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Effect of thiamine pyrophosphate on oxidative damage in the brain and heart of rats with experimentally induced occlusion of the common carotid artery.

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Keywords: carotid artery; ıschemia; occlusion; rat; reperfusion; thiamine pyrophosphate.

Abstract. It is known that a sudden increase in cerebral blood flow (hyperperfusion) with carotid revascularisation may disrupt and damage the blood-brain barrier. This study aimed to explore thiamine pyrophosphate's (TPP) protective effects against potential brain and heart damage resulting from carotid cross-clamping and unclamping in rats. The animals were divided into common carotid cross-clamping and unclamping (CCU), TPP+common carotid cross-clamping and unclamping (TCCU), and sham operation (SG) groups. The TCCU group received an intraperitoneal injection (IP) of 20 mg/kg TPP one hour before anesthesia. The CCU and SG groups received distilled water as a solvent. Ischemia was induced by maintaining the clips closed for 10 min. For the SG group, only a subcutaneous incision was made. Afterward, the clips were removed, the incisions were stitched, and reperfusion was continued for six hours. Subsequently, the rats were euthanized with high-dosage general anesthesia, and heart and brain tissues were removed. TPP significantly suppressed the I/R-induced malondialdehyde (MDA) increase and decreased total glutathione (tGSH) levels in brain and heart tissues. TPP prevented the increase of tumor necrosis factor-alpha (TNF-α), interleukin-1 β (IL-1β), and interleukin-6 (IL-6) levels in both brain and heart tissues. In blood serum, TPP suppressed I/R-induced increase in troponin I (TP I) and creatine kinase-MB (CK-MB) in the blood. TPP was shown to protect the brain and distant cardiac tissues against oxidative and inflammatory damage induced by cerebral I/R.

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Efecto del pirofosfato de tiamina sobre el daño oxidativo en el cerebro y el corazón de ratas con oclusión inducida experimentalmente de la arteria carótida común.

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Palabras clave: arteria carótida; ıschemia; oclusión; rata; reperfusión; pirofosfato de tiamina.

Resumen. Se sabe que un aumento repentino del flujo sanguíneo cerebral (hiperperfusión) con la revascularización carotídea puede causar la alteración y daños de la barrera hematoencefálica. El objetivo de este estudio fue explorar los efectos protectores del pirofosfato de tiamina (TPP) contra los posibles daños cerebrales y cardíacos resultantes del pinzamiento y despinzamiento de la carótida en ratas. Los animales se dividieron en grupos de pinzamiento y despinzamiento de la carótida común (CCU), TPP + pinzamiento y despinzamiento de la carótida común (TCCU) y operación simulada (SG). El grupo TCCU recibió una inyección intraperitoneal (IP) de TPP a una dosis de 20 mg/kg una hora antes de la anestesia. Los grupos CCU y SG recibieron agua destilada como disolvente. La isquemia se indujo manteniendo los clips en posición cerrada durante 10 min. En el grupo SG solo se realizó una incisión subcutánea. Luego se retiraron los clips, se suturaron las incisiones y se mantuvo la reperfusión durante 6 horas. Posteriormente, los animales fueron sacrificados con altas dosis de anestesia y se extrajeron tejidos del corazón y del cerebro. El TPP suprimió significativamente el aumento de malondialdehído (MDA) inducido por I/R y la disminución de los niveles de glutatión total (tGSH) tanto en el tejido cerebral como en el cardíaco. El TPP impidió el aumento de los niveles de factor de necrosis tumoral alfa (TNF-α), interleucina 1β (IL-1β) e interleucina-6 (IL-6) en los tejidos del cerebro y del corazón. En el suero sanguíneo, el TPP suprimió el aumento de la troponina I (TP I) y la creatina quinasa-MB (CK-MB) inducido por I/R en la sangre. Se demostró que el TPP protege el cerebro y los tejidos cardíacos distantes contra el daño oxidativo e inflamatorio inducido por la I/R cerebral.

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INTRODUCTION

In instances of acute occlusion of the extracranial internal carotid artery, 40%-60% of patients experience severe disability, while 16%-55% experience stroke-related complications that lead to death [1]. As known, atherosclerotic carotid artery disease is a significant cause of stroke worldwide [2]. Patients with severe carotid artery stenosis (≥70%) have an increased risk of myocardial infarction and cardiovascular death (22%) [3]. Total occlusion of the common carotid artery is rare. However, endarterectomy and endovascular revascularization are recommended in cases where inadequate cerebral perfusion leads to various neurological symptoms [4]. During carotid endarterectomy, cross-clamp-

ing of the carotid artery may induce local cerebral ischemia, and unclamping may induce ischemia/reperfusion (I/R) injury [5]. A sudden cerebral blood flow (hyper-perfusion) increase following carotid revascularization may disrupt and damage the blood-brain barrier [6]. It is argued that this damage is due to an increased production of reactive oxygen species (ROS) [7]. I/R may cause damage not only in the primary tissue but also in distant organs [8]. ROS, pro-inflammatory cytokines, and polymorphonuclear leukocytes are implicated in I/R-related distant organ injury [9,10]. The neutrophil-to-lymphocyte ratio is recognized as a marker for systemic inflammation and is significantly associated with postoperative complications [11]. Myocardial ischemia has been reported to occur with ST-segment depression during carotid cross-clamping [12]. These data suggest that severe cardiac and systemic complications develop during carotid endarterectomy and endovascular revascularization and that antioxidant and anti-inflammatory medicines are beneficial in curing cardiac and systemic organ injury that may develop due to reperfusion. Myocardial ischemia and hypoxia have been associated with abnormal increases in TPI and CK-MB levels [13]. It is known that elevated levels of TPI and CK-MB are also positively correlated with elevated levels of oxidants and pro-inflammatory cytokines [14].

The current study investigated thiamine pyrophosphate (TPP) for its potential protective effects against cardiac and other organ damage arising from carotid artery cross-clamping and unclamping. TPP, the active metabolite of thiamine [15], is synthesized in the liver through the phosphorylation of thiamine by thiamine pyrophosphokinase [16]. Existing literature suggests that TPP exerts a protective effect by inhibiting the increase in oxidant and pro-inflammatory parameters[17]. Furthermore, TPP protects cardiac tissue from oxidative damage[18]. All these data suggest that TPP is beneficial against possible cardiac and other organ damage resulting from carotid cross-clamping and unclamping. There is a lack of literature investigating the potential impact of TPP on cardiac damage caused by carotid cross-clamping and unclamping procedures. Hence, our study aimed to biochemically explore TPP's protective effects against potential brain and heart damage from animal carotid cross-clamping and unclamping.

MATERIALS AND METHODS

Animals

This experimental study, employed 18 male albino Wistar rats weighing 285-298 g. All experimental rats were sourced from the Erzincan Binali Yıldırım University Experimental Animals Application and Research Center. The rats were housed and fed in groups for one week under standard conditions, including a regular room temperature (22°C) and a 12-h light/12-h dark cycle to facilitate environmental adaptation. All protocols and procedures were confirmed by the Ethics Committee of the Center for Animal Experiments (October 27, 2022, Meeting No. 10/53, Approval No. E-85748827-050.01.04-212799).

Chemicals

The chemicals used in the experiment and ketamine were sourced from Pfizer Pharmaceuticals Inc., Sti (Türkiye), while TPP was sourced from Biofarma (Russia).

Experimental animals

All experimental rats were divided into right and left common carotid cross-clamping and unclamping (CCU), TPP + common carotid cross-clamping and unclamping (TCCU), and sham operation (SG) groups.

Experimental Procedures

Surgical operations were done under sterile conditions in a suitable laboratory environment. The TCCU (n = 6) group received 20 mg/kg TPP intraperitoneally (IP) one hour before anesthesia. The CCU (n = 6) and SG (n = 6) groups received an equiv-

alent volume of distilled water as a solvent via the IP route. General anesthesia was induced with 60 mg/kg of ketamine hydrochloride via the IP route. The period during which the animals remain immobile in the supine position is considered a suitable anesthesia period for surgical intervention [19]. During this period, rats in all groups were secured in the supine position on the operating table, and the midline of the neck was shaved. After disinfecting this shaved area, a midline incision was made. After a superficial microdissection, a deep microdissection was done on the right common carotid artery. The trachea was exposed, paratracheal muscles were dissected to access the common carotid artery, and a clip was placed on the common carotid artery. Ischemia was induced by maintaining the clips closed for 10 min. In the SG group, only a subcutaneous incision was made. At the end of this period, the clips were removed, the incisions were sutured, and reperfusion was sustained for six hours. Subsequently, the rats were euthanized with high-dose anesthesia. The levels of oxidant/antioxidant markers, including malondialdehyde (MDA) and total glutathione (tGSH), as well as pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin 1β (IL-1β), were measured in the extracted brain and heart tissue samples. Troponin I (TP I) and creatine kinase-MB (CK-MB) levels were also investigated in the blood serum. All biochemical results were compared between the groups.

Biochemical analyses

Preparation of Samples

After rinsing the tissue samples with physiological saline, they were ground into powder using liquid nitrogen. Tissue samples were homogenized to determine GSH, thiobarbituric acid reactive substances (TBARS), and protein levels. Clear filtrates were used to analyze MDA, GSH, and protein levels.

Quantification of MDA and GSH, and Protein Levels

MDA and GSH levels in the brain and heart tissues were quantified following the (ELISA) kit instructions of the respective assays (Cayman Chemical Company). Protein was detected according to the Bradford method [20].

TNF-α, IL-1β, and IL-6 Analyses

The tissues were disrupted and homogenized by adding liquid nitrogen. We then added phosphate-buffered saline (pH 7.4) in a 1/10 (w/v) ratio and vortexed, followed by centrifugation for 15 min at 15000×g to collect the supernatant. The samples were maintained at 2-8°C after melting. The TNF-α, IL-1β, and IL-6 levels were determined using a commercial ELISA kit supplied by Eastbiopharm Co. Ltd. ELISA kit, China.

Troponin I (TP I) and Creatine Kinase-MB (CK-MB) Determination

Blood serum TPI levels were measured using the enzyme-linked fluorescent assay technique with the VIDAS TPI Ultra kit and the ELFA (Enzyme-Linked Fluorescent Assay) technique. The test was performed automatically on the VIDAS device using the ready-to-use test reagents provided in the kit. The Roche/Hitachi Cobas C 701 system determined blood serum CK-MB levels. According to the procedure, the test was performed using the immune-UV assay And ready-to-use test reagents.

Statistical Analyses

The experiment results were presented as "mean value ± standard deviation" ($\bar{x} \pm$ SD). The normality test in the groups was determined by the Shapiro-Wilk test, and the Levene test determined the homogeneity of variances. Since the data were normally distributed, one-way ANOVA was used for all analyses, and then the Tukey HSD test was used post-hoc for pairwise. "GraphPad Prism

8 Software" was used. Biochemical results were analyzed using "IBM SPSS 25.00 (Armonk, NY: IBM Corp)". P<0.05 was accepted to be significant.

RESULTS

Biochemical Findings

Oxidant/Antioxidant levels in brain tissue

As seen in Table 1, MDA levels in the brain tissue of animals were significantly higher in the CCU group than in the sham operation (SG) group (p<0.001). TPP significantly inhibited the increase in MDA levels in the CCU group (p<0.001), and there was a statistically significant difference in MDA levels between the TCCU and SG (p = 0.014).

The tGSH, an antioxidant parameter, significantly decreased in the CCU group compared to the SG group (p<0.001). The tGSH level in the TCCU group significantly increased compared to the CCU group (p<0.001). A statistically significant difference in tGSH levels was found between the TCCU and SG groups (p = 0.006).

Pro-inflammatory cytokines levels in brain tissue

According to Table 1, TNF-α, IL-1β, and IL-6 levels in the brain tissues of animals were significantly higher in the CCU group than in the sham group (p<0.001). TPP administration significantly inhibited the increase in pro-inflammatory cytokines levels in the TCCU group compared to the CCU group (p<0.001). TNF-α, IL-1β, and IL-6 levels showed statistically significant differences between the TCCU and SG groups (p = 0.021; p = 0.001; p = 0.034).

Oxidant/Antioxidant levels in heart tissue

MDA levels in the heart tissues of animals were significantly higher in the CCU group than in the SG group (p<0.001).TPP inhibited the increase in MDA levels in the CCU group (p<0.001). MDA levels differed significantly between the TCCU and SG groups (p = 0.009).

Compared to the SG, the tGSH level significantly decreased in the CCU group (p<0.001). Compared to the CCU group, the tGSH level significantly increased in

Table 1
Biochemical analysis results in the brain and heart tissues and blood serum.

	Biochemical parameters	Mean ± Standard Deviation			p values		
		SG	CCU	TCCU	SG vs. CCU	SG vs. TCCU	CCU vs. TCCU
Brain tissue	MDA (µmol/g protein)	3.46±0.22	5.87±0.68	3.96±0.39	<0.001	<0.05	<0.001
	tGSH (nmol/g protein)	4.57±0.12	2.13±0.40	3.95±0.26	<0.001	<0.05	<0.001
	TNF-α (ng/L)	2.13±0.08	4.51±0.13	2.30±0.06	<0.001	<0.05	<0.001
	IL-1β (pg/L)	1.80±0.12	4.26±0.07	2.13±0.08	<0.001	<0.001	<0.001
	IL-6 (pg/L)	2.55±0.05	4.70±0.23	2.79±0.07	<0.001	<0.05	<0.001
Heart tissue	MDA (µmol/g protein)	1.60±0.16	3.05±0.14	1.90±0.16	<0.001	<0.05	<0.001
	tGSH (nmol/g protein)	7.23±0.13	3.65±0.20	6.48±0.23	<0.001	<0.001	<0.001
	TNF-α (ng/L)	3.30±0.09	6.05±0.34	4.37±0.28	<0.001	<0.001	<0.001
	IL-1β (pg/L)	2.41±0.19	4.50±0.22	2.70±0.08	<0.001	<0.05	<0.001
	IL-6 (pg/L)	2.19±0.41	4.77±0.16	2.48±0.16	<0.001	>0.05	<0.001
Blood serum	TP I (µg/L)	0.02±0.002	0.04±0.004	0.02±0.003	<0.001	>0.05	<0.001
	CK-MB (U/L)	40±6.31	82±6.31	44±6.09	<0.001	>0.05	<0.001

MDA; malondialdehyde, tGSH; total glutathione, TNF-α; tumor necrosis factor alpha, IL-1β; interleukin 1 beta, IL-6; interleukin 6, TP I; Troponin I, CK-MB; Creatine kinase-MB. SG: Sham operation group, CCU: Common karotis cross-clamping and unclamping, TCCU: TPP + common karotis cross-clamping and unclamping. All analysis was done by one-way ANOVA and then Tukey HSD test was used as post-hoc for pairwise comparisons (N=6) "p<0.05 was considered significant".

the TCCU group (p = 0.001). A statistically significant difference was observed in tGSH levels between the TCCU and SG groups (p<0.001) (Table 1).

Pro-inflammatory levels in heart tissue

As seen in Table 1, TNF-α, IL-1β, and IL-6 levels in the heart tissues of animals were significantly higher in the CCU than in the sham group (p<0.001). Pro-inflammatory cytokine levels were significantly lower in the TCCU than in the CCU group (p<0.001). TNF-α and IL-1β levels between the TCCU and SG groups were significantly different (p<0.001, p = 0.025, respectively), whereas IL-6 levels showed similar values (p = 0.184).

TP I and CK-MB levels in blood serum

TPI and CK-MB levels in blood serum were significantly higher in the CCU group than in the sham group (p<0.001). TPI and CK-MB levels in the TCCU group significantly decreased compared to those in the CCU group (p<0.001). There was no significant difference in TPI and CK-MB levels between the SG and TCCU groups (p = 0.238; p = 0.550) (Table 1).

DISCUSSION

This study investigated the effect of TTP against oxidative brain and heart damage resulting from experimentally induced common carotid artery occlusion in rats using biochemical methods. The literature indicates that reversing cerebral blood flow during reperfusion after ischemia increases ROS levels [21]. Numerous studies have reported that brain damage resulting from I/R can impact distant tissues, including the heart [22,23]. Although many different mechanisms are responsible for the pathogenesis of distant tissue damage, it has been well established that ROS generation is one of the most frequently observed mechanisms [24]. ROS react with unsaturated fats in biological membranes to form MDA, the end product of lipid peroxidation (LPO) [25]. MDA is a biological sign of tissue damage and one of the most significant markers of oxidative damage [21]. Therefore, our study assessed MDA levels in brain and cardiac tissues using our I/R model induced by the common carotid artery occlusion method in rats. Our experimental findings demonstrated a significant increase in MDA levels in the brain and heart tissues of rats in the cerebral I/R group compared to the sham group. The literature suggests that increased MDA levels in the brain due to cerebral I/R are associated with neuronal damage [21,26]. Ojo *et al.* demonstrated that brain tissue damage impacted heart tissue in an I/R model created by bilateral carotid artery occlusion/reperfusion in rats [24]. They reported cerebral ischemia induces LPO through increased ROS production in cardiac tissue, leading to oxidative damage. Our findings and existing data indicate that cerebral I/R injury increases ROS, affecting cardiac tissue.

Conversely, in our study, the administration of TPP to rats significantly suppressed the I/R-induced increase in MDA levels in both brain and heart tissues. To our knowledge, our study is the first to examine the protective effect of TPP against cardiac damage caused by common carotid artery occlusion. Yasar *et al*. reported that brain damage caused by focal I/R [27], and Polat *et al.* reported that doxorubicin-induced cardiac toxicity [18], TPP has a protective effect against significantly decreasing the increased MDA level. Our experimental results and existing literature data suggest that TPP protects the heart from oxidative damage by significantly reducing LPO during cerebral I/R, owing to its antioxidant properties.

Excessive ROS production during cerebral I/R causes cell damage by surpassing the capacity of endogenous antioxidants [21,28]. Endogenous antioxidants, such as GSH, are responsible for defense against ROS and are crucial in protecting brain and heart tissues against I/R injury [29,30]. GSH is an important antioxidant enzyme that protects cells from

superoxide and hydroxyl radicals [29,31]. Studies have associated decreased GSH levels resulting from cerebral I/R with increased LPO [32]. Sharipov *et al.* reported oxidative damage in the heart mitochondria of rats in the brain focal I/R model due to an increase in superoxide and hydroxyl radicals [33]. However, it was emphasized that decreased GSH levels in cardiac tissue were associated with increased MDA levels following cerebral I/R in rats [24]. Our findings demonstrate reduced tGSH levels in both brain and heart tissue, consistent with the literature. This suggests that tGSH cannot counteract the elevated ROS levels of I/R. However, TPP significantly suppressed the I/R injury-related decrease in tGSH levels in the brain and heart tissues of rats, which is consistent with previous findings [18,30] demonstrating that TPP prevents the reduction in GSH levels, exerts antioxidant effects, and thereby protects heart and brain tissues against oxidative damage. Our findings suggest that TPP protects against distant cardiac tissue damage due to cerebral I/R by inhibiting LPO and preserving the antioxidant system.

Ischemia, followed by reperfusion, disrupts the redox balance in favor of pro-oxidants and prompts the release of pro-inflammatory cytokines such as TNF-α, IL-β, and IL-6 [34]. Existing studies indicate that TNF-α is the primary cytokine responsible for stimulating the synthesis of cytokines, such as IL-β and IL-6, during cerebral I/R. [35,36] TNF-α and interleukins released due to microglia and astrocyte activation exacerbate neuroinflammation, causing secondary I/R damage in distant tissues, such as the heart, ultimately leading to cell death [24]. Consistent with the literature, cerebral I/R significantly increased TNF-α, IL-1β, and IL-6 levels in both brain and distant cardiac tissues in our study. Our findings suggest that I/R injury begins with oxidative stress and persists due to inflammation. We examined the impact of TPP on inflammation and observed that TPP significantly prevented the increase of TNF-α, IL-1β, and IL-6 levels in both brain and heart tissues. While the study by Yasar *et al*. [27] showed that TPP significantly suppressed increased TNF-α and IL-1β levels due to focal I/R injury, there is no study showing the protective effect of TPP against distant cardiac tissue damage induced by I/R injury resulting from bilateral common carotid artery occlusion.

The bilateral common carotid artery occlusion procedure led to an increase in serum TPI and CK-MB levels. The significant increase in TPI and CK-MB levels explains heart tissue damage and reflects the impact of the brain I/R event on the heart tissue. In the literature, abnormal elevation of TPI and CK-MB levels has been associated with myocardial ischemia and hypoxia [13]. Furthermore, increased TPI and CK-MB levels correlate positively with increased oxidants and pro-inflammatory cytokines [14]. The results of the current experimental study align with another study [37], showing that TPI and CK-MB are associated with increased oxidative and pro-inflammatory cytokines.

In our study, bilateral common carotid artery occlusion increased pro-inflammatory cytokine levels in cardiac and brain tissue, accompanied by increased oxidants and decreased antioxidants. Furthermore, TPP protects the brain and distant cardiac tissues against oxidative and inflammatory damage induced by cerebral I/R. This is the first study to demonstrate the effect of TPP against distant tissue heart damage caused by cerebral I/R injury. However, the current study suggests that TPP administration reduces cardiac damage. The most significant limitations of this study include the absence of common carotid occlusion monitoring and the lack of histopathological examinations. Further studies are necessary to clarify the role of TPP in preventing cerebral I/R injury and its protective effects on distant tissue damage.

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Conflict of interest

None.

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Conception and design: IE, HS; Acquisition of data: TAC; Analysis and interpretation of data: ZS; Drafting of the manuscript: IE, HS; Critical revision of the manuscript for important intellectual content: HS; Statistical analysis: ZS; Final approval of the submitted manuscript: IE, ZS, HS, TAC.

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