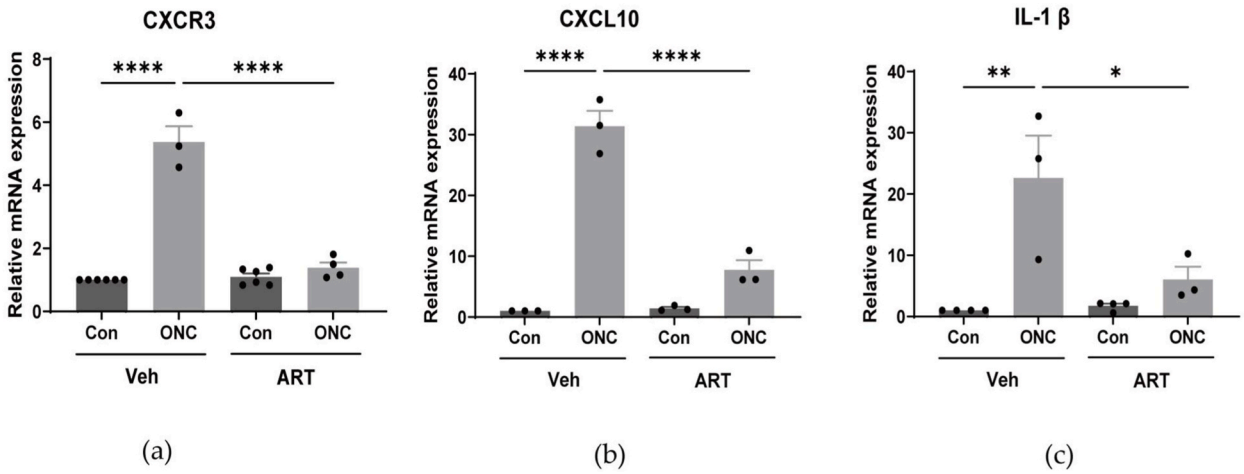


Fig. 2. Changes in the retinal inflammatory factor levels after artemisinin treatment. The bar graphs indicate the relative expression levels of each inflammatory factor: (a) relative expression levels of CXCR3; (b) Relative expression levels of CXCL10; (c) relative expression levels of lL-1β. Compared with those in the Con-Veh group, the changes in the Con-ART group were not significantly different; n = 3–6 retinas; *:$p < 0.05$,**: $p < 0.01$, ****: $p < 0.0001$.

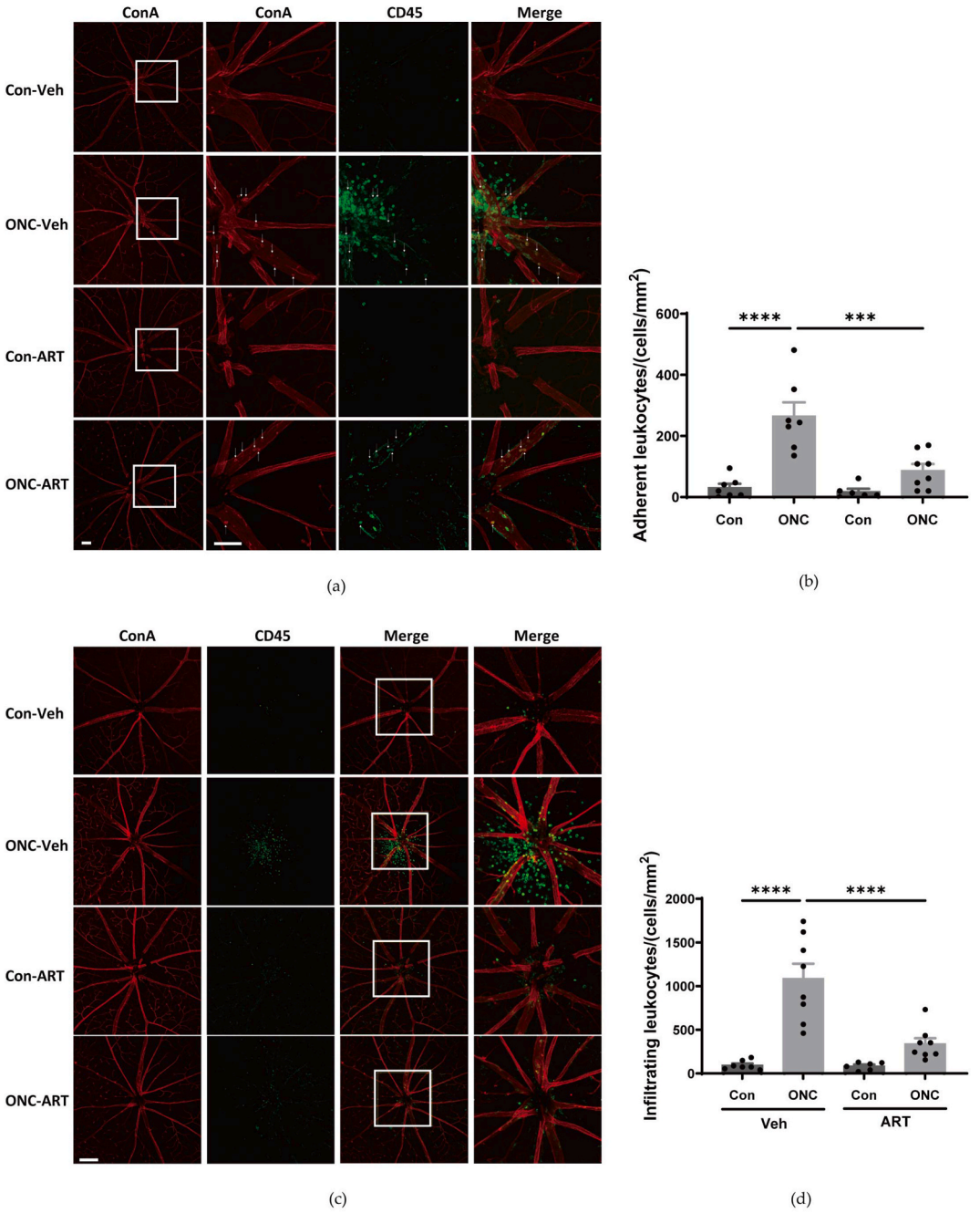


Fig. 3. Intravascular leukocyte adhesion and perivascular leukocyte infiltration in the central region of the retina. (a) Representative images of retinal plains labelled with CD45 antibody (green) and ConA(red) 9 h after ONC, The squares in the third row of images are magnified. Zoom bar: 50 μm. (b) Number of intravascular CD45-positive leukocytes in each field of view, n = 6–8 retinas. **: $p < 0.01$, ***: $p < 0.001$.(c) Representative images of retinal plains labelled with CD45 antibody (green) and ConA (red) at 9 h after ONC, The squares in the third row of images are magnified. Zoom bar: 100 μm. (d) Number of extravascular CD45-positive leukocytes in each visual field. n = 6–8 retinas. **: $p < 0.01$, ***: $p < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.3. Artemisinin reduces apoptosis of RGCs after ONC injury

To investigate whether artemisinin protects RGCs by inhibiting apoptosis, we per-formed TUNEL staining of retinas 3 days after ONC. In the sham operation group, a few apoptotic TUNEL (+) cells were observed in the GCL, whereas many positive cells appeared in the operation group and co-localized with the DAPI-labelled blue nuclei (Fig. 4a). Similarly, the number of apoptotic cells was

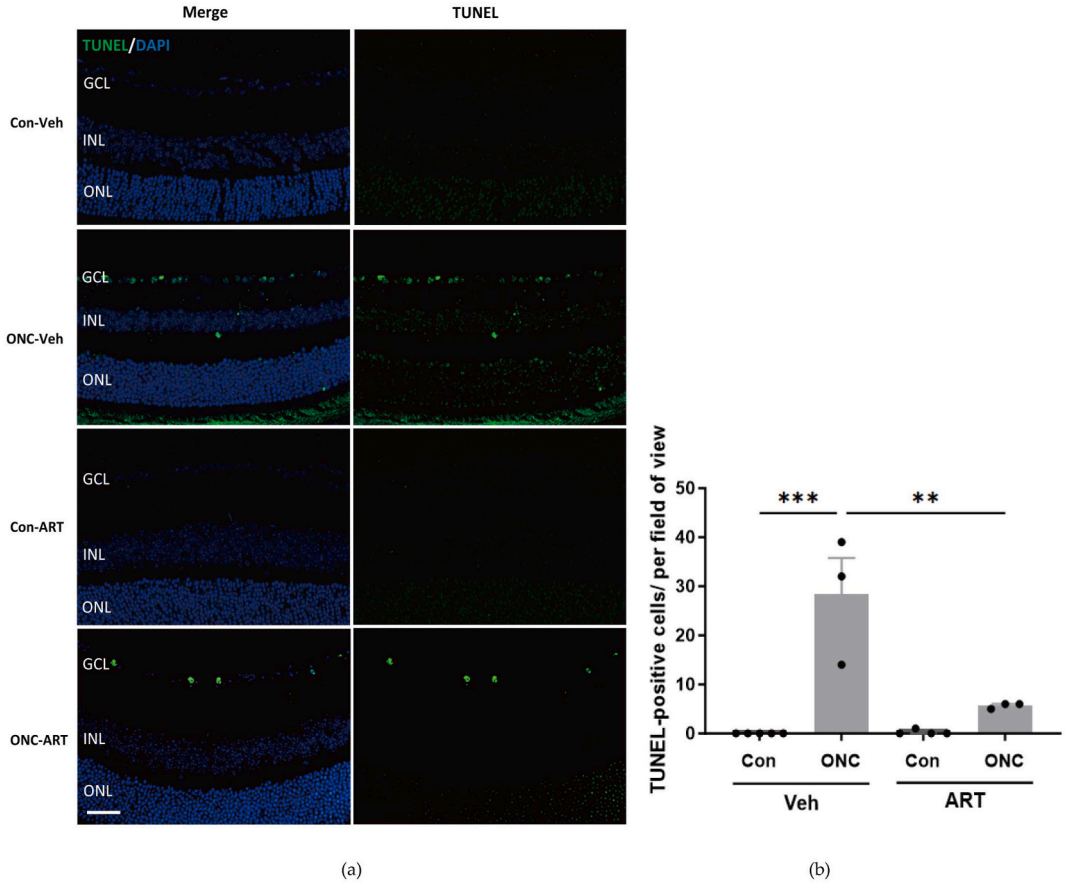
significantly reduced in the ONC-ART group (5.67 ± 0.58 per field of view) compared with that in the ONC-Veh group (28.33 ± 12.90 per field of view), with a significant difference of $p = 0.0018$ (Fig. 4b).

3.4. Artemisinin inhibits ONC-induced tau protein phosphorylation and tau oligomer formation

Recent studies have revealed that tau proteins play a vital role in neurodegenerative diseases, including TON. Related studies have confirmed that tau oligomers can regulate RGC injury; this finding indicates an essential novel mechanism underlying RGC injury [20]. We explored the role of tau proteins in artemisinin therapeutics. Immunofluorescence staining of eye specimens 3 days post-operatively showed that a certain level of tau protein phosphorylation occurred in the retina of the sham-operated group and was primarily concentrated in the GCL, with the expression region being the cytoplasm of RGCs surrounding the nucleus labelled with blue DAPI dye (Fig. 5a). Moreover, 3 days after ONC surgery, the expression level of phosphorylated tau protein in the cytoplasm of RGCs was significantly increased (Fig. 5a). The expression level of phosphorylated tau protein in the artemisinin-treated ONC-ART group (38.00 ± 6.68 AU) was lowered significantly, relative to that in the ONC-Veh group (49.50 ± 5.57 AU); however, it was higher than that in the sham operation group (Fig. 5b). In addition, staining of the GCL revealed green fluorescence in the cytoplasm of the ONC-Veh group 3 days after ONC surgery, suggesting positive tau oligomerisation (Fig. 5c). Similarly, tau oligomer formation was present in the ONC-Art group (Fig. 5c); however, the fluorescence intensity (6.00 ± 1.00 AU) was lower than that of the ONC-Veh group (12.67 ± 3.06 AU) ($p = 0.0103$, Fig. 5d).

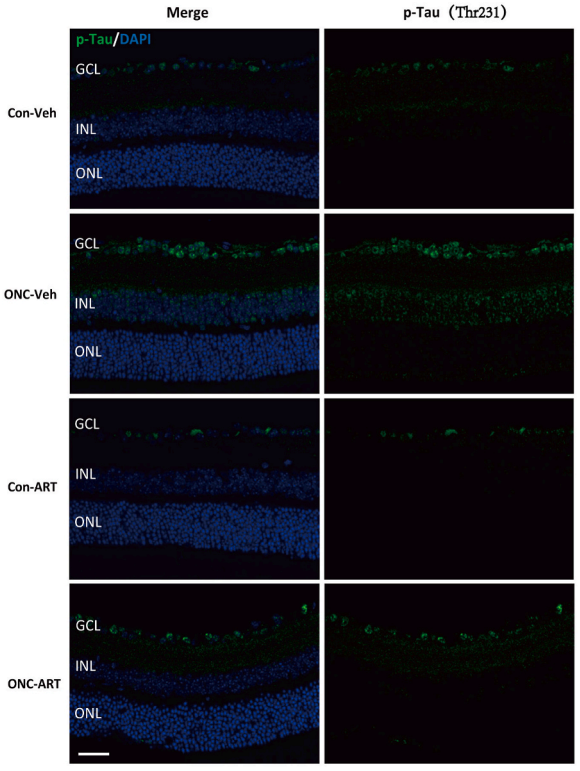
4. Discussion

TON plays an important role in visual loss caused by trauma, but there is currently no recognised effective treatment method. This article explored the role of artemisinin in TON. Our results indicated that daily administration of artemisinin increased the number of surviving RGCs on the seventh day after ONC surgery. At the same time, our assay of the apoptotic level of RGCs indicated a significant decrease in apoptosis of RGCs after artemisinin treatment, indicating that artemisinin has a protective effect on RGCs.

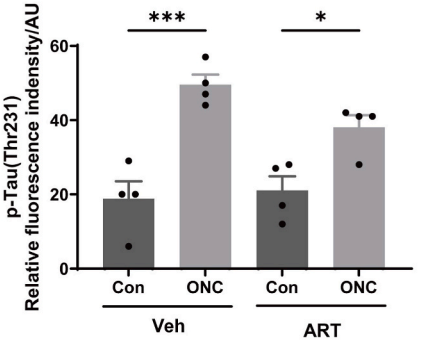


(a) (b)

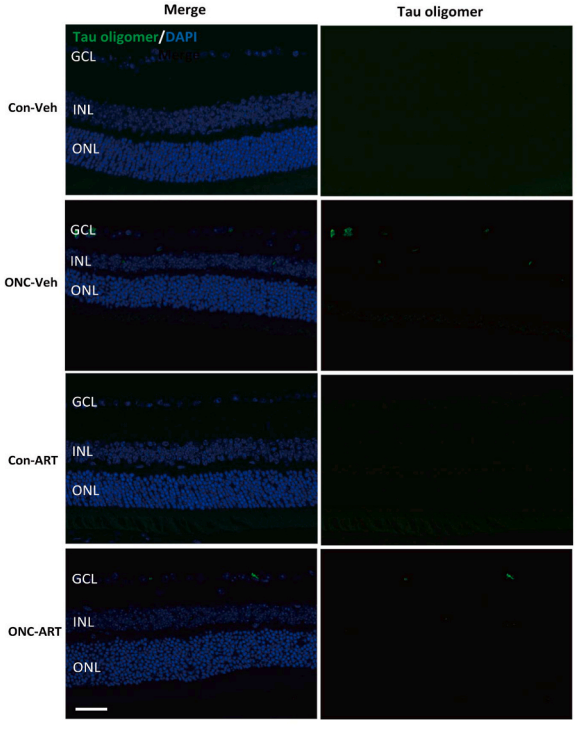
Fig. 4. Apoptosis of RGCs after artemisinin treatment. (a) Apoptosis of RGCs in the retina of each group at three days after ONC. Apoptotic cells are labelled in green (TUNEL) and nuclei in blue (DAP). Scale bars: 50 μm; (b) Number of TUNEL-positive cells, n = 3–5 retinas, **: $p < 0.01$, ***: $p < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)



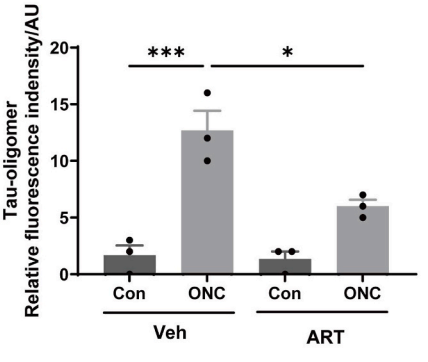
(a)



(b)



(c)



(d)

(caption on next page)

Fig. 5. Phosphorylation levels of tau protein and Levels of tau protein oligomer formation in RGCs after artemisinin treatment. (a) Phosphorylation levels of tau protein in RGCs of each retinal group at three days after ONC. Green (p-tau) indicates the phosphorylated tau protein in the cytoplasm, and blue (DAPl) indicates the nucleus. Scale bar: 50 μm; (b) relative fluorescence intensity of p-tau-positive cells, n = 4 retinas, *: $p < 0.05$. (c) Levels of tau protein oligomers in RGCs of each retinal group at 3 days after ONC. Cytoplasmic tau oligomers are labelled in green (tau oligomer) and nuclei in blue (DAPl). Scale bar: 50 μm; (d) Relative fluorescence intensity of tau oligomer-positive cells, n = 3 retinas, *: $p < 0.05$, ***: $p < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

The protective effect of artemisinin on RGCs may be related to the inhibition of the inflammatory response. Artemisinin has been recently shown to exert anti-inflammatory effects in various disease models [21,22]. In addition, it has been demonstrated that artemisinin can control the inflammatory response by inhibiting various pro-inflammatory factors, such as CXCR3/CXCL10 [23], IL-1β [24–26], NF-κB [27], Nrf2, and ROS-dependent p38 MARK [28]. Moreover, our experiments confirmed that artemisinin reduced the retinal levels of CXCR3, CXCL10, and IL-1β expression. The CXCL10/CXCR3 axis plays an essential role in the inflammatory response by recruiting activated T lymphocytes, monocytes, and macrophages [29–32]. Similarly, IL-1β, a lymphocyte-stimulating factor, facilitates T-cell activation and B-cell proliferation. Therefore, the decreased number of leukocytes observed in this experiment may be related to the inhibitory effect of artemisinin on the CXCR3/CXCL10 axis and inflammatory factors such as IL-1β.

Moreover, the protective effect of artemisinin on RGCs may be related to tau protein, a physiological protein that enhances microtubule assembly and stabilisation in neurons. Phosphorylation of the tau protein plays a crucial role in its function, and hyperphosphorylated tau proteins may cause pathological changes through various pathways, such as promoting oligomer formation [33]. Experiments have demonstrated that artemisinin can inhibit the phosphorylation of tau protein by inhibiting phosphorylation in the 3xtg mouse model of AD [15,16]. Therefore, we speculated that the effect of artemisinin on the survival rate of RGCs might be related to the tau protein.

The limitations of this experiment include a short observation period, failure to explore the specific mechanism of action of artemisinin, and the optimal dosage for achieving protective effects. Also the RT-qPCR in this paper was based on the whole retina and could not identify the cellular origin of the genetic alterations. comparisons of Tunnel, tau, and p-tau staining were also not localized to RGCs. Future experiments should focus on extending the observation cycle, exploring the specific relationship between inflammatory infiltration and tau protein with RGC cell death, as well as the concentration and method of artemisinin administration. Attempts should also be made to localize the study to RGCs. Further studies in this regard are warranted.

5. Conclusions

Our study shows that artemisinin enhances the survival rate of RGCs. The desired effect may be attained by preventing inflammatory reactions, lowering the level of tau protein phosphorylation, and reducing the expression of tau oligomers. Thus, the potential of artemisinin as a therapeutic agent for neuropathy is evident.

Ethical approval statement

The animal study protocol was approved by the Institutional Review Board (or Ethics Committee) of the Experimental Animal Ethics Committee of the Tongji Medical College, Huazhong University of Science and Technology (protocol code 3577, approval date: 31 December 2023).

Funding

This study was supported by National Natural Science Foundation of China (81900860, 82371061); Hubei Provincial Natural Science Foundation of China (2019CFB152, 2023AFB1092); and Science Research Foundation of Union Hospital (2022xhyn046).

Data availability statement

Not applicable, casuse data was included in article.

CRediT authorship contribution statement

Shirui Zhou: Writing – review & editing, Writing – original draft, Data curation. **Wangzi Li:** Software, Methodology, Data curation, Conceptualization. **Ruohan Lv:** Writing – original draft. **MingChang Zhang:** Writing – review & editing, Supervision, Conceptualization. **Wei Liu:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

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.

Acknowledgements

We would like to thank Editage (www.editage.cn) for English language editing.

References

[1] B. Chen, H. Zhang, Q. Zhai, H. Li, C. Wang, Y. Wang, Traumatic optic neuropathy: a review of current studies, Neurosurg. Rev. 45 (3) (2022) 1895–1913, https://doi.org/10.1007/s10143-021-01717-9.
[2] K.K. Gokoffski, P. Lam, B.F. Alas, M.G. Peng, H.R.R. Ansorge, Optic nerve regeneration: how will we get there? J. Neuro Ophthalmol. 40 (2) (2020) 234–242, https://doi.org/10.1097/WNO.0000000000000953.
[3] S. Lin, W. Gao, C. Zhu, et al., Efficiently suppress of ferroptosis using deferoxamine nanoparticles as a new method for retinal ganglion cell protection after traumatic optic neuropathy, Biomater. Adv. 138 (2022) 212936, https://doi.org/10.1016/j.bioadv.2022.212936.
[4] N. Sarkies, Traumatic optic neuropathy, Eye 18 (11) (2004) 1122–1125, https://doi.org/10.1038/sj.eye.6701571.
[5] K.D. Steinsapir, R.A. Goldberg, Traumatic optic neuropathy: an evolving understanding, Am. J. Ophthalmol. 151 (6) (2011) 928–933.e2, https://doi.org/10.1016/j.ajo.2011.02.007.
[6] D.F.V. Pîrşcoveanu, I. Pirici, V. Tudorică, et al., Tau protein in neurodegenerative diseases - a review, Rom. J. Morphol. Embryol. 58 (4) (2017) 1141–1150.
[7] Y. Wang, E. Mandelkow, Tau in physiology and pathology, Nat. Rev. Neurosci. 17 (1) (2016) 5–21, https://doi.org/10.1038/nrn.2015.1.
[8] L. Jiang, P.E.A. Ash, B.F. Maziuk, et al., TIA1 regulates the generation and response to toxic tau oligomers, Acta Neuropathol. 137 (2) (2019) 259–277, https://doi.org/10.1007/s00401-018-1937-5.
[9] J.E. Gerson, A. Mudher, R. Kayed, Potential mechanisms and implications for the formation of tau oligomeric strains, Crit. Rev. Biochem. Mol. Biol. 51 (6) (2016) 482–496, https://doi.org/10.1080/10409238.2016.1226251.
[10] Y. Ha, W. Liu, H. Liu, et al., AAV2-mediated GRP78 transfer alleviates retinal neuronal injury by downregulating ER stress and tau oligomer formation, Invest. Ophthalmol. Vis. Sci. 59 (11) (2018) 4670–4682, https://doi.org/10.1167/iovs.18-24427.
[11] C. Nucci, A. Martucci, A. Martorana, G.M. Sancesario, L. Cerulli, Glaucoma progression associated with altered cerebral spinal fluid levels of amyloid beta and tau proteins, Clin. Exp. Ophthalmol. 39 (3) (2011) 279–281, https://doi.org/10.1111/j.1442-9071.2010.02452.x.
[12] R.M. Fairhurst, A.M. Dondorp, Artemisinin-resistant plasmodium falciparum malaria, Microbiol. Spectr. 4 (3) (2016), https://doi.org/10.1128/microbiolspec.EI10-0013-2016 doi:10.1128/microbiolspec.EI10-0013-2016.
[13] L.H. Stockwin, B. Han, S.X. Yu, et al., Artemisinin dimer anticancer activity correlates with heme-catalyzed reactive oxygen species generation and endoplasmic reticulum stress induction, Int. J. Cancer 125 (6) (2009) 1266–1275, https://doi.org/10.1002/ijc.24496.
[14] F. Yan, H. Wang, Y. Gao, J. Xu, W. Zheng, Artemisinin protects retinal neuronal cells against oxidative stress and restores rat retinal physiological function from light exposed damage, ACS Chem. Neurosci. 8 (8) (2017) 1713–1723, https://doi.org/10.1021/acschemneuro.7b00021.
[15] X. Zhao, S. Li, U. Gaur, W. Zheng, Artemisinin improved neuronal functions in Alzheimer's disease animal model 3xtg mice and neuronal cells via stimulating the ERK/CREB signaling pathway, Aging Dis 11 (4) (2020) 801–819, https://doi.org/10.14336/AD.2019.0813.
[16] S. Li, X. Zhao, P. Lazarovici, W. Zheng, Artemether activation of AMPK/GSK3β(ser9)/Nrf2 signaling confers neuroprotection towards β-amyloid-induced neurotoxicity in 3xTg Alzheimer's mouse model, Oxid. Med. Cell. Longev. 2019 (2019) 1862437, https://doi.org/10.1155/2019/1862437.
[17] H. Oku, T. Kida, T. Horie, et al., Tau is involved in death of retinal ganglion cells of rats from optic nerve crush, Invest. Ophthalmol. Vis. Sci. 60 (6) (2019) 2380–2387, https://doi.org/10.1167/iovs.19-26683.
[18] J. Peng, J. Jin, W. Su, et al., High-mobility group box 1 inhibitor BoxA alleviates neuroinflammation-induced retinal Gan-glion cell damage in traumatic optic neuropathy, Int. J. Mol. Sci. 23 (12) (2022) 6715, https://doi.org/10.3390/ijms23126715.
[19] L.P. Evans, A.W. Woll, S. Wu, et al., Modulation of post-traumatic immune response using the IL-1 receptor antagonist anakinra for improved visual outcomes, J. Neurotrauma 37 (12) (2020) 1463–1480, https://doi.org/10.1089/neu.2019.6725.
[20] Y. Ha, W. Liu, H. Liu, et al., AAV2-mediated GRP78 transfer alleviates retinal neuronal injury by downregulating ER stress and tau oligomer formation, Invest. Ophthalmol. Vis. Sci. 59 (11) (2018) 4670–4682, https://doi.org/10.1167/iovs.18-24427.
[21] Y. Tu, Artemisinin-A gift from traditional Chinese medicine to the world (nobel lecture), Angew Chem. Int. Ed. Engl. 55 (35) (2016) 10210–10226, https://doi.org/10.1002/anie.201601967.
[22] C. Shi, H. Li, Y. Yang, L. Hou, Anti-inflammatory and immunoregulatory functions of artemisinin and its derivatives, Mediat. Inflamm. 2015 (2015) 435713, https://doi.org/10.1155/2015/435713.
[23] H. Liu, Q. Tian, X. Ai, et al., Dihydroartemisinin attenuates autoimmune thyroiditis by inhibiting the CXCR3/PI3K/AKT/NF-κB signaling pathway, Oncotarget 8 (70) (2017) 115028–115040, https://doi.org/10.18632/oncotarget.22854.
[24] W. Qiang, W. Cai, Q. Yang, et al., Artemisinin B improves learning and memory impairment in AD dementia mice by suppressing neuroinflammation, Neuroscience 395 (2018) 1–12, https://doi.org/10.1016/j.neuroscience.2018.10.041.
[25] S.K. Kim, J.Y. Choe, K.Y. Park, Anti-inflammatory effect of artemisinin on uric acid-induced NLRP3 inflammasome activation through blocking interaction between NLRP3 and NEK7, Biochem. Biophys. Res. Commun. 517 (2) (2019) 338–345, https://doi.org/10.1016/j.bbrc.2019.07.087.
[26] H. Long, B. Xu, Y. Luo, K. Luo, Artemisinin protects mice against burn sepsis through inhibiting NLRP3 inflammasome activation, Am. J. Emerg. Med. 34 (5) (2016) 772–777, https://doi.org/10.1016/j.ajem.2015.12.075.
[27] P. He, S. Yan, J. Zheng, et al., Eriodictyol attenuates LPS-induced neuroinflammation, amyloidogenesis, and cognitive impairments via the inhibition of NF-κB in male C57BL/6J mice and BV2 microglial cells, J. Agric. Food Chem. 66 (39) (2018) 10205–10214, https://doi.org/10.1021/acs.jafc.8b03731.
[28] H. Lu, B. Wang, N. Cui, Y. Zhang, Artesunate suppresses oxidative and inflammatory processes by activating Nrf2 and ROS-dependent p38 MAPK and protects against cerebral ischemia-reperfusion injury, Mol. Med. Rep. 17 (5) (2018) 6639–6646, https://doi.org/10.3892/mmr.2018.8666.
[29] H.R. van Weering, H.W. Boddeke, J. Vinet, et al., CXCL10/CXCR3 signaling in glia cells differentially affects NMDA-induced cell death in CA and DG neurons of the mouse hippocampus, Hippocampus 21 (2) (2011) 220–232, https://doi.org/10.1002/hipo.20742.
[30] S. Lacotte, S. Brun, S. Muller, H. Dumortier, CXCR3, inflammation, and autoimmune diseases, Ann. N. Y. Acad. Sci. 1173 (2009) 310–317, https://doi.org/10.1111/j.1749-6632.2009.04813.x.
[31] D.D. Taub, A.R. Lloyd, K. Conlon, et al., Recombinant human interferon-inducible protein 10 is a chemoattractant for human monocytes and T lymphocytes and promotes T cell adhesion to endothelial cells, J. Exp. Med. 177 (6) (1993) 1809–1814, https://doi.org/10.1084/jem.177.6.1809.
[32] G. Flynn, S. Maru, J. Loughlin, I.A. Romero, D. Male, Regulation of chemokine receptor expression in human microglia and astrocytes, J. Neuroimmunol. 136 (1–2) (2003) 84–93, https://doi.org/10.1016/s0165-5728(03)00009-2.
[33] I. Grundke-Iqbal, K. Iqbal, Y.C. Tung, M. Quinlan, H.M. Wisniewski, L.I. Binder, Abnormal phosphorylation of the microtu-bule-associated protein tau (tau) in Alzheimer cytoskeletal pathology, Proc. Natl. Acad. Sci. U.S.A. 83 (13) (1986) 4913–4917, https://doi.org/10.1073/pnas.83.13.4913.