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Research Article

Neuroprotective effects of tryptanthrin in the rat model of transient focal cerebral ischemia

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Abstract

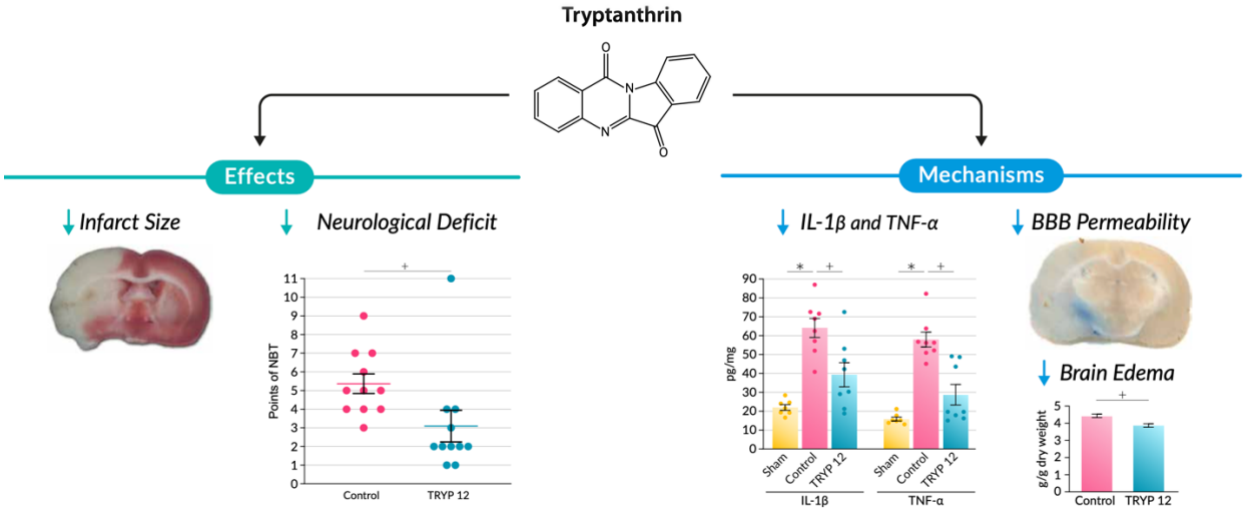
Introduction: The inflammatory response to cerebral ischemia plays a crucial role in stroke outcome. This study focuses on the neuroprotective effects of tryptanthrin, a plant alkaloid with anti-inflammatory activity, in the rat model of focal cerebral ischemia (FCI).

Materials and Methods: The neurological status of animals was assessed at 4, 24, and 48 hours after FCI (1-hour occlusion of the left middle cerebral artery) in male Wistar rats; the infarct size was assessed 48 hours after reperfusion. Rats received intraperitoneal injections of 6 and 12 mg/kg tryptanthrin or vehicle (control group) 30 min and 23 and 47 hours after FCI. The effects of 12 mg/kg tryptanthrin on the IL-1β and TNFα levels in brain tissue, blood brain barrier (BBB) permeability, and cerebral edema were assessed 24 hours after FCI.

Results: Tryptanthrin administration at a dose of 12 mg/kg significantly reduced the neurological deficit and infarct size compared with the corresponding values in control rats. Tryptanthrin administration at a dose of 6 mg/kg was ineffective. The IL-1β and TNF-α levels in cerebral infarction focus, the Evans blue content in the left (affected) hemisphere, and water content in the supra- and subventricular structures of the affected hemisphere increased 24 hours after FCI. Tryptanthrin administration (12 mg/kg) significantly attenuated the TNF-α level in the cerebral infarction site. The values of BBB permeability and water content in the experimental group did not significantly differ from the corresponding values in sham-operated rats.

Conclusion: Obtained data suggested that tryptanthrin may be considered an agent with significant neuroprotective properties, which provides rationale for further studies of this compound as a potential neuroprotector with anti-inflammatory mechanism of action.

Graphical Abstract



Keywords

blood-brain barrier permeability; brain edema; focal cerebral ischemia/reperfusion; IL-1β; infarct size; neurological status; TNFα; tryptanthrin

Introduction

Ischemic stroke is a leading cause of disability and mortality worldwide, which provides rationale for the development of new effective approaches to its prevention and treatment, which still remains challenging (Zhao et al. 2022). Inflammatory response to cerebral ischemia is one of the determinants of stroke outcome (Anrather and Iadecola 2016; Koyama and Shichita 2023). The inflammatory processes, occurring in brain tissue during acute cerebrovascular accident, consist in the activation of microglia and the release of inflammatory mediators from brain cells and ischemic endothelium, which aggravates brain tissue damage (Anrather and Iadecola 2016; Koyama and Shichita 2023). Administration of anti-inflammatory compounds reaching the brain tissue may become a novel therapeutic strategy for patients with ischemic stroke (Haupt et al. 2023).

Tryptanthrin (indolo[2,1-b]quinazolin-6,12-dione, TRYP) is a natural alkaloid found in plants (Kaur et al. 2017). TRYP was shown to exhibit an anti-inflammatory activity in various inflammation models (Kwon et al. 2017; Kirpotina et al. 2020; Zeng et al. 2021; Mostafa et al. 2026). Our earlier pilot studies demonstrated the neuroprotective activity of TRYP at a dose of 10 mg/kg (Chernysheva et al. 2024).

In a single-study framework, **this research aimed to** investigate the dose-dependent effects of TRYP on the neurological deficit and the infarction area and to determine the mechanism of TRYP neuroprotective activity based on assessments of brain edema, BBB permeability, and contents of pro-inflammatory cytokines (IL-1β and TNF-α) in brain tissue using the rat model of focal cerebral ischemia (FCI).

Materials and Methods

Chemical, drugs and kits

The following chemicals, drugs and kits were used: TRYP (98% purity) (Combi-Blocks, San Diego, CA, USA), 2,3,5-triphenyl tetrazolium chloride (St. Louis, MO, USA), diethyl ether (Kuzbassorgkhim, Russia), propofol (Fresenius Kabi Austria GmbH, Austria), 10% neutral formalin (BioVitrum, Russia), Tween 80 (Merck, Germany), dimethyl sulfoxide (St. Louis, MO, USA), rat TNF and rat IL-1β ELISA kits (St. Louis, MO, USA). To obtain a suspension, 20 µL of Tween 80 was added to the TRYP powder (at the calculated dose for each animal), aseptically ground, and 2.0 mL of 0.9% sodium chloride solution was added.

Animals

Adult male Wistar rats weighing 250–280 g (n = 104) were procured from the Department of Experimental Biological Models of the Goldberg Research Institute of Pharmacology and Regenerative Medicine, Tomsk National Research Medical Center (Russia). The animals were housed in groups of five animals per cage (57×36×20 cm) under standard laboratory conditions (ambient temperature of 22 ± 2 °C, 60% relative humidity, and 12:12 hour light–dark cycle) in cages with sawdust bedding and provided with standard rodent feed (PK-120-1, Laboratorsnab Ltd., Russia) and ad libitum water access.

Ethics statement

The study was conducted in strict compliance with the ethical requirements of Directive 2010/63/EU on the Protection of Animals Used for Scientific Purposes. The animal protocols employed were approved by the Animal Care and Use Committee of the E.D. Goldberg Research Institute of Pharmacology and Regenerative Medicine, Tomsk National Research Medical Center (Minutes No. 187092021 of October 10, 2021) (Russia).

Molecular modeling

A substance exerts a direct neuroprotective effect if it crosses the blood-brain barrier (BBB) at an efficient concentration. The Stroke Treatment Academic Industry Roundtable XI (STAIR XI) recommendations for new neuroprotective agents require experimental confirmation of BBB penetration by the candidate molecule or drug (Lyden et al. 2021). The SwissADME web tool was used to assess the ability of TRYP to penetrate the BBB (Daina et al. 2017).

Study design and doses

Four series of experiments (I–IV) involving rats were performed using the FCI model (Plotnikov et al. 2020). Series I of experiments involved assessment of neurological deficit and cerebral infarct size in sham-operated rats, control rats, and rats of two experimental groups receiving TRYP at doses of 6 mg/kg and 12 mg/kg. Experimental series II–IV aimed at studying the mechanisms of TRYP action and included the assessments of IL-1β and TNFα levels in cerebral tissue (Series II), BBB permeability (Series III), and brain edema (Series IV). In series II–IV of experiments, 12 mg/kg of TRYP was administered to rats of the experimental group. One of the requirements of the STAIR XI recommendations for preclinical studies of neuroprotectors is to establish a dose–response relationship. For this reason, the study assessed the effects of two doses, namely 6 and 12 mg/kg; the doses, the route of administration, and the treatment regimen were chosen, taking into account the results of earlier pilot studies where the compound demonstrated its neuroprotective effect (Chernysheva et al. 2024).

Study design is presented in Figure 1. In each series of experiments, the animals were randomly selected from the control and the experimental groups immediately after the FCI period.

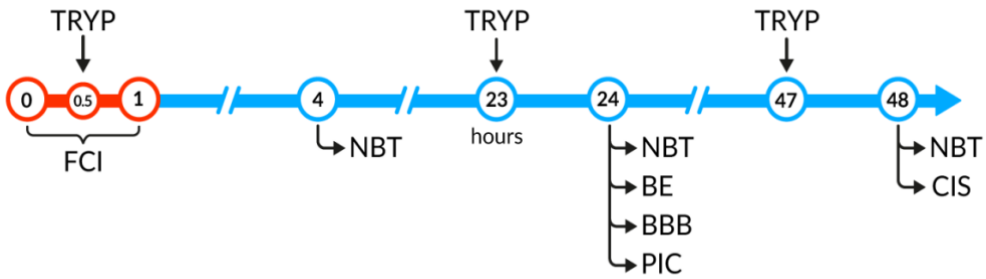


Figure 1. Study design flowchart. ***Note:*** FCI – focal cerebral ischemia; TRYP – intraperitoneal injection of TRYP; NBT – neurobehavioral tests; BBB – blood brain barrier permeability study; PIC – pro-inflammatory cytokine study (IL-1β and TNF-α); BE – brain edema study; CIS – cerebral infarct size assessment.

Модель FCI

FCI was induced by direct occlusion of the middle cerebral artery (MCA) (Chernysheva et al. 2025). In brief, transient FCI was induced by occluding the MCA for 1 h using a filament (silicone tip, 0.35 mm diameter) manufactured by Doccol Corporation (catalogue # 403545PK10Re, Sharon, MA, USA). The filament was introduced through the external carotid artery into the internal carotid artery and advanced into the middle cerebral artery. Fixation of

the filament in the lumen of the artery was achieved by tightening the ligature. After that, temporary stitches were applied, and the rats were allowed to recover from anesthesia. Rats were transferred to cage, and their behavior was observed. Occlusion of the MCA resulted in changes in the animal's posture and stereotypic circling behavior. One hour later, rats were subjected to repeated anesthesia, the filament was removed, the external carotid artery was ligated, and the blood flow through the internal carotid artery resumed. The animals recovered from anesthesia and were transferred to their home cages with free access to food and water.

Studying the neurological deficit and cerebral infarct size

Assessing the ability to weaken neurological deficit and reduce cerebral infarct size is required while studying the neuroprotective activity of a compound under ischemia–reperfusion conditions (Lyden et al. 2021). The degree of neurological deficits was assessed using the neurological severity score based on motor and sensory tests, beam balance tests, and the presence or absence of reflexes and abnormal movements (Chen et al. 2001). The rats were tested at hours 4, 24, and 48 after reperfusion by a researcher who was unaware of the group assignment of the rats. Neurological damage assessment comprised motor, sensory, reflex, and beam balance tests using the Modified Neurological Severity Score (mNSS), as well as the horizontal stability test, the forelimb and hindlimb plantar sensitivity test, the test of holding on an inclined (45°) cage lid, and the negative geotaxis reproduction test. Neurological deficit was assessed according to the sum of points on all 13 tests with a maximum score of 27 points. At the end of the experiment, the rats were euthanized using a CO2 chamber (Open Science, Russia); and the brains were removed to determine the infarct size. The procedure for cerebral infarct size assessment is described elsewhere (Plotnikov et al. 2023).

Studying the mechanism of neuroprotective activity of TRYP

The IL-1β and TNFα levels in cerebral tissue, and brain edema were measured in strict compliance with the previously described methods (Plotnikov et al. 2023; Chernysheva et al. 2025). For assessing BBB permeability, 3 mL/kg of a 2% Evans blue (Sigma-Aldrich, USA) solution in saline was injected into the femoral vein 24 h after reperfusion or sham-operation under short-term anesthesia with diethyl ether. Three hours after dye injection, the animals were euthanized under diethyl ether anesthesia, the chest was opened, and 1 mL of blood was taken from the right ventricle of the heart to assess plasma dye concentration (anticoagulant heparin). Then, the descending aorta was clamped, the right atrium was incised, and the brain was transcardially perfused with 0.9% NaCl in a volume of 200 mL to remove the dye from the vascular system; the pressure in the perfusing system did not exceed 100 mm Hg. After perfusion was completed, the rats were decapitated, the brain was extracted, frozen for 2.5 hours at –12 °C, and then cut into 1.3-mm-thick frontal sections. The slices were placed on slides and scanned to obtain images at 600 dpi using a HP Scanjet 3770 (Hewlett-Packard, China). The localization of dye extravasation areas in brain tissue was assessed in the images. After that, each slice was divided into the left and right hemispheres. The sections of each hemisphere were separately dried to a constant weight at 80 °C. The dry weight of each hemisphere was determined, and both hemispheres were separately homogenized. After that, Evans blue was extracted by incubating the homogenate in 2 mL of N,N-dimethylformamide for 72 h at 80°C. After centrifugation, light absorption of the supernatant was measured at 620 nm using UV-spectrophotometer Cary 50 (Varian, Australia). The dye contents in the samples of the right and left hemispheres and the blood plasma of each rat were estimated using calibration curves. The coefficient of interhemispheric asymmetry was calculated as the ratio of the dye contents in the hemispheres. The extravasation of Evans blue was presented in μg per 1 g of dry brain weight.

Statistical analysis

Statistical processing of data was performed using the STATISTICA 12.0 software (USA). Descriptive data are presented as mean ± standard error of the mean. Normality of distribution was not assessed due to small sample size (n = 5–12). Multiple sample comparisons were performed using the Kruskal–Wallis test and multiple comparisons of mean ranks for all groups. A P value < 0.05 was considered statistically significant.

Results

BBB permeability for TRYP

The predicted pharmacokinetic parameters (WLOGP = 1.93; TPSA = 51.96 Å²; number of rotatable bonds (N_{rot}) = 0) indicate that TRYP meets the criteria for BBB permeability (Daina et al. 2017).

TRYP improves the neurological status

The neurological status of sham-operated animals did not change after surgical intervention. In control animals, the maximum neurological deficit score was observed at 4 hours after FCI and remained unchanged for the next 48 hours. Neurological deficit in rats administered with 6 mg/kg TRYP did not differ from that in control rats. A 24% decrease in the neurological deficit score occurred 4 hours after FCI in rats administered with 12 mg/kg TRYP. The neurological deficit score further declined by 36% (at 24 hours) and 43% (at 48 hours) compared to the corresponding value in control rats (Fig. 2).

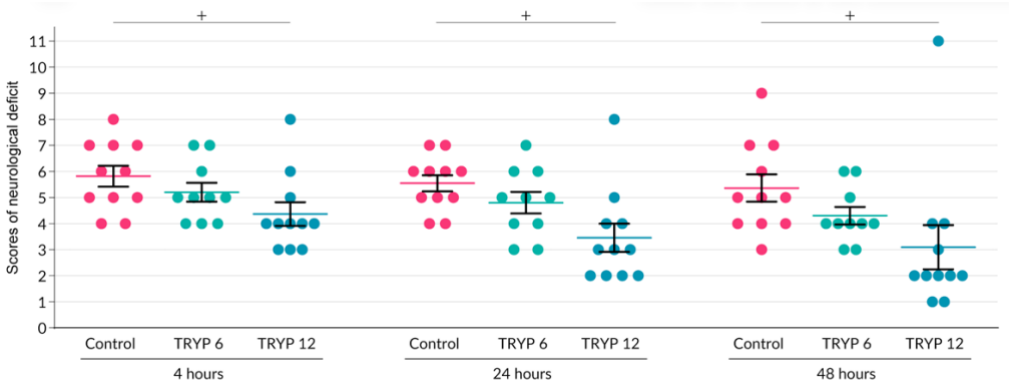


Figure 2. The mean and individual scores of neurological deficit in control rats (n = 11), 6 mg/kg TRYP-treated rats (n = 10), and 12 mg/kg TRYP-treated rats (n = 11) at hours 4, 24, and 48 after FCI. ***Note:*** Data are presented as mean ± SEM and individual values and analyzed using the Kruskal–Wallis test. $^{+}P < 0.05$ *vs*. control animals.

TRYP reduces the infarct size

Figure 3 shows the effects of TRYP on the cerebral infarct size. The cerebral infarct size of control rats was 28 ± 1% of the total area of brain sections. Administration of 6 mg/kg TRYP did not significantly reduce the infarction zone. A protective effect was observed when TRYP was administered at a dose of 12 mg/kg: the infarct size decreased by 28% compared to that in control rats.

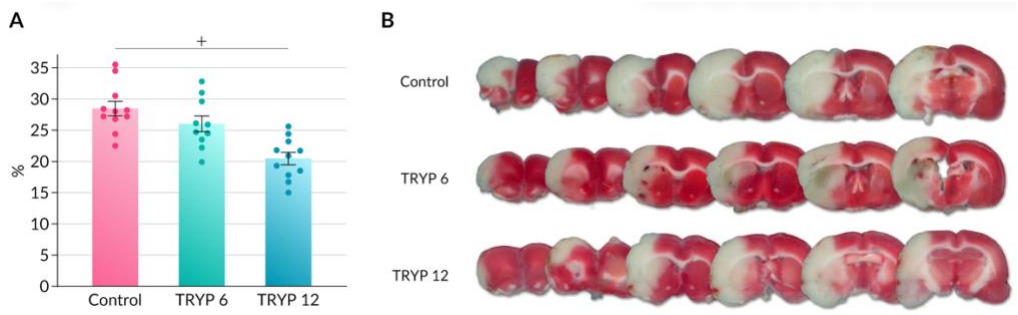


Figure 3. The effect of TRYP on cerebral infarct sizes 48 hours after FCI. ***Note:*** **A.** The mean and individual scores. The data are presented as mean ± SEM and individual values and analyzed using the Kruskal–Wallis test. $^{+}P < 0.05$ *vs*. control animals. **B.** Representative samples of 2,3,5-triphenyl tetrazolium chloride (TTC)-stained rat brain sections in the sham-operated, control, and experimental groups. White areas represent the infarcted regions.

TRYP reduces the IL-1β and TNF-α levels in cerebral tissue

Cytokine levels were determined 24 hrs after FCI in the sham-operated (n = 7), control (n = 8), and experimental groups (n = 8). In the control group, the IL-1β and TNFα levels in the ischemic brain tissue exceeded the corresponding values in the sham-operated group by 114% and 281%, respectively. In the experimental group, the level of TNF-α in the infarction zone was by 50% lower than the corresponding value in the control group (Fig. 4).

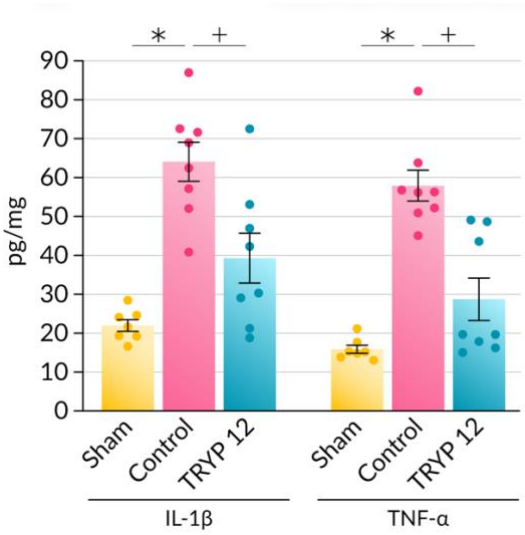


Figure 4. The cytokine levels (pg/mg protein) in the ischemic brain focus in sham-operated rats (n = 7), control rats (n = 8), and rats treated with 12 mg/kg TRYP (n = 8). ***Note:*** The data are presented as mean ± SEM and individual values and analyzed using the Kruskal-Wallis test. *P < 0.05 *vs*. sham-operated animals; ^{+}P < 0.05 *vs*. control animals.

TRYP reduces BBB permeability

The Evans blue dye content in blood plasma did not significantly differ between the groups and ranged within 0.85–0.91 mg/mL. In sham-operated rats, Evans blue staining was not visually detected in brain sections of the middle cerebral artery territory. In control group, a focus of Evans blue extravasation was detected in the infarction area on brain sections 24 hours after FCI (Fig. 5). The dye contents were significantly higher in the left (affected) hemisphere compared with those in the right hemisphere and increased 3.9- and 2.5-fold in sham-operated and control groups, respectively. The dye contents in the left and the right hemispheres in TRYP-treated rats did not significantly differ from the corresponding values in the sham-operated rats.

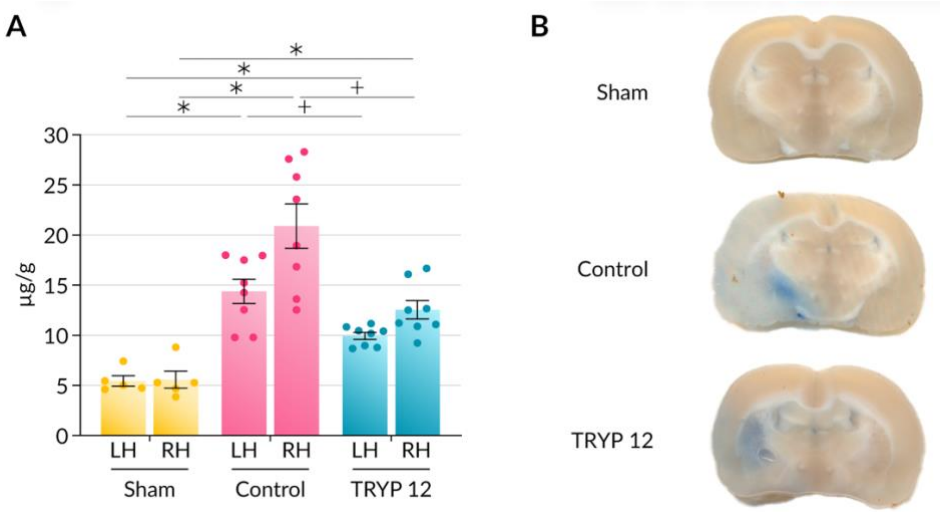


Figure 5. The effect of TRYP (12 mg/kg) on the BBB. **A.** BBB permeability for Evans blue in brain tissue (μg/g) in sham-operated rats (n = 5), control rats (n = 8), and TRYP-treated rats (n = 8) 24 hours after FCI. ***Note:*** Data are presented as mean ± SEM and individual values. Significance of differences was assessed using the Kruskal–Wallis test. *P < 0.05 *vs*. sham-operated animals; ^{+}P < 0.05 *vs*. control animals. **B.** Representative samples of rat brain sections with Evans blue penetration (the blue areas of brain tissue) in the sham-operated, control, and experimental groups.

TRYP reduces brain edema

In the control group of rats, the water content in the left (affected) hemisphere was significantly higher by 30% 24 hours after FCI compared with that in sham-operated rats (Table 1). In the left hemisphere, water content increased by 40% in the supraventricular structures (P < 0.05) and by

19% in the subventricular structures ($P < 0.05$), leading to edema development. Water content in the contralateral hemisphere also increased due to an increase in the subventricular structures, but the change was three times less prominent. The interhemispheric asymmetry coefficient was 1.20 ± 0.03 ($P < 0.05$). Water content in the cerebellum of control rats did not differ from that in sham-operated rats. TRYP at a dose of 12 mg/kg reduced water content in the left (affected) hemisphere, and the water content in the supra- and subventricular structures of the left and right hemispheres did not either significantly differ compared with the corresponding values in sham-operated animals. No intergroup differences in water content in the cerebellum were observed.

Table 1. Brain water content (g/g dry weight) in sham-operated, control, and TRYP-treated rats

Brain Hemisphere		Sham-Operated ($n = 9$)	Control ($n = 11$)	TRYP ($n = 8$)
Left Hemisphere	Total	3.40 ± 0.07	4.42 ± 0.11*	3.76 ± 0.08
	Supraventricular part	3.53 ± 0.07	4.93 ± 0.14*	3.94 ± 0.11
	Subventricular part	3.09 ± 0.08	3.67 ± 0.12*	3.26 ± 0.12
Right Hemisphere	Total	3.37 ± 0.08	3.70 ± 0.09	3.45 ± 0.07
	Supraventricular part	3.53 ± 0.05	3.66 ± 0.05	3.63 ± 0.04
	Subventricular part	3.10 ± 0.09	3.38 ± 0.07	3.33 ± 0.09
Cerebellum		3.35 ± 0.06	3.39 ± 0.04	3.33 ± 0.04

Note: *$P < 0.05$ *vs.* values in sham-operated rats.

Discussion

Sufficient BBB permeability is an essential quality of promising neuroprotectors (Roleira et al. 2010; Lyden et al. 2021). Numerous neuroprotectors showed promise for treatment of ischemic stroke, but their delivery to the brain remained challenging (D'Souza et al. 2021). The ability of TRYP to cross the BBB was previously evaluated in three *in vitro* human and animal BBB models (Jähne et al. 2016). Our calculations showed that TRYP can penetrate the BBB. The obtained *in silico* evidence of BBB permeability for TRYP, as well as the data from the pilot study of TRYP (Chernysheva et al. 2024), formed the basis for conducting an integrated single study of the effects of TRYP on the neurological deficit and the infarction zone with an assessment of the dose–response relationship and an investigation of the mechanism of neuroprotective activity including the effect of TRYP on cerebral edema, BBB permeability, and the content of pro-inflammatory cytokines (IL-1β and TNF-α) in brain tissue in the rat model of FCI. The dynamic changes in the neurological abnormalities and infarct size represent important criteria for the assessment of novel neuroprotector efficacy in an experimental stroke model (Lyden et al. 2021). Another essential requirement of the STAIR XI recommendations is establishing the dose–response relationship for a neuroprotector. Accordingly, two doses of TRYP (6 and 12 mg/kg) were used in this study. For the first time, we demonstrated that the course administration of 12 mg/kg TRYP significantly reduced the infarct size and accelerated the neurological recovery. TRYP administered at this dose decreased neurological deficit in rats at hours 4, 24, and 48 after the middle cerebral artery occlusion. The 6 mg/kg dose was ineffective.

Inflammatory response to cerebral ischemia is one of the key pathophysiologic factors responsible for ischemic stroke outcome (Anrather and Iadecola 2016; Haupt et al. 2023). The inflammation caused by impaired cerebral blood circulation supply is accompanied by the activation of intravascular leukocytes (Hermann et al. 2018) followed by infiltration of the damaged brain by polymorphonuclear neutrophils and macrophages (Liesz et al. 2011), pro-inflammatory mediator release from the ischemic brain endothelium and parenchyma, and cytokine hyperproduction, which may aggravate tissue damage (Sarwal et al. 2012; Kuriakose and Xiao 2020; Koyama and Shichita 2023).

Neuroinflammation suppression may be considered an essential element of the neuroprotective strategy in ischemic stroke treatment (Chamorro et al. 2021; Tao et al. 2020). Successful phase II–IV clinical trials of novel neuroprotectors including those with anti-neuroinflammatory activity (Paul and Candelario-Jalil 2021; Haupt et al. 2023) as well as the ongoing studies of other promising neuroprotectors (Tao et al. 2020; Haupt et al. 2023) imply that neuroprotection remains a relevant strategy in stroke (Yeh et al. 2020). The obtained results provide rationale for continuous search for new candidates to neuroprotectors among the compounds with an anti-inflammatory activity in the context of ischemic brain injury.

Selection of TRYP for the neuroprotection study was based on the previously described anti-inflammatory properties of the compound. In mouse arthritis models, TRYP reduced the levels of proinflammatory cytokines produced by lymph node cells (Kirpotina et al. 2020). Zeng et al. (2021) demonstrated the ability of TRYP to reduce the levels of proinflammatory cytokines (IL-2, IL-10, and TNF-α) in serum of tumor-bearing mice. In this context, data about anti-inflammatory TRYP in LPS-induced BV2 microglial cells are of particular interest (Kwon et al. 2017). Authors demonstrated that TRYP downregulated the production of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) in LPS-stimulated BV2 microglial cells. TRYP significantly attenuated inflammation by reducing the expression of M1 markers (IL-6, IL-1β, TNF-α, and CD86) and increasing the expression of M2 markers (CD206, arginase-1) in the immortalized murine microglial cell line BV-2 (Fan et al. 2025). These studies provided rationale for elucidation of the mechanisms of action of TRYP. Rat model of FCI allowed us to demonstrate significant attenuation of the TNF-α and IL-1β levels in the ischemic zone 24 hours after ischemia–reperfusion suggesting that TRYP administration led to the suppression of neuroinflammation in the ischemic focus.

Neuroinflammation in ischemic stroke leads to increased BBB permeability and cerebral edema (Martínez-Coria et al. 2021; Wang et al. 2025). Our earlier pilot study suggested that TRYP reduced BBB permeability at delayed stages (48 and 72 hours) after FCI. In this study, TRYP exerted a significant protective effect on the BBB 24 hours after FCI, reducing BBB permeability to albumin-bound Evans blue. Observed decrease in severity of cerebral edema may be explained by TRYP ability to reduce the production of pro-inflammatory cytokines in brain tissue and attenuate disturbances in the BBB permeability. Figure 6 shows the mechanistic profile of TRYP in focal cerebral ischemia in rats.

Limitations

The STAIR XI recommendations highlight certain limitations of the present work and indicate prospects for further TRYP research, which include the need to confirm neuroprotective activity of TRYP in experimental animals of both sexes and aged animals, as well as to obtain data on TRYP permeability through the BBB *in vivo*.

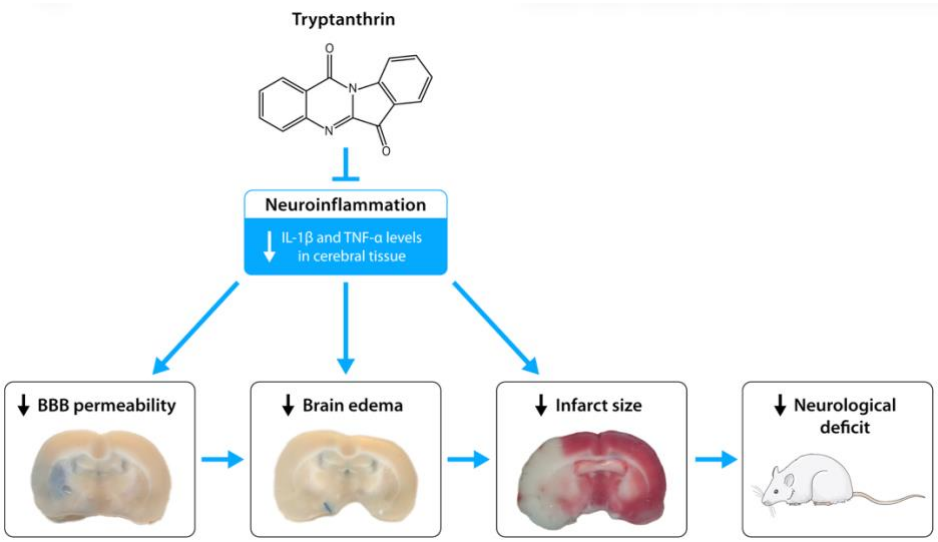


Figure 6. The mechanistic profile of TRYP in focal cerebral ischemia in rats.

Conclusion

The results of our study demonstrated the ability of TRYP to exert a neuroprotective effect in the FCI rat model. TRYP at a dose of 12 mg/kg significantly reduced neurological deficit compared with the corresponding neurological severity score in control rats and reduced the infarct size. Compared with control group, 12 mg/kg TRYP significantly decreased the IL-1β and TNF-α levels in the cerebral infarction focus, BBB permeability, and water content in the supraventricular part of the affected hemisphere. The obtained data provide a rationale for further study of TRYP as a candidate neuroprotector with an anti-inflammatory mechanism of action.

Additional Information

Conflict of interest

The authors declare that they have no conflicts of interest.

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Ethics statement

The animal protocols employed were approved by the Animal Care and Use Committee of the E.D. Goldberg Research Institute of Pharmacology and Regenerative Medicine, Tomsk National Research Medical Center (Minutes No. 187092021 of October 10, 2021).

Acknowledgments

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