ORIGINAL ARTICLE

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Investigation of the Effects of Piceatannol on Endoplasmic Reticulum Stress on Brain in Rats with Experimental Subarachnoid Hemorrhage

Deneysel Subaraknoid Kanama Modeli Oluşturulmuş Sıçanlarda Piseatannolün Beyinde Endoplazmik Retikulum Stresi Üzerine Etkilerinin Araştırılması

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ABSTRACT

Aim: Subarachnoid hemorrhage (SAH) is a common neurologic disorder that accounts for brain injury, diminished neuron function, and neuronal death. Due to various options, SAH treatment remains lacking. Endoplasmic reticulum stress (ERS) in the brain is known as the disruption of the blood-brain barrier and triggered neuronal apoptosis, and contributes to SAH pathogenesis. This study aims to investigate the effects of piceatannol (PST) on ERS and neuronal apoptosis in an experimental SAH model in rats.

Materials and Methods: For this purpose, 24 Wistar Albino male rats (8-10 w) were randomly divided into three groups (n=8); SHAM, SAH, and PST. SAH model was induced via injection of 120 µL of autologous blood into pre-chiasmatic cisterna. 30 mg/kg PST was injected intraperitoneally after 60 minutes from SAH inducement. Garcia's neurologic examination, rotarod, and horizontal bar tests were applied for neurological evaluation. Frontal cortex specimens were harvested for histological and gene expression analysis.

Results: Our results indicated that PST treatment significantly improved Garcia scores ($p<0.01$; $p<0.05$). In addition, PST decreased pyknosis ($p<0.001$) and edema ($p<0.001$) levels, and the number of damaged cells ($p<0.01$) and apoptotic cells ($p<0.05$). GRP78 (78-kDa glucose-regulated protein; $p=0.01$), ATF4 (Activating transcription factor 4; $p=0.01$), and CHOP (C/EBP homologous protein; $p<0.05$) gene expression levels of the SAH group were increased compared to SHAM. Moreover, PST significantly decreased the expression levels of p53 ($p<0.01$).

Conclusion: Our results showed that PST indicated protective effects on ERS after SAH. It could be suggested that PST might be a supportive adjuvant agent in SAH management.

Keywords: Subarachnoid hemorrhage, early brain injury, piceatannol, endoplasmic reticulum stress, apoptosis

ÖZ

Amaç: Subaraknoid kanama (SAK) insanlarda beyin hasarına ve ölüme yol açan, günümüzde henüz kesin bir tedavisi olmayan bir hastalıktır. SAK'ın patogenezi tam olarak aydınlatılmamış olmakla birlikte erken beyin hasarı (EBH) en önemli neden olarak gösterilmektedir. EBH'nin nedenlerinden bir tanesi de endoplazmik retikulum stresidir (ERS). ERS beyin hücrelerinde apoptoza ve kan-beyin bariyerinin bozulmasına yol açmaktadır. Bu çalışmada deneysel SAK modeli oluşturulmuş sıçanlarda piseatannolün (PST) frontal korteksteki ERS ve apoptoz üzerine etkilerinin araştırılması amaçlanmıştır.

Gereç ve Yöntem: Çalışmada 8-10 haftalık Wistar Albino sıçanlardan SHAM (n=8), SAK (n=8) ve PST (n=8) olmak üzere üç grup oluşturuldu. SAK ve PST gruplarında, 120 µL otolog arteriyel kan prekiazmatik sisternaya enjekte edilerek SAK modeli oluşturuldu. PST grubuna SAK sonrası 60. dakikada 30 mg/kg PST intraperiteonal uygulandı. Tüm gruplarda SAK öncesi ve sonrası Garcia nörolojik muayene skorlaması yapıldı. SAK'tan 24 saat sonra frontal korteks dokuları alınarak histopatolojik ve genetik analizler gerçekleştirildi.

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Bulgular: PST uygulamasının istatistiksel olarak anlamlı düzeyde olmak üzere nörodavranışsal test sonuçlarında iyileşmeye (p<0,01; p<0,05) ve histopatolojik düzeyde piknoz (p<0,001), ödem (p<0,001) ve TUNEL+ apoptotik hücre sayısında (p<0,05) azalmaya sebep olduğu gözlendi. SAK grubunda GRP78 (p=0,01), ATF4 (p=0,01) ve CHOP (p<0,05) gen ekspresyon seviyeleri SHAM grubuna göre yüksek bulundu. PST uygulaması SAK'ta artan tüm ERS göstergelerini azaltıcı etki gösterdi. Bu azalma GRP78 için istatistiksel olarak anlamlı bulundu (p<0,05). PST ayrıca SAK'ta artan p53 (p<0,01) gen ekspresyon değerlerini azaltıcı etki gösterdi.

Sonuç: SAK sonrası artan ERS üzerine PST'nin koruyucu etki gösterdiği anlaşıldı. Bu bulgulardan yola çıkarak, SAK tedavisinde PST'nin destekleyici bir adjuvant ajan olarak kullanım potansiyeli olduğu anlaşıldı.

Anahtar Kelimeler: Subaraknoid kanama, erken beyin hasarı, piseatannol, endoplazmik retikulum stresi, apoptoz

INTRODUCTION

Subarachnoid hemorrhage (SAH) is blood flow to the subarachnoid area between the arachnoid and pia mater filled with cerebrospinal fluid. SAH is associated with high morbidity and mortality rates and is responsible for 3-10% of all strokes[1]. SAH is commonly seen in 40-60 ages, with a mortality rate of up to 50% in the 1st month after hemorrhage[2].

The main underlying mechanism of SAH pathogenesis is early brain injury (EBI) which occurs within the first 3 days following SAH. EBI is a complex pathophysiological process that includes cerebral ischemia, blood-brain barrier (BBB) leakage, brain edema, oxidative stress, activation of inflammatory pathways signaling, and neuronal apoptosis[3]. Recent studies claimed that dysfunction of intracellular organelles such as endoplasmic reticulum (ER) and mitochondria may also contribute to the pathophysiology of SAH[4]. It is well known that ER stress (ERS) is responsible for cell death after SAH and ERS triggers the apoptosis of endothelial cells and disruption of the BBB[5]. Therefore, further studies are still required for reducing ERS in SAH.

ERS has been defined as an imbalance between the protein folding capacity and the overload of protein production in ER[6]. Synthesized polypeptides are constantly at risk of misfolding or aggregating into cytotoxic complexes. Molecular chaperones counteract this cytotoxicity by protein refolding and prevention against protein aggregation[7]. Proteins are under chaperone control under normal conditions[8]. Protein folding is facilitated by ER chaperone proteins such as BiP, or GRP78 (78-kDa glucose-regulated protein) and GRP94 (94-kDa glucose-regulated protein), and enzymes such as protein disulfide isomerase and peptidyl-prolyl isomerase[9]. Accumulation of misfolded proteins in ER lumens causes alteration in the ER homeostasis and stimulates the ERS[10]. Misfolded or unfolded proteins are degraded by the ER-associated protein degradation (ERAD) control system within the ER and maintain the protein balance[11]. However, sometimes the ERAD mechanism becomes insufficient to maintain protein balance, and in such cases, the unfolded protein response (UPR) signaling is activated in the cell[12-14]. This pathway is an important signaling mechanism required to restore homeostasis in ER function[15]. Activation of this pathway is mediated by three key sensor proteins, called (a) protein kinase RNA-like ER kinase (PERK), (b) inositol-requiring enzyme-1 (IRE1), and (c) activating transcription factor 6 (ATF6).

It has been reported in many studies that ERS and oxidative stress contribute to the pathogenesis of EBI in SAH[16,17]. Therefore, the reduction or complete elimination of ERS in SAH is considered among the treatment options. For this purpose, research on many molecules continues intensively. One of the candidate molecules considered for the reduction of ERS is piceatannol (PST).

PST is commonly found in blueberries, grapes, and passion fruit seeds, which is a hydroxyl analog of resveratrol (RES)[18]. PST has a higher bioavailability rate than RES[19-21]. This has led to the evaluation of PST as an alternative molecule to RES. The therapeutic aspect of PST is realized thanks to its anti-inflammatory, anti-oxidative, and anti-proliferative properties[22].

In addition, studies have also demonstrated that PST reduces or prevents ERS[13,23]. Although the effects of PST on ERS have been demonstrated in cells and organs such as the liver, osteoblast cells, and endothelial cells, its effect on ERS in SAH has not yet been investigated.

Therefore, our study aimed to investigate whether PST has an effect on ERS levels in the frontal cortex in SAH.

MATERIALS AND METHODS

This study was established with the approval of Çanakkale Onsekiz Mart University Animal Experiments Local Ethics Committee with a number of 2021/02-08. The animal experimentation process of the study was performed at Çanakkale Onsekiz Mart University Experimental Research Application and Research Center.

Animals

In this study, 24 male Wistar rats (8-10-week-old and 200-300 g) were used. The rats were fed ad-libitum during the

experimental period at room temperature of approximately 20±2 °C on a 12-hour light-dark cycle. The rats were randomly divided into three groups (n=8) as follows;

SHAM (n=8): The sham SAH model was established for this group. The PST solvent was administered intraperitoneally (i.p.).

SAH (n=8): The SAH model was created with 120 µL of non-heparinized fresh autologous arterial blood injected into the pre-chiasmatic cisterna for 10 seconds. PST solvent was administered i.p.

PST (n=8): PST (dissolved in distilled water containing 2% ethanol) at a dose of 30 mg/kg was injected i.p. after 60 minutes of SAH inducement.

Preparation and Administration of Piceatannol

PST (Cayman Chemical; Cat. no: 10083-24-6) was first dissolved in 99% ethanol and then diluted with distilled water. The final ethanol concentration of the carrier solution was 2%. The solution was prepared just before injection and freshly administered i.p. at a dose of 30 mg/kg 60 minutes after the SAH inducement. The SHAM and SAH groups were injected with PST dissolved solution via the same route and at the same time points.

Establishment of the SAH Model

For the SAH model, first, the anterior regions of the skulls of rats were shaved under general anesthesia (60-80 mg/kg ketamine hydrochloride and 5 mg/kg xylazine hydrochloride). The tail was opened with a vertical incision and 120 µL arterial blood was collected from the tail artery. The skin and muscles were opened with a vertical incision in the frontal region and the bregma bone junction was reached. Using the stereotaxy device, the needle was tilted 30 degrees 2 mm to the right of the sagittal plane and placed 7 mm anterior to the bregma in the midline with a 1.5 mm diameter burr hole. 120 µL of non-heparinized fresh autologous arterial blood was injected into the pre-chiasmatic cisterna with a 30 G needle in 10 seconds. The incision site was sutured and closed.

In the SHAM group, all stages of the SAH model were performed, 30 G needle was kept for 10 seconds into the pre-chiasmatic cisterna, but no blood was injected into the area[24].

Neurobehavioral Assessment

All rats were subjected to neurologic tests, the day before the start of experimental procedures. The same tests were repeated 24 hours after SAH. For this purpose, Garcia's neurologic examination was performed.

Evaluation of Garcia's Neurological Assessment

Garcia's neurological assessment was used to evaluate neurologic and sensory functions. The evaluation was performed 24 hours before and after SAH by two blind observers[25].

Six tests including spontaneous activity (0-3 points), symmetry in four limb movements (0-3 points), forepaw extension (0-3 points), climbing (1-3 points), body proprioception (1-3 points) and vibration sense (1-3 points) were evaluated with a total score between 3 and 18.

Euthanasia and Tissue Harvesting

At the end of the study (24 hours after SAH), all rats were euthanized by cervical dislocation under general anesthesia, and the frontal cortex specimen was rapidly removed on an ice block. Total brain tissues from 3 of the 8 animals in each group were harvested to determine brain edema. Brain tissues of the remaining animals were used for histopathologic (kept in 4% formaldehyde) and genetic (stored at -80 °C) analysis.

Brain Water Content

Brain water content was evaluated to determine brain edema levels. For that purpose, the wet weight of total brains was rapidly measured and kept in an oven at +70 °C for 72 hours. After 72 hours, the tissue residues were removed from the oven and dry weights were measured. The ratio of wet and dry weight was determined as (%) described previously[26];

Brain Fluid Content=[(Wet Weight - Dry Weight) / Wet Weight] x 100%

Genetic Analysis

Total RNA Isolation

The frontal cortex samples were used for genetic analysis. 25-30 mg frontal cortex samples were weighed and homogenized. Total RNA was isolated from homogenates using PURE Link RNA MiniKit. The concentration and purity of total RNAs were determined by NanoDrop Spectrophotometer and samples with a purity ratio between 1.8-2.1 were considered usable for cDNA production. The obtained RNAs were stored at -80 °C until use[27].

cDNA Yield

cDNA yield was obtained according to the kit procedure (High Capacity cDNA Revere Transcription Kit, USA). The obtained cDNAs were run on a polymerase chain reaction (PCR) device (The Applied Biosystems®, 2720 Thermal Cycler 96-Well PCR).

Real-Time PCR (qRT-PCR) Application

The cDNA samples were amplified and then used for quantitative real-time PCR (qRT-PCR, StepOnePlus™ real-time PCR System). Gene expression levels were analyzed using TaqMan (TaqMan™ Fast Advanced Master Mix, Ampliqon, Lithuania).

Normalization was performed for GRP78, PERK, ATF4, CHOP (C/EBP homologous protein), p53 (tumor protein 53), and NF-κB (nuclear factor kappa b) genes, whose gene expression levels were analyzed in the qRT-PCR method. β-actin was used for normalization.

Histopathological Analysis

The frontal cortex tissue samples were first fixed in a 4% paraformaldehyde solution. The fixed tissue samples were placed in cassettes and washed under water for 2 hours. The tissues were passed through a series of alcohols (60%, 70%, 80%, 90%, 90%, 96%, and 100%) at increasing degrees respectively to remove water from the tissues. The tissues were then passed through xylol and embedded in paraffin. The sections were subjected to routine hematoxylin-eosin (HE) staining procedure and the stained sections were scored for cellular pyknosis and edema under a microscope (x100) according to HE staining criteria. The scoring was performed using 5 different grades Grade 0 (no findings), Grade 1 (mild), Grade 2 (moderate), Grade 3 (severe), and Grade 4 (highly severe).

TUNEL Assay

TUNEL assay was performed on 4 µm thick sections taken from paraffin blocks on slides. For this purpose, deparaffinization, rehydration, blocking of endogenous peroxidase activity, and diaminobenzidine chromogen staining procedures were performed brown stained cells were accepted as apopototic cells and apoptotic index (AI) was calculated according to below mentioned formula: AI=(apoptotic cell number/total cell number)x100.

Statistical Analysis

To determine the changes in gene expression levels, crossing point values were first obtained from qRT-PCR analysis. The results were normalized according to β-actin and gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ formula[28].

Statistical significance levels of other data were analyzed using IBM SPSS 26 software. The Kruskal-Wallis test was used for multiple group comparisons. A comparison between the two groups was performed with the Mann-Whitney U test. The value of $p<0.05$ was considered statistically significant.

RESULTS

This study was performed with 24 rats and 8 animals in each group. No death was observed in the groups during the experiment. Severe hemorrhage in the subarachnoid region was observed in rats subjected to SAH. In the PST group, it was observed that bleeding in the subarachnoid region decreased. No calculation was made for bleeding levels. Only observationally, the presence and amount of bleeding were evaluated. Brain edema data were 77.1% in the SHAM group, 77.8% in the SAH group, and 77.5% in the PST group. There was no significant difference between the groups in terms of brain edema findings.

Garcia's Score

The neurologic functions of the rats were evaluated by Garcia's neurologic examination. There was no statistically significant difference between the groups 24 hours before SAH ($p>0.05$). However, SAH and PST groups indicated significant differences 24 hours after SAH (Figure 1).

ERS Related Genes Expression Levels

In our study, GRP78, PERK, ATF4, and CHOP gene expression levels were examined. While no significant change was observed in PERK gene expression levels, statistically significant changes were observed in GRP78, ATF4, and CHOP gene expression levels ($p<0.05$ or $p<0.01$; Figure 2a-2d).

p53 and NF-κB Gene Expression Levels

The results of the study showed that p53 mRNA levels increased dramatically in the SAH group and PST strongly reduced p53 mRNA levels ($p<0.01$). However, no significant change was

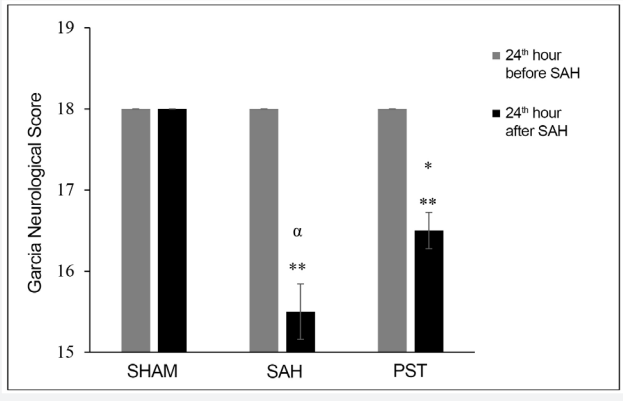

Figure 1. Comparison of Garcia's neurological scoring data
*Compared to SAH ($p<0.05$)
**Compared to SAH ($p<0.01$)
α: Comparison of before and after SAH group ($p<0.01$)

found in NF-κB gene expression levels between the groups (Figure 3a, 3b).

Hematoxylin-Eosin Staining

Frontal cortex tissue samples were evaluated for cellular pyknosis and edema by HE staining. No cellular pyknosis and edema occurred in the SHAM group. Cellular pyknosis and edema levels were significantly increased in both SAH ($p<0.001$ for both parameters) and PST (pyknosis: $p<0.01$; edema: $p<0.001$) groups compared to the SHAM group. HE staining samples of the groups are represented in Figures 4a, b, and c. The statistical significance levels of these changes are represented in Figures 5a and b.

TUNEL Results

Apoptotic cell numbers were determined by the TUNEL staining method. According to the findings, there was a statistically significant increase in the amount of TUNEL-positive cells in both SAH and PST groups compared to the SHAM group ($p<0.01$ and $p=0.05$, respectively). PST treatment significantly decreased the number of apoptotic cells after SAH (Figure 6a-6d).

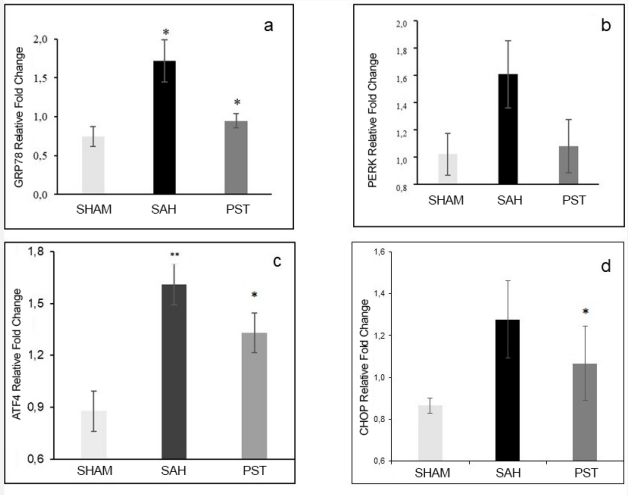

Figure 2. Representative fold changes of ERS related gene expression levels. (a) GRP78, (b) PERK, (c) ATF4, (d) CHOP.

*Compared to the SHAM group ($p<0.05$).

**Compared to the SHAM group ($p=0.01$).

DISCUSSION

In this study, the ameliorative effects of PST on ERS in the frontal cortex were demonstrated for the first time with an experimental SAH model in rats. It was determined that PST administration at 60 minutes after SAH a) improved Garcia's neurological score, b) decreased gene expression levels of ERS markers such as GRP78, PERK, ATF4, and CHOP, c) decreased p53 and NF-κB gene expression levels as inflammatory and apoptotic targets, d) altered pyknosis and edema score at the histopathological level and (e) decreased the number of TUNEL positive cells. These are the first experimental findings clarified that PST administration after SAH may improve SAH-induced ERS and reduce cell damage.

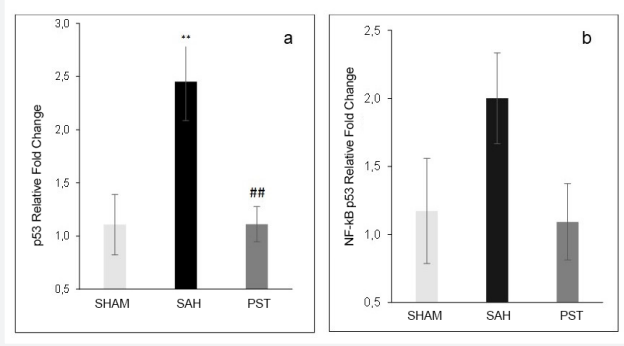

Figure 3. Representative fold changes of apoptosis and inflammatory related gene expression levels. (a) p53, (b) NF-κB. ($p>0.05$)

**Compared to the SHAM group ($p<0.01$).

##: Compared to the SAH group ($p<0.01$).

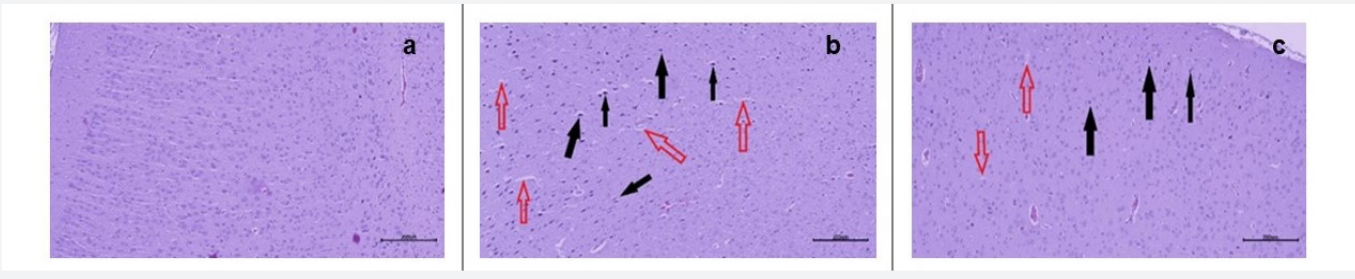

Figure 4. (a) SHAM, (b) SAH and (c) PST groups frontal corteks sections (HE, 100x). Red arrows represent edema and black arrows indicate cellular pyknosis.

In our study, the SAH model modified from Prunell et al.[24] (2003) was applied. At the end of the study, after removing the brain tissues of the animals, the subarachnoid space of all rats was checked for blood accumulation. We proved the success of our SAH model by blood clots in the subarachnoid space, neurologic score, genetic and histopathologic results.

It was observed that our results counteracted previous studies about the wet/dry brain weight ratio. Xiong et al.[29] (2020) removed the brain tissue of rats 48 hours after SAH inducement, dried at 100 °C for 48 hours, and determined the wet/dry brain ratio. The study showed that the wet/dry brain ratio increased in the SAH group and L-cysteine administration reduced brain edema. Yan et al. (2017)[30] reported that brain tissue was removed at 72 hours after SAH and divided into 4 sections. After each section was dried separately at 105 °C for 72 hours, brain edema was determined using the wet/dry brain ratio formula. Researchers claimed that this rate increases with the SAH model. In the study by Qi et al.[31] (2018), the brain tissues were removed 24 hours after SAH and dried at 105 °C for 24 hours. In this study, it was revealed that brain edema increased with SAH, and atorvastatin administration decreased brain edema. In our study, the highest wet/dry brain ratio was determined in the SAH group and PST administration decreased this ratio. However, there was no statistical difference between the groups. The reason may be the small amount of blood injection or the fact that brain edema data were recorded in only 3 rats. On the other hand, we detected edema at a histopathologic level in the brain tissue samples of the SAH group. These results suggest that our SAH model caused brain edema at the histopathologic level, but not severe enough to cause a significant increase in total brain water content.

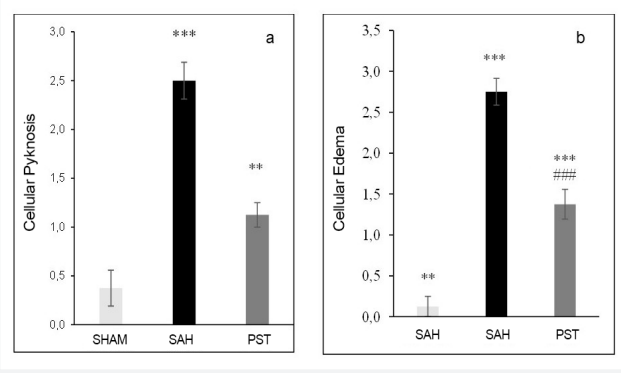

Figure 5. HE staining evaluation results of (a) cellular pyknosis and (b) edema.

**Compared to the SHAM group (p<0.01).

***Compared to the SHAM group (p<0.001).

###Compared to the SAH group (p<0.001).

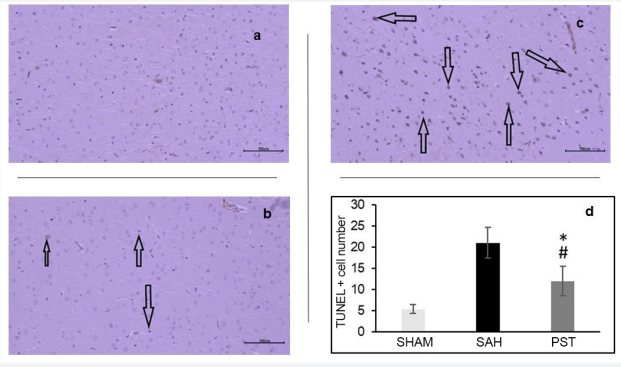

Figure 6. Apoptotic cell images of groups. (a) SHAM, (b) SAK and (c) PST (TUNEL, 200x). Black arrows represent apoptotic cells. (d) TUNEL (+) cell numbers according to AI index.

*Compared to the SAH group (p=0.05).

**Compared to the SHAM group (p<0.01).

#Compared to the SHAM group (p=0.05).

In our study, neurobehavioral test findings of all rats were recorded before the SAH model was created and compared with the results 24 hours after SAH. The neurobehavioral test was performed with Garcia's neurological assessment. Garcia's neurologic scores of all rats before SAH were 18. In the SAH group, the scores decreased to 15.5 at 24 hours. It was determined as 16.5 in the SAH group treated with PST. These results show that PST improves the Garcia neurologic score, which worsened after SAH (Figure 1). Post-SAH neurological score records are determined in almost all SAH studies. The level of deterioration in neurologic scores provides information both about the occurrence of SAH and the level of SAH. On the other hand, the effects of preventive or therapeutic agents on neurologic scores in SAH are also examined. Neurologic score data after SAH are usually recorded at 24 hours because the most significant changes occur at this time. Tian et al.[32] (2020) determined Garcia scores at the 3rd, 6th, 12th, 24th, 48th, and 72nd hours in rats in which they created a SAH model and measured the lowest score at the 24th hour. In their study, the Garcia score, which started to decrease at the 6th hour, decreased to 10 at the 24th hour and then started to increase again. They suggested that this decrease in Garcia score may be related to neuronal apoptosis at this time point[32]. Similarly, in our study, we found that the SAH group with the lowest Garcia score had the highest number of frontal cortex apoptotic cells.

The pathologic events that occur in the brain in the 24th-72nd hours after SAH are defined as EBI. Neuronal apoptosis is the most important underlying mechanism of EBI. Neuronal apoptosis is considered to be the main cause of neurologic deterioration and loss of function after SAH. Many mechanisms are known to stimulate neuronal apoptosis. These include increased ROS, excitotoxicity, synaptic dysfunction, impaired

protein degradation systems, ERS, DNA damage, mitochondrial dysfunction, and inflammation[33]. ERS, one of these factors, has recently been associated with neuronal apoptosis after SAH. Impaired ER function leads to the accumulation of unfolded proteins and consequently to ERS. ERS leads to the activation of the UPR pathway. The UPR activates ERS-responsive proteins such as PERK and ATF4. Activation of PERK stimulates the activation of ER-derived chaperones and cytokines. Moderate ERS leads to cell survival, whereas severe and sustained ERS leads to nerve cell death. A study performed by Nakka et al.[17] (2016) showed that ERS was involved in ischemia/reperfusion-induced nerve cell death. In their study, they determined that GRP78, CHOP, and ATF4 gene expression levels increased in brain tissue after ischemia/reperfusion, and high levels of neuronal damage. When they applied salubrinal, a selective eIF2α inhibitor, to reduce UPR, significant reductions in neuronal cell death. In another study, the effect of zonisamide on neuronal cell death via ERS was examined. The results revealed that zonisamide decreased ERS via CHOP and caspase-3 and prevented neuronal damage in rats with Parkinson's model[34].

In addition to neurodegenerative diseases, increased ERS after SAH has also been associated with neuronal cell death. Jiang et al.[35] (2021) showed that GRP78, CHOP, and caspase-12 gene expression levels increased after SAH in cerebral cortex tissue in rats, thus increasing neuronal cell death. Findings show that neuronal apoptosis is improved in rats given hydrogen-rich saline. Tian et al.[32] (2020) showed that the number of TUNEL-positive apoptotic cells increased at 24 hours after SAH. The researchers suggested that the increase in neuronal apoptosis may be caused by increases in GRP78, CHOP, caspase-12, and ASK1 gene expression levels. In our study, increases in GRP78, PERK, ATF4, and CHOP gene expression levels, which are indicators of ERS, were also observed after SAH and PST administration showed a therapeutic effect against these increases. We observed that both p53 and NF-κB and apoptotic nerve cell numbers were increased in the groups in which ERS indicators were also increased. These findings show that increased ERS after SAH triggers neuronal apoptosis in parallel with the reports in the literature. PST administration showed an ameliorative effect on neuronal damage by decreasing ERS.

In our study, NF-κB gene expression levels were analyzed as a parameter that may indicate inflammatory processes. The fact that NF-κB gene expression levels, which increased after SAH, decreased by approximately 50% in the PST group suggested that PST inhibited inflammatory processes. The inhibitory effect of PST on NF-κB was demonstrated in a study[36]. According to the results of the study, it was suggested that the antioxidant and anti-inflammatory effects of PST, which occur in brain tissue and are thought to be protective against brain damage, were associated with NF-κB inhibition. In the study, the effect of PST on brain cells was examined in lipopolysaccharide-induced inflammation. In an *in vitro* study, it was reported that PST might protect the brain against damage by reducing the disruption in the BBB. In another study, the protective effects of PST in a brain ischemia/reperfusion model were examined[37]. PST is known as a SIRT1 activator. SIRT1 activation protects cells against damage by stimulating the synthesis of antioxidant and antiapoptotic factors[38,39]. Based on the study results, the antioxidant and anti-inflammatory effects of PST in a brain ischemia/reperfusion model were associated with SIRT1 activation[37]. Although the above-mentioned studies show the protective effects of PST on brain injury, only one study examining the effects of PST in SAH was found. However, PST is known as a RES analog and there are studies on the role of RES in both brain injury and SAH. In a study in which RES was administered at both very high and low doses in an SAH model, it was found that its antiapoptotic effect only appeared at high doses. In the study, RES showed this effect by inhibiting the Akt pathway[40]. In another study, RES also showed a decreasing effect on ERS with increased oxidative stress after SAH[41]. The effect of RES was examined in the prefrontal cortex similar to our study. RES significantly decreased the increased CHOP and GRP78 levels after SAH. Increased ROS accumulation in SAH triggers processes leading to ERS and apoptosis. RES, an antioxidant, shows a decreasing effect on ERS thanks to this property. In our study, similar to RES, PST decreased CHOP, GRP78, ATF4, and PERK gene expression levels, which are among the increased ERS indicators after SAH. PST also decreased the number of TUNEL-positive cells. These data suggest that PST also decreased apoptosis after SAH.

Study Limitations

In our study, the protein levels of the markers were not evaluated. In addition, only 3 rats were used for brain edema because of ethical issues.

CONCLUSION

Our results showed that PST improved the deteriorated neurologic scores after SAH, decreased apoptosis, and produced a protective effect against the ERS. These results are the first findings that PST has a protective effect on ERS after SAH. Based on these findings, PST has the potential to be used as an adjuvant agent in the treatment of SAH. However, further studies are required to elucidate other underlying mechanisms.

Ethics

Ethics Committee Approval: This study was approved by Çanakkale Onsekiz Mart University Animal Experiments Local Ethics Committee with a number of 2021/02-08.

Informed Consent: Animal experiment.

Authorship Contributions

Surgical and Medical Practices - Concept - Design - Data Collection or Processing - Analysis or Interpretation - Literature Search - Writing: B.Ö., M.U.

Conflict of Interest: No conflict of interest was declared by the authors.

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