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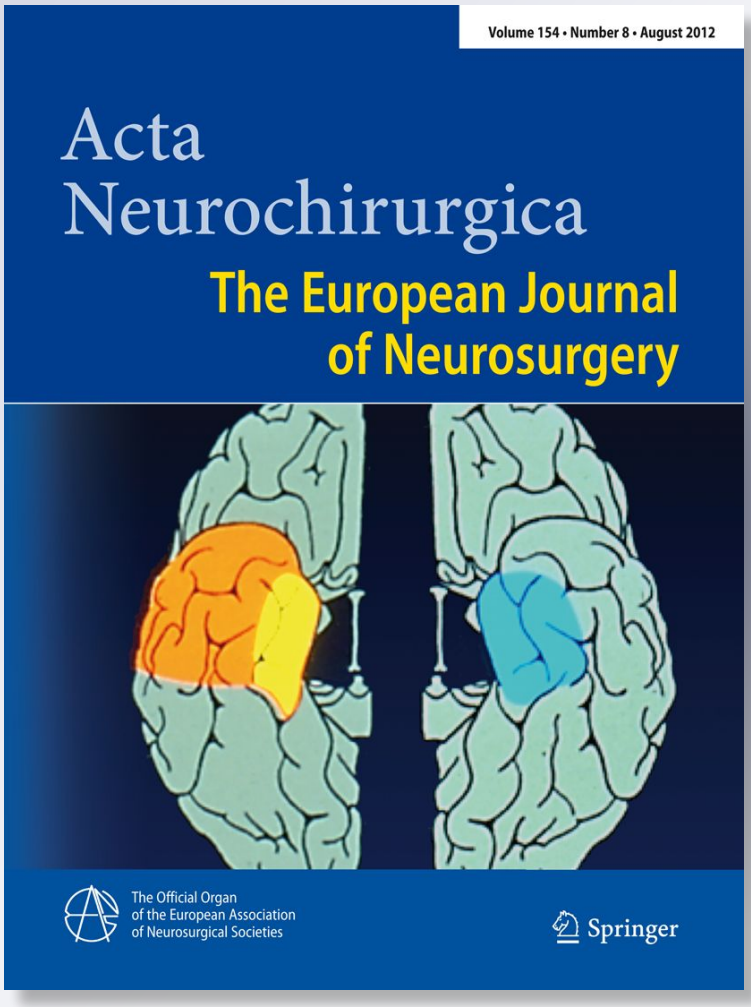

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EXPERIMENTAL RESEARCH

The effect of thiocolchicoside on cerebral vasospasm following experimental subarachnoid hemorrhage in the rabbit

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Abstract
Background This study investigated the effects of thiocolchicoside to prevent cerebral vasospasm in a rabbit model of subarachnoid hemorrhage.
Methods Twenty-four adult male New Zealand white rabbits were randomly divided into three groups of eight rabbits each: group 1 (control), group 2 (subarachnoid hemorrhage), group 3 (treatment). Thiocolchicoside (4 mg/kg, intraperitoneally) was administered just before intracisternal blood injection and continued for 72 h once a day in the same dose for group 3. Animals were killed 72 h after subarachnoid hemorrhage. Basilar artery cross-sectional areas and arterial wall thicknesses were measured in all groups.
Results Intraperitoneal administration of thiocolchicoside was found to attenuate cerebral vasospasm after subarachnoid hemorrhage in rabbits. Thiocolchicoside treatment was determined to be effective in increasing the luminal area and reducing the wall thickness of the basilar artery.
Conclusions Our findings, for the first time, showed that TCC can prevent vasospasm induced by SAH. Our results also showed that GABAergic activity may play an important role in cerebral vasospasm etiopathogenesis. In conclusion, the thiocolchicoside treatment might be beneficial in preventing vasospasm after subarachnoid hemorrhage, thus showing potential for clinical application.

Keywords GABA · Rabbit · Subarachnoid hemorrhage · Thiocolchicoside · Vasospasm

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Introduction

Cerebral vasospasm after subarachnoid hemorrhage (SAH) is characterized by prolonged and reversible contraction of the cerebral arteries, which is a major leading cause of mortality and morbidity. The exact pathogenesis of cerebral vasospasm is still unclear. The breakdown products of blood are considered to play an important role in the pathogenesis of cerebral vasospasm. Inflammatory processes, free radical formation, an imbalance between vasoconstrictive and vasodilator substances, neuronal mechanisms that regulate vascular tone, apoptosis, and endothelial proliferation have all been accused as causative and pathogenic factors [17].

The existence of γ-aminobutyric acid (GABA) receptors in cerebrovascular system is well documented [1, 11, 14, 18]. Studies showed that these GABA receptors play an important role in regulating vascular tone.

Thiocolchicoside (TTC) is a semi-synthetic sulfur derivate of colchicoside, which was widely used clinically more than a half decade as a muscle relaxant, anti-inflammatory, and analgesic drug [15]. TTC has been reported to have activity along GABA receptors [5, 7, 8, 10, 15]. Because of this GABAergic

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activity, we hypothesized that TCC may have potential effects on SAH-induced cerebral vasospasm.

Materials and methods

Experimental groups

All experimental procedures used in this investigation were reviewed and approved by the ethical committee of Ministry of Health Refik Saydam Hıfzıssıha Institution. Twenty-four adult male New Zealand white rabbits weighing 2,500–3,850 g were randomly divided into three groups of eight rabbits each:

Group 1 Control group (n=8); was a sham surgery group, in which SAH was not induced. In this group, after induction of anesthesia, the cisterna magna was punctured as described below and 1 ml/kg of physiological saline (0.9 % NaCl) was slowly injected into the cisterna magna after removal of the same amount of cerebrospinal fluid (CSF).

Group 2 SAH group (n=8); the SAH protocol was used to induce vasospasm as described below.

Group 3 Treatment group (n=8); cerebral vasospasm was induced by SAH protocol described below and the animals received TTC (Muscoril, Sanofi-Aventis, Turkey). In experimental studies, the effective dose of TTC on myorelaxation and antiinflammation was determined as 2.5–4.7 mg/kg [15]. As a result, the dose of TTC in this group was accepted as 4 mg/kg and administered intraperitoneally. The treatment was started just before intracisternal blood injection and continued for 72 h once a day in the same dosage.

Anesthesia and surgical procedure

The animals were kept at optimal (18–21 °C) room temperature and fed with standard diet where 12-h light-dark cycle was implemented. Free access to food and water was allowed. The animals were anesthetized by intramuscular administration of 70 mg/kg ketamine (Ketalar, Parke Davis Eczacıbaşı, Turkey) and 5 mg/kg xylazine (Rompun, Bayer, Turkey) combination. All animals breathed spontaneously throughout the procedures. Arterial blood samples of PO_2 and PCO_2 were taken from each animal from the catheterized ear arteries for blood gas analysis during the procedures, and only those animals with PO_2 greater than 70 mmHg and PCO_2 lesser than 40 mmHg were included in the study. Heart rate and arterial blood pressure were measured with the use of an ear arterial catheter. Physiological parameters of the experimental groups were summarized in Table 1. Core body temperature was monitored rectally and maintained at 37 °C± 0.5 °C with a heater.

Cerebral vasospasm model

The head of the rabbit was extended in the prone position. A midline nuchal incision was made, and dermal and subdermal tissues, fascia and paravertebral muscles were dissected to expose the atlanto-occipital membrane. A 25-gauge needle was inserted through the dura mater and the arachnoid membrane into the cisterna magna; 1 ml/kg of CSF was withdrawn and equal volume of fresh, non-heparinized autologous arterial blood which was obtained from the ear artery injected into cisterna magna within 2 min. The animals were then placed in a head-down position at 30° for 30 min to hold the blood in the basal cisterns. After the recovery from anesthesia and confirmation of vital signs, the rabbits were left to their cages for the establishment of cerebral vasospasm.

Perfusion-fixation

All animals were euthanized by perfusion-fixation 72 h after procedures. The animals were anesthetized as described above. The ear artery was catheterized for monitoring blood pressure and for blood gas analysis. When satisfactory respiratory parameters were obtained, a thoracotomy was performed, the left ventricle cannulated, the right atrium opened widely, and the descending thoracic aorta clamped. After perfusion with 300 ml of physiological saline, a fixative was perfused (10 % formaldehyde, 200 ml). Perfusion was performed at a standard height of 100 cm from the chest. The brains were then removed and stored in formaldehyde solution at 4 °C overnight.

Histological morphometric analysis of basilar artery

Each brainstem specimen was embedded in paraffin. The entire basilar artery was sectioned into five segments 2 mm in length (Fig. 1), and stained with hematoxylin and eosin (H&E). The morphometric measurements on all five segments of the basilar were performed using the BAB-Bs200ProP Image Processing and Analysis System (Ankara, Turkey). The luminal area was calculated as the area contained within the boundaries of the internal elastic lamina. The size of cross-sectional area for each basilar artery was obtained by averaging these measurements. The mean ± SD value obtained from each artery was used as the final value for a particular vessel.

The wall thickness between the lumen and the external border of the muscle layer was measured at four quadrants of each segment of basilar artery. If an undulating luminal border was encountered, an extra measurement was done from the internal elastic membrane to the external border of the muscle layer. The vessel wall thickness for each basilar artery segment was obtained by averaging these measurements. The mean±SD value obtained from each artery was

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Table 1 Physiological parameters of the experimental groups

Group	*n*	pH	PO_2	PCO_2	MABP	HR
Control	8	7.46±0.01	94±3.0	35.2±1.0	98±2.2	170±5.5
SAH	8	7.44±0.02	93±2.4	34.3±1.1	95±3.1	172±4.6
Treatment	8	7.44±0.01	95±2.1	35.5±0.9	96±2.0	168±3.8

Data are expressed as mean±SD. All the values were not statistically significant between the groups ($p>0.05$). *SAH* subarachnoid hemorrhage; *MABP* mean arterial blood pressure (mmHg); *HR* heart rate (beats per minute)

used as the final value for a particular vessel. All measurements were repeated three times for each artery in a blinded fashion by two pathologists and the conclusive values were obtained by averaging these measurements. Inter-observer reliability levels for area and wall thickness were found as 0.876 (95 % CI: 0.754–0.939) and 0.924 (95 % CI: 0.846–0.963), respectively.

Statistical analysis

Data analysis was performed using SPSS for Windows, version 11.5 (SPSS Inc., Chicago, IL, USA). Whether the distributions of continuous variables were normal or not was determined by using Shapiro–Wilk test. Levene test was used for the evaluation of homogeneity of variances. Data were shown as mean ± standard deviation or median (IQR), where applicable, while the mean differences among the groups were analyzed by one-way ANOVA following post hoc Tukey test. Otherwise, Kruskal–Wallis test and Conover's multiple comparison tests were applied for the comparisons of the median values.

The intra-class correlation coefficient (ICC) for area and wall thickness was calculated for determining the inter-observer reliability levels. A p value less than 0.05 was considered statistically significant.

Results

The mean basilar artery cross-sectional area in the control group was 310,245.5±56,697.34 μm^2. In the SAH group, the mean basilar artery cross-sectional area decreased to 155,021.3±70,167.51 μm^2. This decrease was statistically significant ($p<0.001$). In the treatment group, the basilar

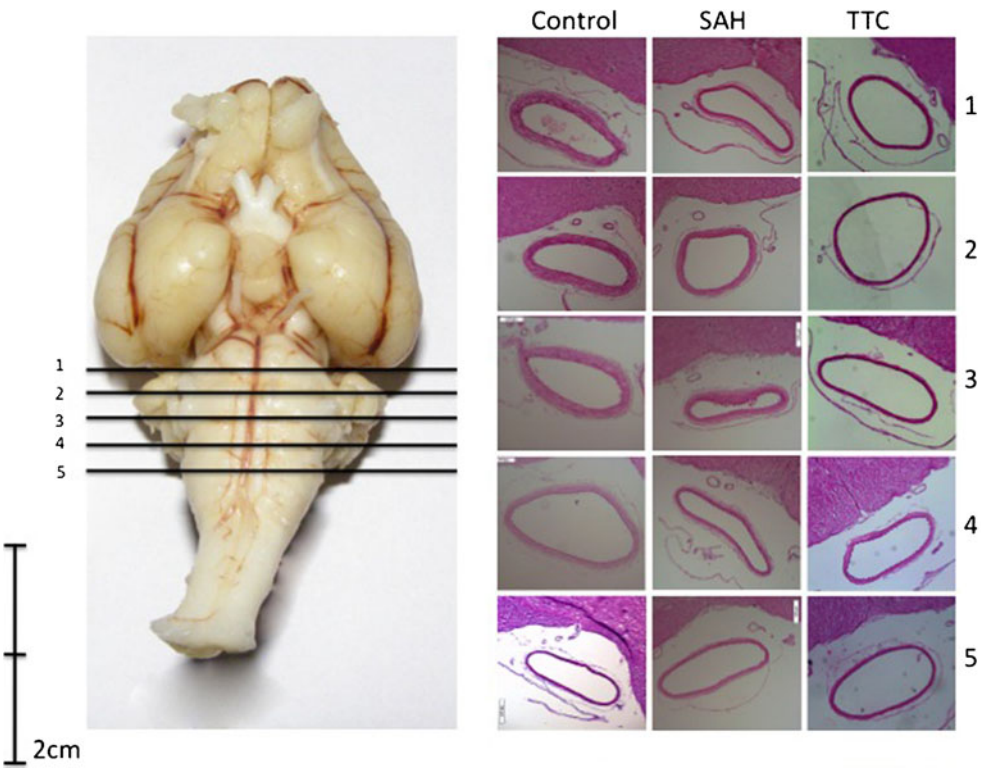

Fig. 1 **a–e** Macroscopic view of a basis of the rabbit brain from the control group (*left*) and representative histological cross sections of the basilar artery (H&E; 40x obj.) corresponding to all groups (*right*) (*SAH* subarachnoid hemorrhage, *TTC* thiocolchicoside)

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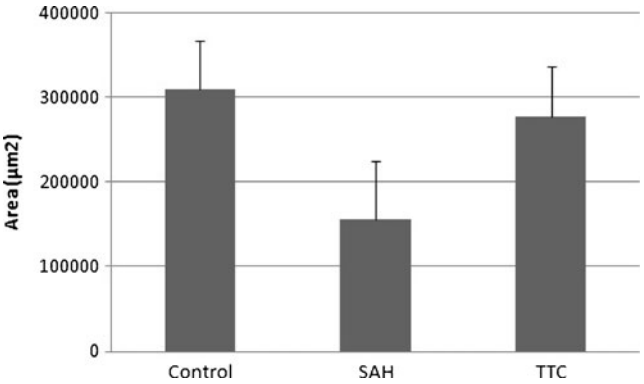

Fig. 2 Mean basilar artery cross-sectional areas of the study groups. Values are expressed as a mean ± SD. (*SAH* subarachnoid hemorrhage, *TTC* thiocolchicoside)

artery cross-sectional area was 276,809.5±60,471.55 μm^2, which was statistically significant compared to the SAH group (p=0.002) (Fig. 2).

The mean value of the basilar artery wall thickness was 24.7±3.27 μm in the control group, and 44.4±3.86 μm in the SAH group. The treatment group had an average value of 29.7±5.94 μm of the basilar artery wall thickness. When compared to the control group, after SAH, basilar artery wall thickness was increased significantly (p<0.001). Basilar artery wall thickness in the treatment group was smaller than SAH group and this was statistically significant at p<0.001 (Fig. 3).

Mean basilar artery cross-sectional area and arterial wall thickness values are provided in Table 2. Detailed results of each experimental group were also provided in Table 3.

Discussion

Cerebral vasospasm is one of the most important clinical problems of the SAH. Symptomatic vasospasm is reported in 20–30 % of the SAH patients, where radiological vasospasm is reported in 70 % of patients [16]. The pathogenesis of cerebral vasospasm is complex, multifactorial, and is not fully understood. The catastrophic problem due to cerebral vasospasm is ischemic neurological deficit both the treatment strategies and research are focused on these parameters.

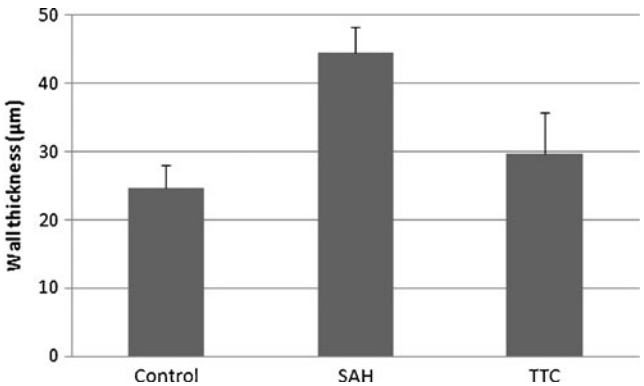

Fig. 3 Mean basilar artery wall thicknesses of the study groups. Values are expressed as a mean ± SD (*SAH* subarachnoid hemorrhage, *TTC* thiocolchicoside)

Table 2 Mean basilar artery cross-sectional area and arterial wall thickness values

Group	CSA (μm^2)	AWT (μm)
Control	310,245.5±56,697.34	24.7±3.27
SAH	155,021.3±70,167.51	44.4±3.86
Treatment	276,809.5±60,471.55	29.7±5.94

Data are expressed as mean±SD. *SAH* subarachnoid hemorrhage; *CSA* cross-sectional area; *AWT* arterial wall thickness

Table 3 The detailed basilar artery cross-sectional area and arterial wall thickness values of each experimental group

	CSA (μm^2)	AWT (μm)
Control		
Rabbit #1	250,333.3	24.7
Rabbit #2	263,196.2	24.5
Rabbit #3	306,020.8	21.4
Rabbit #4	269,566.9	19.7
Rabbit #5	346,383.9	25.2
Rabbit #6	302,313.5	25.5
Rabbit #7	426,900.9	24.6
Rabbit #8	317,247.9	26
SAH		
Rabbit #1	118,669	43.7
Rabbit #2	136,495.3	44
Rabbit #3	133,493.6	43.1
Rabbit #4	165,167.7	44.7
Rabbit #5	94,849.5	45.7
Rabbit #6	143,429.4	48.3
Rabbit #7	125,528	47.6
Rabbit #8	321,537.8	42.3
TTC		
Rabbit #1	276,524.2	25.4
Rabbit #2	157,962.3	26.1
Rabbit #3	251,024.2	29
Rabbit #4	265,441.8	30.5
Rabbit #5	297,278.8	33
Rabbit #6	339,704.2	31.8
Rabbit #7	355,962.8	30.4
Rabbit #8	270,577.6	24

Data are expressed as mean. *SAH* subarachnoid hemorrhage; *CSA* cross-sectional area; *AWT* arterial wall thickness

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In the present study, we firstly investigated the effects of TCC on cerebral vasospasm. We used the single-SAH model in the rabbit. Due to past literature, injection of fresh blood into the cisterna magna is one of the most commonly used protocols to establish SAH in rabbits. It has been found that this protocol is successful in obtaining a vasospasm that accounts for 32–55 % of the baseline in the basilar artery [6]. It has also been shown that cerebral vasospasm reaches its maximum level during the third day of SAH in rabbits [22]. Thus, in this study, the treatment was stopped and the animals were killed 72 h after the induction of SAH. Due to past studies, the measurements were done in the basilar artery, not in an artery of the anterior circulation [6, 19]. On the other hand, it would have been interesting if the comparison between the basilar artery and an anterior circulation artery, in terms of the dimensions, before and after treatment was calculated and compared. This issue may be the subject of a future study.

The existence of GABA receptors in cerebrovascular system is well known and many studies reported that GABA had vasodilator effects on cerebral arteries [1, 11, 14, 18]. It was also shown that GABA was able to induce a dose-dependent cerebral vasodilatation in many species [1, 2, 11, 12]. On the other hand, Cetas et al. [9] reported that rostral ventromedial medulla (RVM) modulates the acute cerebrovascular response to SAH. They also reported that, following experimental SAH, an acute reduction of cerebral blood flow was markedly potentiated by inactivation of RVM. It was shown that GABA antagonist bicuculline activates the neuronal activity of RVM. Activating the neurons of RVM by GABA receptor antagonist bicuculline resulted in a 20–30 % increase in cerebral blood flow by vasodilatation. As a result, past studies demonstrated that both GABAergic agonism and antagonism may have vasodilatory effects on cerebral vasculature.

In the present study, we investigated the effects of TCC on cerebral vasospasm following SAH. TCC is a semi-synthetic sulfur derivate of colchicoside, a naturally occurring glucoside present in the plant Gloriosa superb. TCC has been used clinically for more than half a decade as a muscle relaxant, anti-inflammatory and analgesic drug [15]; but its molecular targets and mechanisms of action are still under investigation. It was shown that TCC seems to bind to the GABA binding sites in both brain and spinal cord [4, 5, 13]. TCC has been thought to act as GABA receptor agonist in the central nervous system [3, 7, 15, 20, 21]. Conversely, Carta et al. [8] showed that TCC is a potent antagonist of GABA receptor function.

Both antagonist (via RVM) and agonist (via direct effect of GABA) GABAergic activity of TCC may explain the vasodilator effects of this drug against vasospasm induced by SAH, as shown in this study. Both further investigations to explain the vasodilator mechanism of TCC are needed.

On the other hand, there are some limitations of this study. Dose-dependent results with delayed histopathological assessment of the vasospasm after SAH will increase the potential value of TTC in treatment. Many patients are admitted to the hospital several days after SAH with signs of already-established vasospasm. Whether TTC could reverse already-established vasospasm in this group of patients still remains unclear. Thus to address the effect of TTC for both prevention and reversion of vasospasm has to be justified with additional studies.

Conclusions

Our findings, for the first time, showed that TCC can prevent vasospasm induced by SAH. Our results also showed that GABAergic activity may play an important role in cerebral vasospasm etiopathogenesis. We suggest that TCC might be a part of the preventive therapy for vasospasm and is worthy of further investigation.

Conflicts of interest None.

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