doi.org/10.36721/PJPS.2026.39.10.289.1

Neuropharmacological assessment of intrathecal thyrotropin-releasing hormone in cerebral ischemia

Onur Cicek[1]*, Haci Hasan Esen[2], Sait Sami Erdem[3] and Bahadir Feyzioglu[4]
[1]Department of Neurosurgery, Special Akademi Meram Hospital, Konya, Turkey
[2]Department of Pathology, Necmettin Erbakan University Meram Medicine Faculty, Konya, Turkey
[3]Department of Biochemistry, Konya City Hospital, Konya, Turkey
[4]Department of Microbiology, Necmettin Erbakan University Meram Medicine Faculty, Konya, Turkey

Abstract: **Background**: In experimental models of central nervous system damage, thyrotropin-releasing hormone (TRH) has been shown to have neuromodulatory, antioxidant and anti-inflammatory effects. Its neuroprotective efficacy in embolic cerebral ischemia remains unknown. **Objectives**: This study evaluated the effects of intrathecal TRH administration on cerebral infarct volume, oxidative stress and inflammatory markers in a rabbit model of embolic cerebral ischemia. **Methods**: Twenty adult female New Zealand white rabbits were randomly divided into two groups (n = 10 per group): a control group and a TRH-treated group. Injecting broken autologous blood clots into the right common carotid artery caused cerebral ischemia. Forty-five minutes after embolization, the treatment group received intrathecal TRH (0.20 mg/kg) via the cisterna magna. Serum interleukin-1β (IL-1β) concentrations and levels of lactate and malondialdehyde (MDA) in the cerebrospinal fluid (CSF) were assessed at baseline and 24 hours after embolization. Computerized histopathological image analysis was used to measure the volume of the cerebral infarct. **Results**: The mean cerebral infarct volumes at 24 hours varied slightly between the TRH-treated group (122.84 ± 15.84 mm^3) and the control group (126.79 ± 14.04 mm^3) ($p = 0.496$). Serum levels of IL-1β, MDA and CSF lactate increased statistically significantly ($p < .05$) in both the TRH-treated and control groups. Following embolization, the TRH-treated group had decreased serum levels of CSF lactate, CSF MDA and IL-1β; nevertheless, none of the group comparisons were significant ($p > .05$). **Conclusion**: Although no statistically significant differences were observed, consistent directional reductions in infarct volume and biochemical markers suggest potential biological activity of TRH that may require optimization of dosing and study design. At the dosage and duration employed in this study, TRH showed limited efficacy under the experimental conditions.

Keywords: Thyrotropin-releasing hormone; Cerebral ischemia; Neuroprotection; Oxidative stress; Animal model; Interleukin-1β

Submitted on 19-01-2026 – Revised on 08-03-2026 – Accepted on 04-06-2026

INTRODUCTION

A stroke, also known as a cerebral vascular accident (CVA), is defined by the sudden onset of neurological dysfunction caused by the interruption of blood supply to either part of the brain or the entire brain. The primary mechanisms through which this interruption occurs include thrombotic, embolitic, or haemorrhagic mechanisms. Cerebral ischemia results in substantial morbidity and mortality worldwide. Therefore, it remains to be seen whether oxidative stress and inflammation contribute to the increased incidence of ischemic brain injury (Iadecola *et al*., 2020; Jayaraj *et al*., 2019; Kuriakose and Xiao, 2020). Edaravone, a free radical scavenger, has demonstrated neuroprotective properties by inhibiting lipid peroxidation, decreasing inflammatory mediator release and limiting infarct expansion in experimental and clinical studies. Although edaravone has demonstrated neuroprotective effects in experimental and clinical studies, it was not included as a comparator treatment group in the present exploratory study. The current investigation was designed to evaluate the independent neuropharmacological effects of intrathecal thyrotropin-releasing hormone (TRH) in an embolic cerebral ischemia model. In addition to edaravone, the hypothalamic neuropeptide thyrotropin-releasing hormone (TRH) had previously been identified as having potential neuropharmacological properties and to exert antioxidant and anti-inflammatory effects in different animal models of central nervous system injury. However, while TRH shows promise as a neuroprotective agent, its effectiveness remains unclear, particularly in embolic models of cerebral ischemia. Unlike prior studies that predominantly used rodent middle cerebral artery occlusion (MCAO) models and systemic routes of administration, the present study uniquely evaluates the effects of intrathecal TRH administration in an embolic cerebral ischemia model. The embolic model more closely mimics the pathophysiology of human ischemic stroke, particularly thromboembolic events, thereby enhancing translational relevance. Furthermore, intrathecal delivery allows direct central nervous system exposure while minimizing peripheral metabolism,

**Corresponding author:* e-mail: dronurcicek@hotmail.com

providing a distinct pharmacological context that has been insufficiently explored in previous studies. The purpose of this study was to determine the effects of intrathecally administered TRH on cerebral infarct volume and on biochemical markers of oxidative stress and inflammation.

The shortage of oxygen and glucose resulting from this interruption will trigger a series of biochemical reactions in the affected area of the brain, causing immediate neuronal injury and initiating an Ischemic Cascade that culminates in complete neuronal death or irreversible damage.

An increasing problem for the global community, a stroke now ranks as the third leading cause of death in the developed nations of the world, after cardiovascular disease and cancer and is responsible for approximately 10% of all deaths worldwide. Stroke is also a significant health problem and is the subject of intense scientific investigations to identify novel pharmacologic or interventional strategies that may be useful in the treatment of this disease, both in preclinical and clinical settings (Chamorro *et al*., 2021; Campbell *et al*., 2020).

Thyrotropin-releasing hormone (TRH) is a tripeptide (5-oxo-L-prolyl-L-histidyl-L-proline amide) that has been identified as a primary endocrine regulator of the release of both thyroid-stimulating hormone (TSH) and prolactin (PRL) from the anterior pituitary gland. TRH also has neuromodulatory and neurotropic properties and is found in many peripheral and central nervous system (CNS) tissues.

TRH has been researched for therapeutic uses in many neurological diseases (such as amyotrophic lateral sclerosis, spinal cord injury, spinocerebellar atrophy, endogenous depression and epilepsy) over the last few decades.

This study assesses the neuroprotective effect of intrathecal protirelin (TRH analogue) in a rabbit embolic cerebral ischemia model created with homologous blood embolism. Specifically, this study aims to see if TRH protects against cerebral infarction by decreasing total infarct volume and regulating 3 different biochemical markers of ischemic damage: cerebrospinal fluid lactate, cerebrospinal fluid malondialdehyde and serum interleukin-1 beta.

Several experimental studies have investigated the neuroprotective potential of TRH and TRH analogues in experimental models of central nervous system injury and cerebral ischemia. These studies suggest that TRH may attenuate neuronal injury by modulating oxidative stress, excitotoxicity and inflammatory responses, although the reported efficacy varies depending on the experimental model, route of administration and treatment protocol (Zhang *et al*., 2022; Zhou *et al*., 2021). Despite these findings, evidence regarding the neuroprotective potential of TRH in embolic models of cerebral ischemia remains limited. Therefore, further experimental investigations are required to clarify the potential neuroprotective role of TRH in embolic cerebral ischemia.

Therefore, this study provides a focused evaluation of TRH in a clinically relevant embolic ischemia model using a central delivery route. Importantly, the study also aims to clarify whether previously reported neuroprotective effects of TRH are reproducible under these conditions, thereby contributing critical evidence to the ongoing evaluation of TRH as a potential neuroprotective agent.

MATERIALS AND METHODS

Study design

Between April and July 2006, an experimental study was undertaken at Selçuk University's Experimental Animal Laboratory. The study included 20 adult New Zealand white rabbits weighing 2–3 kg. Only female New Zealand white rabbits were included in order to minimize inter-animal variability associated with body weight differences, aggressive behavior and handling-related stress that are more commonly observed in male rabbits. The use of a single sex also improved experimental standardization in this preliminary investigation. However, sex-specific biological responses to cerebral ischemia and neuroprotective interventions cannot be excluded and future studies should include both sexes to improve translational relevance.

A formal a priori power analysis was not conducted because this study was designed as a preliminary exploratory investigation intended to evaluate the potential neuropharmacological effects of intrathecal thyrotropin-releasing hormone in an embolic cerebral ischemia model. The selected sample size of ten animals per group was determined based on ethical considerations regarding animal experimentation, feasibility constraints and consistency with previously published exploratory ischemia studies using rabbit models. Although the sample size may have limited the statistical power to detect modest treatment effects, the study was intended to generate preliminary data for future adequately powered investigations.

The rabbits were randomly allocated into two equal-sized groups (n=10 per group) using a simple randomization method. The selected dose of TRH (0.20 mg/kg) and administration timing (45 minutes post-embolization) were based on prior experimental studies suggesting potential neuroprotective effects in the early post-ischemic window. The study was designed as an

exploratory investigation to evaluate the independent pharmacological effects of TRH without confounding influences from combination therapies or comparator drugs. All procedures were approved by the Institutional Animal Care and Use Committee and performed in accordance with institutional guidelines for laboratory animal welfare.

Preparation of autologous clot embolus

From the auricular vein, 1.5 mL of blood from each rabbit was allowed to clot for 3 hours at room temperature. After separation of the serum, the fragmented clots were then pushed through a 21-gauge needle. The remaining fragments of clot (0.4 mL) were re-suspended in isotonic sodium chloride and microscopic measurements showed that the suspension consisted of clot fragments measuring between 0.3 and 2 mm long.

To improve reproducibility of embolization, clot fragments were prepared using a standardized protocol that included identical clotting duration, mechanical fragmentation technique and suspension preparation for all experimental animals. Microscopic confirmation of clot fragment size ranging from 0.3 to 2 mm was performed before embolization. Nevertheless, minor variability in embolus distribution and vascular occlusion severity could not be completely eliminated and may have contributed to variability in infarct volume between animals.

Surgical procedure and embolization

Animals were anesthetized with an intramuscular injection of 35 mg/kg ketamine hydrochloride (Ketalar® - Eczacıbaşı – Parke Davis) and 5 mg/kg xylazine hydrochloride (Rompun® 2% - Bayer). Body temperature was maintained using an infrared lamp throughout anesthesia.

After the skin was sterilized, a vertical incision was made in the neck, providing access to the right side of the common carotid artery.

The proximal segment was temporarily clamped and a 21-gauge intravascular catheter was inserted into the carotid artery and advanced to the bifurcation. The prepared clot suspension was injected through the catheter over 2 minutes. Upon completion of the injection, the catheter was withdrawn and the vascular puncture site was sealed with absorbable gelatin sponge (Curaspon®) or, if necessary, sutured with 5-0 Vicryl to ensure hemostasis. The arterial clamp was then removed to restore blood flow.

For the second group, an intrathecal dose of 0.2 mg/kg thyrotropin-releasing hormone (TRH; TRH Ferring amp, 0.2 mg/mL; Er-Kim) was administered via cisterna magna 45 minutes after embolization.

Post-procedure monitoring and tissue harvesting

Cerebral ischemia was caused in female adult New Zealand white rabbits by embolizing fragmented autologous blood clots through the right common carotid artery under a ketamine-xylazine anesthetic. Each rabbit was randomly assigned to either a control group (receiving no pharmacological treatment) or a treatment group that received thyrotropin-releasing hormone (TRH) intrathecally at a dose of 0.20 mg/kg via the cisterna magna at 45 minutes after embolization. Blood and cerebrospinal fluid samples were collected before the experimental procedure and again 24 hours after embolization to measure serum interleukin-1β and cerebrospinal fluid lactate and malondialdehyde levels. Brains were collected at the conclusion of the experimental period and computerized image analysis was used to examine histopathology and to determine volumes of infarcted tissue.

Following embolization, animals were observed for 24 hours. Blood (1.5 mL) and cerebrospinal fluid (CSF, 1.5 mL) samples were collected preoperatively and at the 24th postoperative hour. Animals were euthanized under deep anesthesia using intracardiac air injection in accordance with institutional ethical approval and laboratory animal welfare guidelines. Any animal that died within the first 24 hours following surgery was not included in the analyses of the results obtained from this study. In compliance with institutional regulations, brain tissue was removed from all animals and immersed in 10% buffered formalin for one week before processing and further analysis.

Biochemical analysis

Samples collected were kept frozen at -70°C until analysis. The following were performed on the collected samples:

- Lactate in Cerebrospinal Fluid (CSF): 1 ml of CSF was measured by Spectrophotometry using the Kodak Vitros Ektachem 700XR system along with the compatible reagents from this system.
- Malondialdehyde (MDA) in CSF: The measurement of MDA was done using a double heating method via spectrophotometry. 0.5 ml of CSF was placed into a 15 ml glass centrifuge tube containing 2.5 ml of 10 percent trichloroacetic acid (TCA) and heated in water at 90°C for 15 minutes. The tube was then centrifuged at 3000 rpm for 10 minutes. 2 ml of supernatant was removed from the tube and it was placed into another tube with 1.0 ml of 0.675 percent thiobarbituric acid (TBA) solution. The tube was then re-heated for 15 minutes at 90°C and cooled. The absorbance of MDA was measured at 532 nm and calculated using the molar extinction coefficient. (ε = 58.27 $mM^{-1} \cdot cm^{-1}$):

MDA concentration (nmol/mL) was calculated using the molar extinction coefficient (ε = 58.27 $mM^{-1} \cdot cm^{-1}$).

Measurement of IL-1β in Serum: The concentration of IL-1β in serum was measured using a 'sandwich ELISA kit' made by BioSource International, USA. The sample serum was placed onto a 96-well plate coated with anti-IL-1β antibodies. After three serial incubation steps at 37°C with biotinylated antibodies and Streptavidin-HRP, color developed in the wells due to a stabilized chromogen and the absorbance was measured at 450 nm. Concentrations are calculated using a comparison with a standard curve.

Histopathological evaluation

Following fixation, the brains were hemisected and each hemisphere was divided into three coronal sections (anterior, middle and posterior) using predefined anatomical landmarks (anterior to the genu of the corpus callosum, anterior to the temporal pole and parieto-occipital sulcus). Three standardized coronal sections were obtained from each hemisphere to ensure consistency between animals. Infarcted areas were measured in each section using computerized image analysis software and total infarct volume was calculated by multiplying the infarct area by section thickness and summing the values obtained from all analyzed sections. Tissue processing was performed using a standard automated tissue processor. Paraffin blocks were sectioned at 5 µm thickness and stained with hematoxylin and eosin. Histopathological examination demonstrated characteristic ischemic alterations including neuronal degeneration, eosinophilic cytoplasm, tissue edema and focal infarct regions within affected cerebral areas. Quantitative infarct volume measurements were performed using computerized image analysis by a pathologist blinded to the experimental group allocation to minimize observer bias.

Infarct volumes were calculated using computerized image analysis. The infarcted cross-sectional areas from each coronal section were measured using the image analysis software and multiplied by section thickness to estimate total infarct volume.

The infarct volume was calculated using the following formula:

$V = \Sigma (Ai \times d)$

Where:

V = Total infarct volume (mm^3)

Ai = Infarct area of each section (mm^2)

d = Distance between sections (mm)

Histopathological assessment and infarct volume measurements were performed by a pathologist who was blinded to the experimental group allocation.

Statistical analysis

Statistical analyses were performed using non-parametric methods due to the small sample size. Data are expressed as mean ± standard deviation (SD). Differences between the two groups (control and TRH-treated groups) were assessed using the Mann–Whitney U test. Within each group, Baseline values and 24-hour post-treatment values were compared using the Wilcoxon signed-rank test. A p-level <0.05 was considered significant. In addition to hypothesis testing, effect size trends were evaluated using percentage differences between groups to assess the magnitude of observed effects. Although statistical significance was not achieved, percentage differences between groups were calculated to provide additional insight into potential biological effects and to account for the limited sample size.

RESULTS

A total of 20 rabbits were equally divided into control and TRH-treated groups (n = 10 per group). No animal died before the 24-hour evaluation time point, and all animals were included in the final analysis. The results for cerebral infarct volume and biochemical parameters in serum and cerebrospinal fluid (CSF), including interleukin-1β (IL-1β), lactate, and malondialdehyde (MDA), are summarized in table 1. Representative histopathological sections demonstrating infarcted brain tissue in the experimental model are shown in fig. 1.

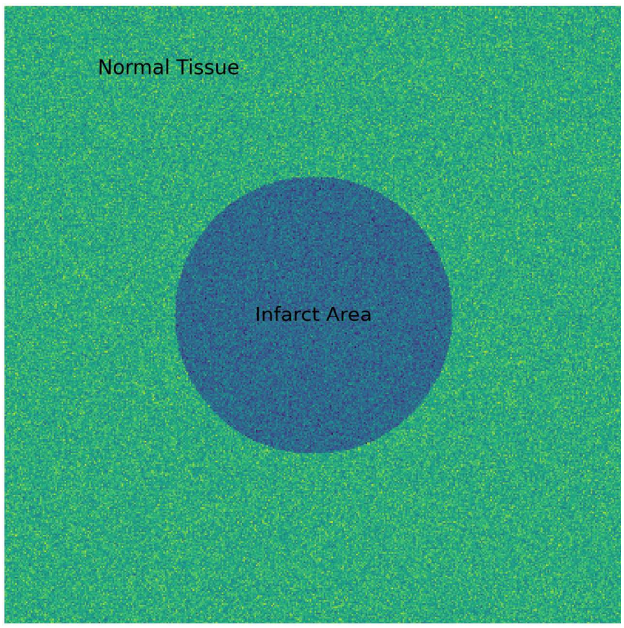

Fig. 1: Representative histopathological section of rabbit brain following embolic cerebral ischemia showing the infarcted region and surrounding tissue.

Cerebral infarct volume

The mean infarct volume was slightly lower in the TRH-treated group (122.84 ± 15.84 mm^3) compared to the control group (126.79 ± 14.04 mm^3), although this difference did not reach statistical significance (Mann–Whitney U = 41.000, p = 0.496) (Fig. 2).

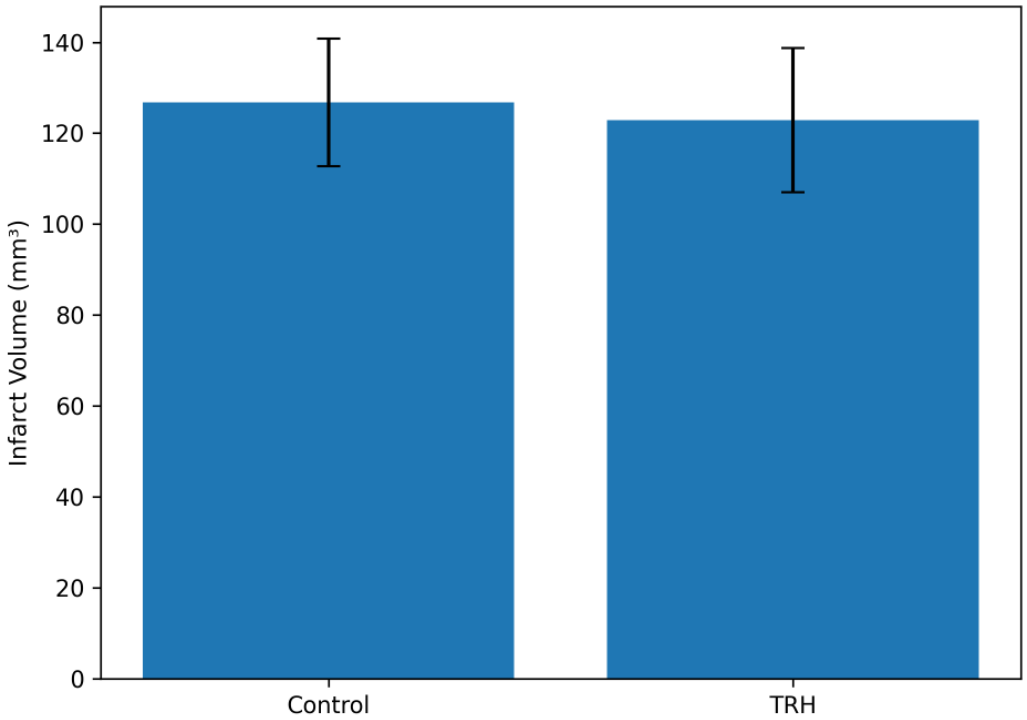

Fig. 2: Comparison of cerebral infarct volume between control and TRH-treated groups at 24 hours after embolization. Data are presented as mean ± SD.

Table 1: The effect of intrathecal TRH hormone on cerebral infarct volume and biochemical parameters studied in rabbits with embolic cerebral ischemia.

Parameter	Time (hr)	Control group (n=10)	TRH group (n=10)	P value
Cerebral infarct volume (mm^3)	24	126.79 ± 14.04	122.84 ± 15.84	0.496
CSF lactate (mg/dL)	Baseline	20.99 ± 2.03	20.83 ± 4.27	0.762
	24	32.64 ± 9.87	26.74 ± 4.58	0.151
CSF malondialdehyde (nmol/mL)	Baseline	3.54 ± 0.25	3.63 ± 0.28	0.426
	24	6.51 ± 2.13	5.49 ± 1.88	0.121
Serum IL-1β	Baseline	193.83 ± 54.28	220.98 ± 49.27	0.406
	24	432.23 ± 78.43	411.76 ± 59.08	0.496

CSF lactate levels

At baseline (pre-embolization), no significant difference was observed in CSF lactate levels between the control (20.99 ± 2.03 mg/dL) and TRH (20.83 ± 4.27 mg/dL) groups ($p = 0.762$). At 24 hours post-embolization, lactate levels increased in both groups but were significantly lower in the TRH group (26.74 ± 4.58 mg/dL) compared to controls (32.64 ± 9.87 mg/dL), although the intergroup difference did not reach statistical significance ($p = 0.151$).

Within-group comparisons using the Wilcoxon signed-rank test showed a significant increase in CSF lactate from baseline to 24 hours in both groups ($p = 0.005$ for control; $p = 0.017$ for TRH).

CSF Malondialdehyde (MDA) levels

Baseline MDA levels were similar between the control (3.54 ± 0.25 nmol/mL) and TRH (3.63 ± 0.28 nmol/mL) groups ($p = 0.426$). After 24 hours, MDA levels were lower in the TRH group (5.49 ± 1.88 nmol/mL) than in the control group (6.51 ± 2.13 nmol/mL), but this difference did not reach statistical significance ($p = 0.121$).

However, intra-group analysis revealed a significant increase in MDA levels at 24 hours in both the TRH and control groups ($p = 0.005$).

Serum IL-1β levels

No statistically significant difference in baseline serum IL-1β levels was found between the control (193.83 ± 54.20 pg/mL) and TRH (220.98 ± 49.27 pg/mL) groups ($p = 0.406$). At 24 hours, IL-1β levels were slightly lower in the TRH group (411.76 ± 59.08 pg/mL) than in controls (432.23 ± 78.43 pg/mL), but again the difference was not statistically significant ($p = 0.496$).

Despite no significant between-group differences, Wilcoxon signed-rank tests showed a significant increase in IL-1β levels from baseline to 24 hours in both groups ($p = 0.005$ for both).

In summary, intrathecal administration of TRH did not produce a statistically significant reduction in ischemic brain injury to tissue (infarct), although numerically lower values were observed in the TRH-treated group. These differences did not reach statistical significance and should therefore be interpreted with caution, as the numerical differences were small and do not support a definitive conclusion regarding the pharmacological activity of TRH. Nevertheless, consistent directional reductions across infarct volume and biochemical parameters were observed in the TRH-treated group, suggesting a potential biological effect that may not have reached statistical significance due to limited sample size. Previous studies have demonstrated significant neuroprotective effects of antioxidant agents such as edaravone in experimental models of cerebral ischemia. In contrast, under the experimental conditions of the present study, TRH did not produce statistically significant improvements in infarct volume or biochemical markers of oxidative stress and inflammation.

DISCUSSION

Cerebral ischemia initiates a complex cascade of biochemical and cellular processes that result in neuronal damage, synaptic failure and ultimately neurocognitive impairment. Excessive neurotransmitter and peptide release occurs after synaptic activity ceases, which can lead to excitotoxicity and functional deficits during the post-stroke period. Thyrotropin-releasing hormone (TRH) was initially characterized as a hypothalamic peptide responsible for stimulating thyroid-stimulating hormone production; however, TRH has also been identified as a neuromodulator and neurotransmitter with widespread distribution throughout the CNS.

The central effects of TRH include regulation of autonomic, behavioral and somatic systems as well as those influenced by its analeptic properties. Therefore, it has been proposed that the endogenous TRH system may protect against ischemic and traumatic injury to the CNS. One major contributor to ischemic neuronal damage is oxidative stress, caused by oxygen-free radicals generated during reperfusion (Chen *et al*., 2021; Wang *et al*., 2023; Kim *et al*., 2022). Oxygen free radicals can damage biological membranes by causing oxidative damage to lipids. The peroxidation of lipid molecules produces malondialdehyde (MDA), a common marker of oxidative stress measured by the thiobarbituric acid reactive substances (TBARS) assay. Despite being an imprecise marker, levels of MDA correlate with the amount of lipid peroxidation and peak within an hour or less following neuronal injury.

The present study used the thiobarbituric acid reactive substances assay as a conventional method to assess malondialdehyde levels and lipid peroxidation. However, the thiobarbituric acid reactive substances method has limited specificity because other aldehydes and reactive compounds may contribute to absorbance values. More specific oxidative stress biomarkers, including 4-hydroxynonenal, F2-isoprostanes, glutathione-related enzymes and superoxide dismutase activity, were not evaluated because of methodological and resource limitations. Future studies incorporating these biomarkers would provide a more comprehensive characterization of oxidative injury associated with cerebral ischemia.

In the present study, CSF MDA and lactate levels were significantly elevated 24 hours after embolization, indicating increased oxidative and metabolic stress. The 24-hour endpoint was selected in order to evaluate early biochemical and histopathological alterations following embolic cerebral ischemia. However, certain inflammatory, apoptotic and tissue remodeling processes may continue to evolve beyond the acute phase of injury. Therefore, longer observation periods may be necessary to evaluate delayed neuroprotective effects and long-term histopathological outcomes associated with thyrotropin-releasing hormone administration.

However, the use of an intrathecally administered TRH analogue, protirelin, was not able to significantly reduce either of these elevations. This contrasts with previous studies that indicated a preventative effect of TRH on oxidative stress and cerebral edema in models of traumatic and hemorrhagic brain injury. Previous experimental studies have shown that antioxidant and neuroprotective interventions may reduce oxidative stress and lipid peroxidation following cerebral ischemia. However, the reported efficacy varies among different therapeutic agents and experimental models, highlighting the complexity of ischemic brain injury and the need for further mechanistic investigations (Chen et al., 2021; Wang *et al*., 2023; Zhang *et al*., 2022).

To the best of current knowledge, few studies have directly examined the relationship between intrathecal administration of thyrotropin-releasing hormone (TRH) and lipid peroxidation in experimental models of cerebral ischemia. The present study contributes additional evidence in this area. However, protirelin did not produce significant changes in either MDA or lactate levels under the dose and administration regimen used in the present study. Furthermore, only selected biochemical markers of oxidative stress and inflammation were evaluated. Other important mechanisms involved in ischemic brain injury, including excitotoxicity, mitochondrial dysfunction,

apoptotic signaling pathways and microglial activation, were not investigated and remain areas for future research. Therefore, the present study provides only limited mechanistic insight into the potential neuroprotective actions of TRH in cerebral ischemia. Mechanistically, TRH exerts its effects through activation of TRH receptors (TRHR1 and TRHR2), which regulate intracellular calcium signaling and downstream neuroprotective pathways. However, the rapid degradation of TRH in cerebrospinal fluid and its short biological half-life may limit sustained receptor activation. Additionally, ischemic injury involves complex pathways including excitotoxicity, mitochondrial dysfunction and microglial activation, which were not directly addressed in this study.

After ischemia, secondary damage and growth of the infarct zone are caused by the increase of inflammatory cytokines, neuroinflammatory mediators and infiltration and activation of free radicals, creatinine and polyunsaturated fatty acids (PUFAs) (Maida *et al*., 2020; Liu *et al*., 2020; Li *et al*., 2022).

Furthermore, cytokines such as TNF-α, IL-1β and IL-6 increase following ischemic stroke and can be detected in both the central nervous system and systemic circulation. Interleukin-1β was selected as the primary inflammatory biomarker because it is recognized as an important early mediator of ischemic neuroinflammation and neuronal injury. However, cerebral ischemia involves a complex inflammatory cascade that includes additional cytokines such as tumor necrosis factor-alpha, interleukin-6 and chemokines associated with microglial activation. The absence of a broader inflammatory cytokine panel represents a limitation of the present study and should be addressed in future experimental investigations.
These, as a collection of mediators, are thought to help create new structures in the post-ischemic nervous tissue and CNS (e.g., the damaged area), thereby encouraging neuroinflammation (Jayaraj *et al*., 2019; Maida *et al*., 2020).

In accordance with previously published findings, significant elevations in serum IL-1β concentrations were observed in both the control and TRH-treated groups. While the TRH group had lower serum IL-1β concentrations than the control group, this was not statistically significant. This finding may indicate that the selected protirelin dosing regimen was insufficient to produce a measurable modulatory effect on the inflammatory response under the experimental conditions used in this study.

According to experimental findings, TRH has been shown to modulate immunity, which may occur either directly via the TRH receptor or indirectly through stimulation of prolactin secretion and its related actions. Synthetic TRH analogue: YM-14673 has been reported to produce neuroprotective effects. Previous experimental data indicate that YM-14673 produces less cerebral edema and reduced neuronal tissue damage compared with related rodent models of cerebral ischemia.

In the present study, intrathecal administration of protirelin did not reproduce the neuroprotective effects reported in some previous experimental studies. The results suggest that TRH did not demonstrate a measurable neuroprotective effect under the specific experimental conditions and dosing regimen used in this study. This finding may reflect limitations related to the pharmacokinetic profile of TRH, including its short biological half-life and rapid degradation in cerebrospinal fluid. The present findings suggest that the selected dosing strategy did not produce a statistically significant reduction in infarct size. Future studies should evaluate different dosing regimens and repeated administration strategies to better determine the optimal therapeutic potential of TRH. Therefore, the lack of significant differences between the two groups suggests that the standard treatment protocol used in this study may not reduce the size of the infarcted area or improve functional outcomes after injury.

Several pharmacological and methodological factors may explain the absence of any substantial neuroprotective effects of intrathecal TRH. TRH is also known to exert endocrine effects through stimulation of thyroid-stimulating hormone (TSH) and prolactin secretion. These endocrine effects represent an important translational consideration when evaluating TRH as a potential therapeutic agent for ischemic stroke. From a translational perspective, the absence of significant neuroprotection despite direct central nervous system delivery suggests that pharmacokinetic limitations and insufficient target engagement may hinder clinical applicability. These findings highlight the importance of developing TRH analogues with improved stability, prolonged half-life and enhanced receptor specificity. Although intrathecal administration was selected to target the central nervous system directly, potential systemic endocrine effects cannot be completely excluded. For this reason, several experimental studies have investigated TRH analogues with improved pharmacokinetic properties and reduced endocrine activity. While TRH can regulate neuronal excitability and inflammatory pathways, its biological half-life is very short and it is rapidly broken down within cerebrospinal fluid, possibly preventing adequate prolonged therapeutic concentrations in ischemic areas. Furthermore, both timing and single-dose administration of intrathecal TRH in this study may not have been the most advantageous for modulating the complex series of oxidative and inflammatory reactions attributable to obtaining ischemia induced by embolization. Conversely, agents like edaravone demonstrate direct scavenging

capabilities on free radicals and have shown consistent efficacy across different types of ischemia; thus, making stability, target specificity and the type and timing of dosing essential for effective neuroprotective drug therapy. Therefore, future TRH research will need to concentrate on developing an analogue to TRH; determining an optimal dosage schedule or delivery route; and/or combining TRH with another compound with the intention of increasing TRH's ability to reach the central nervous system and have a greater mechanistic effect (Zhang *et al*., 2024).

Several limitations of the present study should be acknowledged. First, the relatively small sample size limited the statistical power of the study and increased the possibility of Type II error. Second, the absence of a positive control group, such as edaravone, restricted comparative interpretation of therapeutic efficacy. Third, only female rabbits were included in the experimental design and therefore potential sex-related biological differences could not be evaluated. Fourth, neurological and behavioral functional outcomes were not assessed, limiting translational interpretation of the histopathological findings. Fifth, only selected biochemical markers were evaluated, whereas additional oxidative stress and inflammatory biomarkers may have provided a more comprehensive mechanistic understanding of ischemic injury. Finally, only a single dose and a single 24-hour observation period were investigated, which may not represent the optimal therapeutic conditions required to observe delayed neuroprotective effects of thyrotropin-releasing hormone.

CONCLUSION

Intrathecal administration of TRH did not produce statistically significant neuroprotective effects under the conditions tested; however, consistent directional improvements across multiple parameters suggest potential biological activity that warrants further investigation. While there have been prior reports indicating that TRH and analogs have protective effects for neural tissue damage, protirelin demonstrated limited efficacy at the dose and experimental conditions used in this study. Therefore, further studies should investigate how different relative types of TRH analogs administered via other routes (i.e., systemic intravenous administration or intrathecal spaces), combinations of dosages, or time points for administration might impact their efficacy as a neuroprotectant against ischemic stroke. The evidence suggests that, in contrast with traditional neuroprotective drugs like edaravone (that is, proven to provide neuroprotection), the use of TRH at the current dose and time frame has minimal therapeutic benefits. Additionally, long-term histopathological outcomes and functional recovery after ischemic stroke should be further investigated to better define the potential of TRH as an effective treatment option through the use of molecular and genetic factors directly associated with neuroinflammation and the development of oxidative damage following ischemia (including the development of cerebral infarction).

Acknowledgements
This work was supported by the Special Akademi Meram Hospital, Neurosurgery, Necmettin Erbakan University Meram Medicine Faculty, Pathology, Konya City Hospital, Biochemistry and Necmettin Erbakan University Meram Medicine Faculty, Microbiology.

Authors' contribution
Onur Cicek: Contributed to study design, data collection and experimental procedures; Hacı Hasan Esen: Performed histopathological analyses and interpretation of results; Sait Sami Erdem: Conducted biochemical analyses and contributed to data interpretation; Bahadır Feyzioglu: Assisted with manuscript preparation and critical revision of the manuscript. All authors reviewed and approved the final version of the manuscript.

Funding
There was no funding.

Data availability statement
The datasets generated and/or analysed during the current study are available from the corresponding author upon reasonable request.

Ethical approval
The experimental protocol was reviewed and approved by the Ethics Committee of Special Akademi Meram Hospital, Konya, Turkey (Decision No. 2025/19-176; approved on 18 January 2025). All animal experimental procedures were conducted in accordance with institutional ethical guidelines for the care and use of laboratory animals. This study was performed in adherence with the ARRIVE guidelines. See supplementary file for the ARRIVE checklist.

Conflict of interest
The authors declare no conflict of interest.

Supplementary data
https://www.pjps.pk/uploads/2026/07/SUP1783770543.pdf

REFERENCES

Campbell BCV, Khatri P and Saver JL (2020). Advances in stroke reperfusion therapy. *Nat. Rev. Neurol.*, **16**(5): 267-278.

Chamorro A, Dirnagl U, Urra X and Planas AM (2021). Neuroprotection in acute stroke: Targeting excitotoxicity, oxidative stress and inflammation. *Lancet Neurol.*, **20**(5): 425-436.

Chen H, Yoshioka H, Kim GS and Jung JE (2021). Oxidative stress in ischemic brain injury: Mechanisms and therapeutic strategies. *Antioxidants*, **10**(2): 190.

Iadecola C, Buckwalter MS and Anrather J (2020). Immune responses to stroke: Mechanisms, modulation and therapeutic implications. *Cell*, **181**(1): 62-73.

Jayaraj RL, Azimullah S, Beiram R, Jalal FY and Rosenberg GA (2019). Neuroinflammation: Friend and foe for ischemic stroke. *J. Neuroinflamm.*, **16**(1): 142.

Kim JS, Kim YJ and Kim JT (2022). Oxidative stress and neuroinflammation in stroke pathophysiology and treatment. *Cells*, **11**(11): 1800.

Kuriakose D and Xiao Z (2020). Pathophysiology and treatment of stroke: Present status and future perspectives. *Int. J. Mol. Sci.*, **21**(20): 7609.

Li X, Yang R, Zhang Y and Chen J (2022). Inflammatory signaling pathways in ischemic stroke and therapeutic targets. *J. Neurochem.*, **161**(4): 418-435.

Liu X, Zhang Z, Sun W and Yang Y (2020). Emerging roles of inflammatory cytokines in ischemic stroke. *J. Neuroimmunol.*, **346**: 577304.

Maida CD, Norrito RL, Daidone M, Tuttolomondo A and Pinto A (2020). Neuroinflammatory mechanisms in ischemic stroke: Focus on cytokines and immune response. *Int. J. Mol. Sci.*, **21**(18): 6451.

Wang J, Zhang Q, Zhang Y and Wang J (2023). Targeting oxidative stress in ischemic stroke: Molecular mechanisms and therapeutic strategies. *Front. Pharmacol.*, **14**: 1160042.

Zhang Y, Wang H, Li X and Liu J (2022). Neuroprotective effects of thyrotropin-releasing hormone analogues in central nervous system injury models. *Front. Neurosci.*, **16**: 843219.

Zhang W, Wang H and Li X (2024). Recent advances in neuroprotective strategies for ischemic stroke. *Brain Sci.*, **14**(2): 158.

Zhou Y, Yang X, Zhang H and Liu J (2021). Experimental models of cerebral ischemia and their application in neuroprotective drug research. *Brain Res. Bull.*, **173**: 82-92.

Zhu H, Jian Z, Zhong Y, Ye Y and Zhang Y (2021). Role of oxidative stress and inflammation in ischemic stroke. *Oxid. Med. Cell Longev.*, **2021**: 663831.