Available online at www.medicinescience.org

ORIGINAL ARTICLE

Medicine Science International Medical Journal

Medicine Science 2020;9(4):817-22

Assessment of sertraline activity in a vasospasm model following experimental subarachnoid haemorrhage

Veysel Kiyak[1], Mustafa Namik Oztanir[2], Nese Basak Turkmen[3], Asli Tasdemir[4], Osman Ciftci[5]

[1]Surgeon, Tokat State Hospital, Tokat, Turkey
[2]Associate Professor, Department of Neurosurgery, Faculty of Medicine, Bezmialem Vakif University, Istanbul, Turkey
[3]Assistant Professor, Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Inonu University, Malatya, Turkey
[4]Associate Professor, Department of Histology and Embryology, Faculty of Medicine, Inonu University, Malatya, Turkey
[5]Full Professor, Department of Medical Pharmacology, Faculty of Medicine, Pamukkale University, Denizli, Turkey

Received 09 August 2020; Accepted 15 September 2020
Available online 27.09.2020 with doi: 10.5455/medscience.2020.08.158

Abstract

Vasospasm following subarachnoid haemorrhage (SAH) is a process yet to be fully clarified in terms of its aetiology and results. According to one of the many theories about vasospasm developing after SAH, the process results from an increase of pro-inflammatory agents and decrease in antioxidant agents. Other hand experimental studies on rats found a significant decrease in the pro-inflammatory parameters TNF-α and IL-1β in the blood values obtained after the use of sertraline. In this study, the findings regarding the effectiveness of sertraline in the treatment of vasospasm developing an experimental SAH model are presented. In this study, adult males of Spraque-Dawley breed, not used in any previous study and weighing between 250–350 g, were used. Rats were divided into 4 groups with the control group (n=5) and other groups (n=6 in each). Group 1 was the control, and Group 3 was the sertraline group. In Groups 2 and 4, SAH was initiated by giving rats autologous arterial blood in the cisterna magna. The tissues were examined in terms of mononuclear cell infiltration, vascular congestion, and neuron degeneration. In the experimental SAH model based on these values, it was found that the use of sertraline significantly reduced mononuclear cell infiltration, vascular congestion, and neuron degeneration. Moreover, in animal studies, it was shown that SSRIs increased neurogenesis and release of neurotrophins from the hippocampus. In our study, it was concluded that sertraline was effective in dissolving vasospasm in the experimental SAH model. However, we further believe that more experimental studies to investigate other SSRI compounds of the same family can contribute to the knowledge and understanding of this process.

Keywords: Sertraline, vasospasm, subarachnoid haemorrhage

Introduction

Subarachnoid haemorrhage (SAH) is the bleeding in the subarachnoid space of the brain usually due to arterial reasons, albeit it may occasionally occur for venous reasons. Its incidence varies from 10 to 16 per year in a population of 100,000, but it is known that these rates increase with age. Subarachnoid haemorrhages can occur as a result of trauma, aneurysm, vascular malformations, bleeding disorders, brain tumours, or as a complication of anticoagulant treatment; in 20% of all cases, however, no reason can be found [1]. Cerebral vasospasm (CVS) is shown as the most important cause of mortality and morbidity following a SAH. The severity of vasospasm is directly related to the amount of blood in the subarachnoid space [2]. Despite all the developments in medical sciences, a cure is yet to be found for vasospasm following a SAH and its complications. The fact that it is based on multiple reasons further complicates the development of a definitive cure. CVS was the subject of many studies after it was first shown radiologically in 1951. According to studies, even though the rate of radiological vasospasm was 70% in patients who experienced a SAH, only 30–40% developed symptomatic cerebral ischemia [3]. Unlike other ischemic strokes, vasospasm following an aneurysmal SAH is a condition where mortality and morbidity can be reduced by taking necessary precautions in terms of predictability, prevention, and treatment, by following the patient very closely on critical days, and delivering treatment from the moment it is suspected [4].

Sertraline is one of the selective serotonin reuptake inhibitors (SSRI) and increases the levels of extracellular serotonin and brain-derived neurotrophic factor (BDNF). It also has antioxidant and anti-inflammatory properties [5, 6]. Sertraline is a potent serotonin reuptake inhibitor. It binds to serotonin reabsorption (reuptake) sites with a high affinity. It has a weak effect on noradrenaline and dopamine reabsorption [7]. In a study with sertraline administration for 21 days, it was found that repeated doses of antidepressants stimulated brain dopaminergic receptors [8]. In an experimental study with rats, a significant decrease in the blood levels of the pro-inflammatory parameters TNF-α and IL-1β was observed following sertraline use [9]. In this ischemic model

*Coresponding Author: Veysel Kiyak, Surgeon, Tokat State Hospital, Tokat, Turkey, E-mail: vyslkyk86@gmail.com

study, the effects of SSRIs were considered in terms of preventing inflammation by triggering neurogenesis in the hippocampal dentate gyrus and stopping the migration of microglial cells [10]. Our study aimed to investigate the efficacy of the sertraline used towards the prevention mortality-morbidity caused by cerebral vasospasm following a SAH, and to this end, it was used following the experimentally induced SAH.

Materils and Methods

In this study, a total of 40 rats were used, and the study was performed with 23 male rats of the Spraque-Dawley breed that were divided into 4 groups: Group 1 control (n=5), Group 2 with SAH induced (n=6), Group 3 only sertraline (10 mg/kg) administration (n=6), and Group 4 SAH and sertraline (10 mg/kg) (n=6). In our study, 50-mg tablets of Lustral (sertraline) were used. Dosage was prepared by dissolving the calculated amounts of sertraline in chemically modified curcumin (CMC). After anaesthesia, 0.3 mL of intracardiac blood was taken from the rats in the supine position using a PPD injector. The rats were then placed in the prone position. The head was brought to hyperflexion, and the cisterna magna was entered from the atlanto-occipital distance using a PPD injector. The blood taken from the rats in the amount of 0.3 mL was injected into the SAH and SAH + Sertraline groups. The rats were placed in the Trendelenburg position for 15 minutes for the blood to spread to their cisterna. The rats were caged after they woke up completely.

Group 1 (n=5) rats were sacrificed at the end of day 14 without any treatment. Group 2 (n=6) rats were sacrificed on day 14 following the induction of SAH. Four lost rats were excluded from the study. Group 3 (n=6) rats were given only sertraline and were sacrificed at the end of day 14. Lost rats were excluded from the study. Group 4 (n=6) rats were sacrificed on day 14 following induced SAH and administration of sertraline. Four lost rats were excluded from the study. In the study, 40 adult male rats of the Spraque-Dawley breed, weighing between 250–350 g and not used in any previous study were used with the permission obtained from our university, Experimental Animals Ethics Committee on January 15, 2016, no. 2016/A-04. All subjects were kept in cages at room temperature, in conditions of 12 hours' light and 12 hours' dark throughout the test period. Standards for care and use of laboratory animals were followed. They were given standard nutrition. All animals were subjected to large craniectomy with the total removal of the brain, cerebellum, and brain stems. Then sections of brain tissue were photographed at 40× magnification. Sacrification process following the collection and preparation of tissue samples: At the end of day 14, all subjects were anaesthetized in spontaneous breathing with the intraperitoneal injection of a mixture of ketamine hydrochloride (30 mg/kg) (Alfamine 10%) and xylazine hydrochloride (10mg/kg) (Alfazyne 2%). Cerebrum, cerebellum, and brainstem remaining on the foramen magnum were removed in total to maintain anatomical integrity. Preparation of tissue samples: they were stored in an aluminium foil at –30 degrees in 10% formaldehyde. Test subjects were randomly distributed to 4 groups of 10 rats each. Preparates and their histopathological examinations were finalized in the Histology and Embryology Department Laboratory, and the immunochemical measurements were performed in the Biochemistry Division Research Laboratory. Statistical evaluations were carried out in the SPSS for Windows 21.0 software package. Following the normality test, the differences between groups were compared, using the one-way variance analysis (One-Way ANOVA). Results were expressed as mean ± standard error, and $p<0.05$ was considered statistical significance.

Results

All animals were subjected to large craniectomy with the total removal of the brain, cerebellum, and brain stems. Then sections of brain tissue were photographed at 40× magnification. Sacrification process following the collection and preparation of tissue samples: Afterwards, biochemical levels of thiobarbituric acid reactive substance (TBARS), superoxide dismutase (SOD) activity, glutathione peroxidase (GPx) activity, catalase (CAT) enzyme activity, and reduced glutathione (GSH) were measured.

Histopathological Findings

From the paraffin blocks, sections of 5 µm thickness were obtained with the help of a microtome. The prepared sections were dyed using the hematoxylin-eosin (HE) staining method and observed and photographed using a Leica DFC 280 light microscope, Leica Q Win Image Analysis System (Leica Microsystems Imaging Solutions, Cambridge, UK). Brain tissue was observed in normal histological appearance in the control group (Figure 1A). The neurons in the brain cortex were observed as normal in histological appearance (Figure 1B). In the SAH group, significant histopathological changes were observed in brain tissue samples. These histopathological changes were in the pia mater layer of cell infiltration and congestion (arrows, Figure 2A), mononuclear cell infiltration (arrows, Figure 2B), vascular congestion (arrows, Figure 2C, D), and neuron degeneration (Figure 2E). However, it was found that the administration of sertraline reduced histopathological damage in the group of SAH model and remedied negative effects significantly. In the SAH + sertraline group, on the other hand, a decrease in cell infiltration and congestion was detected in the pia mater layer (arrows, Figure 3A), in addition to decreased haemorrhage (arrows, Figure 3B), decreased mononuclear cell infiltration (arrows, Figure 3C), and a significant decrease in neuron degeneration (Figure 3D). In the sertraline group, brain tissue (Figure 1C) and neurons (Figure 1D) were in a normal histological appearance. An examination of the cerebellum tissue in all groups revealed Purkinje cells (arrows) of normal histological appearance in the control (Figure 4A) and sertraline (Figure 4D) groups. As opposed to the pronounced presence of degenerate Purkinje cells in the SAH group (Figure 4B), a significant decrease was observed in the degenerated Purkinje cells in the SAH + sertraline group (Figure 4C).

Biochemical Findings

Before the subjects were sacrificed, blood samples were collected from all of them to check serum TBARS (thiobarbituric acid reactive substance), GSH (reduced glutathione), SOD (superoxide dismutase), CAT (catalase) and GPX (glutathione peroxidase) levels (Table 1).

Immunological Findings

Before the subjects were sacrificed, blood samples were collected from all of them to check serum IL-1β (interleukin 1 beta) and TNF-α (tumour necrosis factor alfa) levels (Table 2).

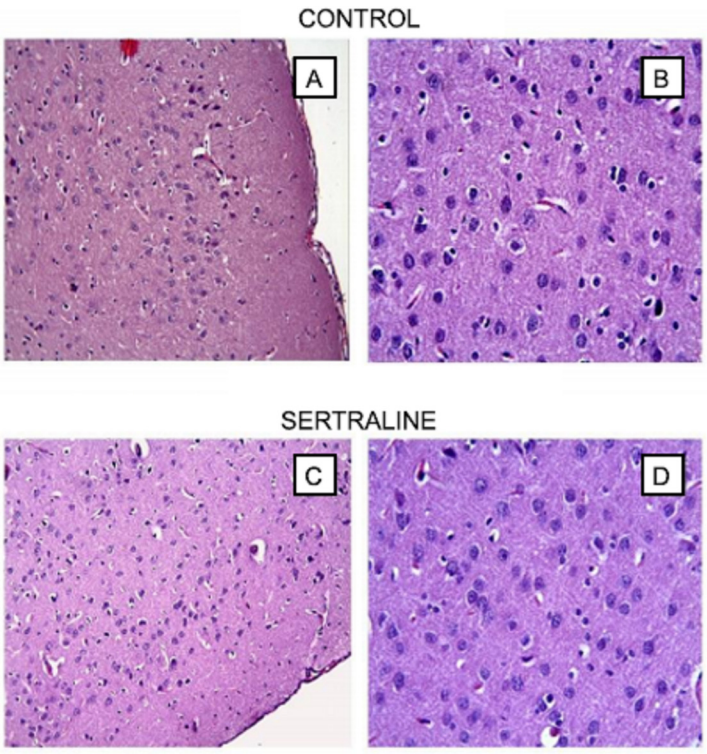

Figure 1. Control (A) and sertraline (B) groups were observed in normal histological appearance. A, C: H-E: ×20, B, D: H-E; ×40

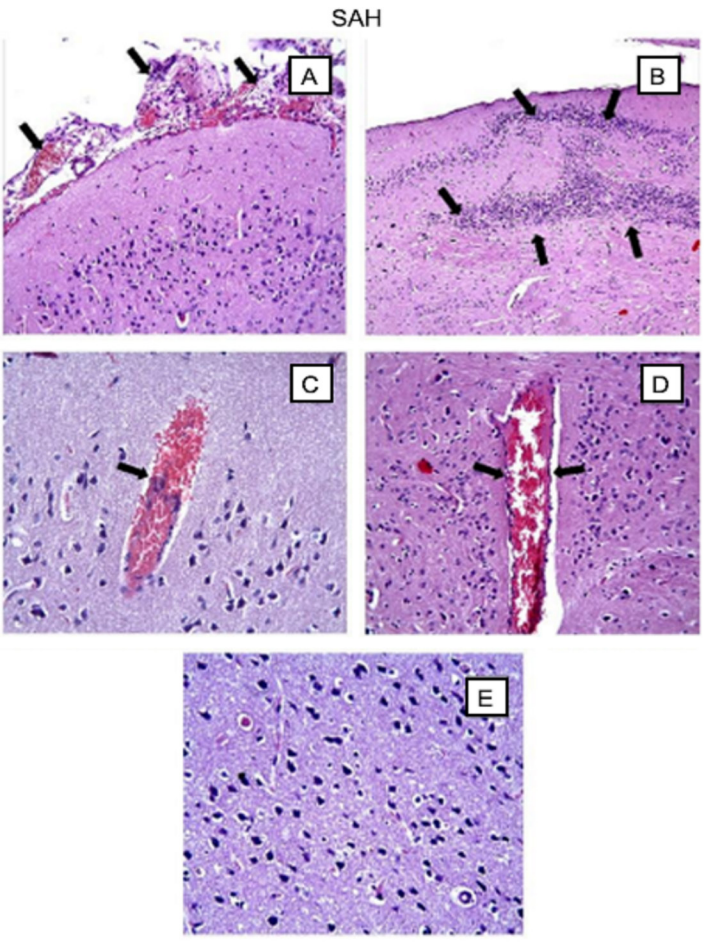

Figure 2. SAH group: Cell infiltration and congestion (arrows) (A), mononuclear cell infiltration (arrows) (B), vascular congestion (arrows) (C), and neuron degeneration (E) were observed in the pia mater layer. A: H-E; ×10, B: H-E; ×20, C: H-E; 40, D: H-E; 20, E: H-E; ×40

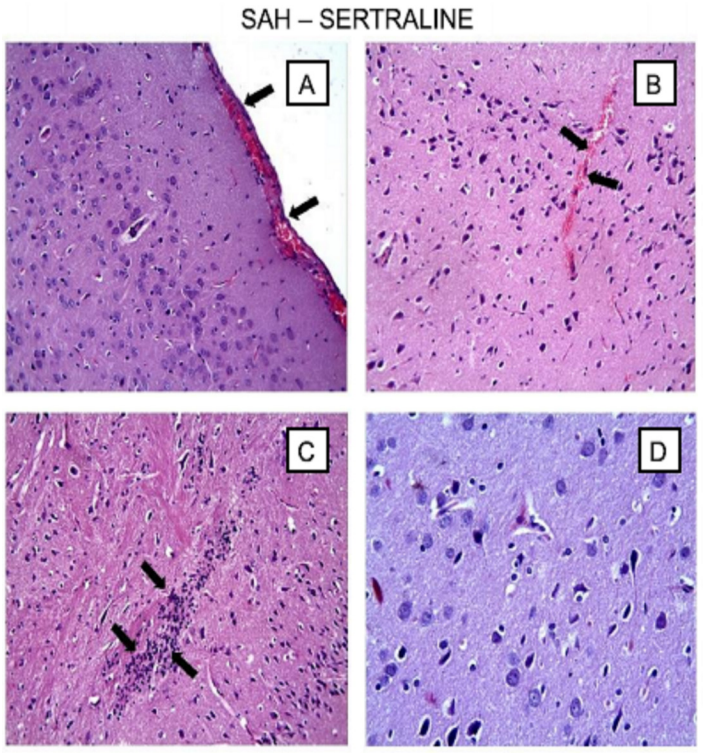

Figure 3. SAH + sertraline group: Decrease in cell infiltration and congestion (arrows) (A), slight haemorrhage (arrows) (B), decrease in mononuclear cell infiltration (arrows) (C), and a pronounced decrease in neuron degeneration (D) was detected in the pia mater layer. A, B, C: H-E; ×20; D: H-E; ×40

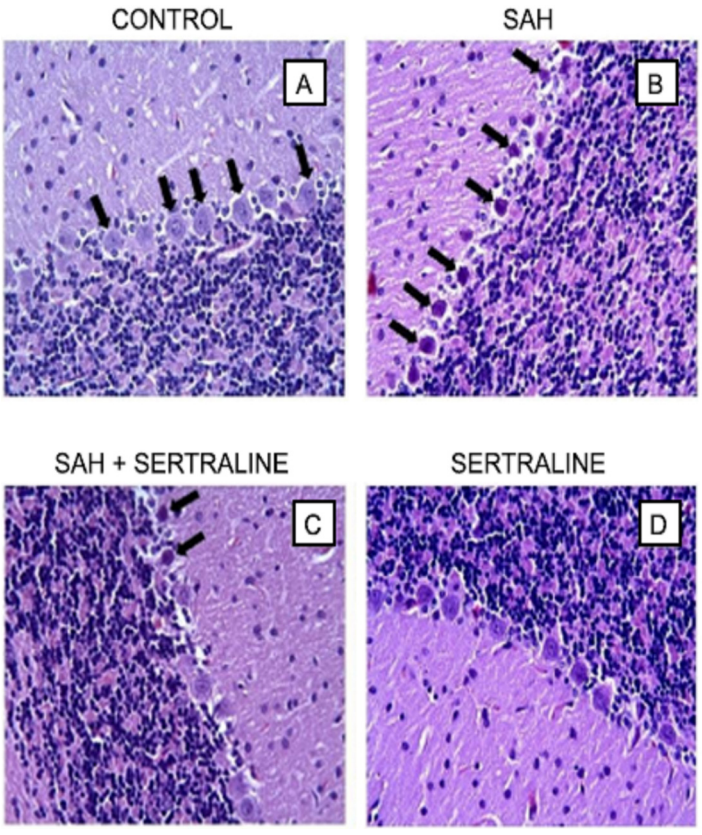

Figure 4. Purkinje cells (arrows) of normal histological appearance in the control (A) and sertraline (D) groups, in addition to a large number of Purkinje cells (arrows), detected in the SAH group. As for the SAH + sertraline group, a pronounced decrease was observed in the degenerated Purkinje cells (arrows). A, B, C, D: H-E; ×40

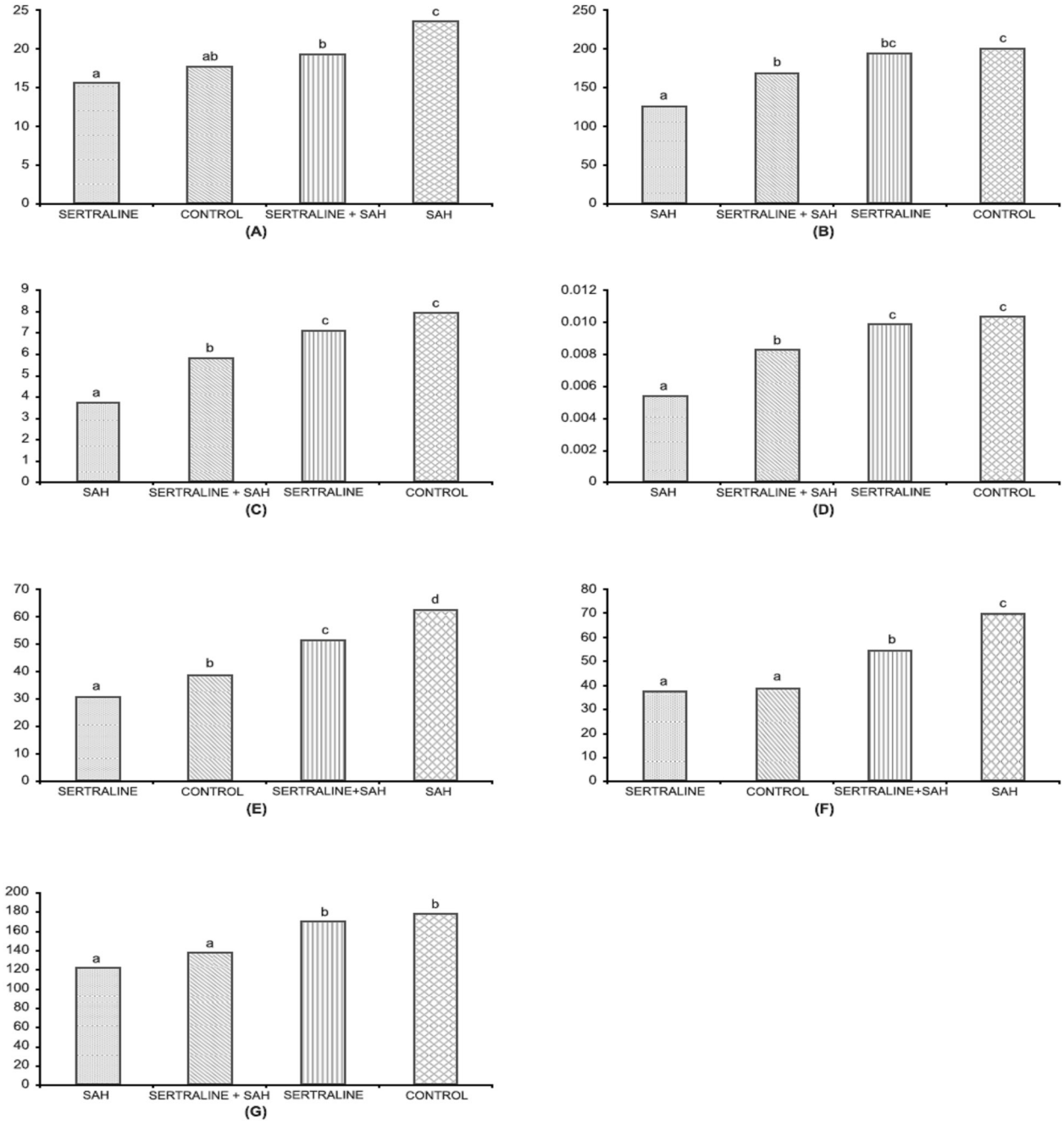

Figure 5. (A) Serum TBARS levels—no statistically significant difference was found between same letters based on the threshold of $p<0.05$, (B) Serum GSH levels—no statistically significant difference was found between same letters based on the threshold of $p<0.05$, (C) Serum SOD levels—no statistically significant difference was found between same letters based on the threshold of $p<0.05$, (D) Serum CAT levels—no statistically significant difference was found between same letters based on the threshold of $p<0.05$, (E) Serum IL-1β levels, (F) Serum TNF-α levels—no statistically significant difference was found between same letters based on the threshold of $p<0.05$, and (G) Serum GPX levels—no statistically significant difference was found between same letters based on the threshold of $p<0.05$

Table 1. Serum TBARS (thiobarbituric acid reactive substance), GSH (reduced glutathione), SOD (superoxide dismutase), CAT (catalase) and GPX (glutathione peroxidase) levels

Cadaver	TBARS	GSH	SOD	CAT	GPX
CONTROL	17.82 ± 1.72^{ab}	202.0 ± 33.3^{c}	7.97 ± 0.65^{c}	0.0104 ± 0.0008^{c}	178.1 ± 12.8^{b}
SERTRALINE	15.73 ± 3.36^{a}	195.0 ± 12.6^{bc}	7.13 ± 0.90^{c}	0.0099 ± 0.0006^{c}	170.6 ± 12.6^{b}
SAH	23.70 ± 2.53^{c}	127.5 ± 32.5^{a}	3.75 ± 0.45^{a}	0.0054 ± 0.0012^{a}	122.6 ± 20.4^{a}
SERTRALINE+SAH	19.39 ± 2.19^{b}	170.0 ± 5.69^{b}	5.87 ± 0.65^{b}	0.0083 ± 0.0018^{b}	138.3 ± 8.80^{a}

Table 2. Serum IL-1β (interleukin 1 beta) and TNF-α (tumour necrosis factor alfa) levels

	TNF-α pg/ml	IL-1β pg/ml
CONTROL	38.8±5.9[a]	31.0±5.9[b]
SAH	69.9±4.8[c]	62.9±4.2[d]
SERTRALINE	37.6±5.4[a]	39.1±9.6[a]
SERTRALINE+SAH	54.9±6.6[b]	51.6±5.8[c]

Discussion

Many studies were conducted on vasospasm after it was first radiologically shown in 1951. Studies concluded that despite 70% of patients experiencing a SAH had radiological vasospasm, only 30–40% of them developed symptomatic cerebral ischemia [3]. Serotonin is an important monoamine with complex activity on brain arteries. SSRIs narrow large brain arteries while widening small ones [6]. This characteristic strengthens the thesis that they may play an important part in the vasospasm process. Sertraline is a pharmacological agent of the group of selective serotonin reuptake inhibitors (SSRI). It increases the levels of extracellular serotonin and brain-derived neurotrophic factor (BDNF), as well as having antioxidant and anti-inflammatory properties [5, 6]. There are many attempts to explain vasospasm and related changes, but they have not yet been fully clarified. Following a SAH, intracranial pressure increases, and thus, cerebral perfusion pressure decreases. This leads to malnutrition of the brain tissue. Problems such as acute inflammation, free radical formation, decreased antioxidant agents, and lipid peroxidation that occur in later stages can give an idea of the enormous complexity of the entire process. Many studies are performed on the subject, as it does not only involve the changes occurring in the cerebral vessels, but a large number of morphological and biochemical changes occur, as well. It was shown on animal brain that the increases in oxidative damage and free radical production take place after ischemia/reperfusion [14]. The pronounced increase in antioxidant parameters in this study confirms that sertraline may have an antioxidant effect. These findings are also compatible with previous studies which demonstrated the antioxidant effect of sertraline in neurodegenerative diseases [15]. It also has an antioxidant-like effect in sertraline ischemia-reperfusion injury. In another experimental study with rats, a significant decrease was detected in TNF alpha and IL-1β levels, as pro-inflammatory parameters [9]. In another study with the SSRI fluoxetine, it was shown that rats that were given a middle cerebral artery (MCA) occlusion had less ischemic damage although the drug was administered postischemic at the 9th hour [11]. Fluoxetine's anti-inflammatory effects providing neural protection by reducing late-term postischemic inflammation led to the thinking that other SSRIs such as sertraline could have similar effects. The most important factors in the ischemic process are the duration of ischemia and early restoration of cerebral blood flow. The literature offer examples as HIF 1 (hypoxia-inducible factor, a secretion closely related to cellular oxygen concentration) play an important part in neuronal survival following ischemia [11]. In the literature, in a model of mice with photothrombotic cortical ischemia, positive effects were detected on the autoregulation of cerebral blood flow as a result of postischemic treatment with SSRI derivatives, fluoxetine and sertraline, and a significant difference was observed in infarct areas. In the same study, it was found that the expression of HIF 1 and HO-1 proteins increased. It is thought that the increase in these proteins activates VEGF (vascular endothelial growth factor), which in turn has an important part to play in oxygen homeostasis through gene expression [12]. Furthermore, small brain arteries may be interacting with calcium signaling mechanisms in smooth muscle cells through SSRIs, which in turn leads to vasodilatation. This suggests that they contribute to the early recovery of cerebral microcirculation. Treatment with postischemic sertraline and fluoxetine enables cerebral autoregulation with a lower mean blood pressure. Experimental studies also report that SSRIs help maintain the integrity of blood-brain barrier (BBB). In these studies, brain edema severity was found to be significantly lower in groups with SSRI [11]. Animal studies showed that chronic treatment with SSRIs stimulated beta adrenergic regulation in caudate putamen and frontal cortex. This led to desensitization of the physiological responses of postsynaptic 5-HT1A receptors [13]. In animal studies, SSRIs were demonstrated to increase neurogenesis and release of neurotrophins from the hippocampus [6]. Many studies showed that SSRI-induced neurogenesis limited postischemic damage.

H-E staining was applied on the tissue samples obtained in the experimental study, and it was observed that the brain tissue maintained its normal histological appearance in the control group (Figure 1A). The neurons in the brain cortex were observed as normal in histological appearance (Figure 1B). In the SAH group, significant histopathological changes were observed in brain tissue samples. These histopathological changes were detected on the pia mater layer as cell infiltration and congestion (arrows) (Figure 2A), mononuclear cell infiltration (arrows) (Figure 2B), vascular congestion (arrows) (Figure 2C, D), and neuron degeneration (Figure 2E). However, it was found that sertraline administration reduced histopathological damage in the SAH model group, as well as significantly eliminating these negative effects. In the SAH + sertraline group, reduced cell infiltration and congestion (arrows) (Figure 3A), slight haemorrhage (arrows) (Figure 3B), decrease in mononuclear cell infiltration (arrows) (Figure 3C), and a significant decrease in neuron degeneration (Figure 3D) were detected. In the sertraline group, brain tissue (Figure 1C) and neurons (Figure 1D) were in a normal histological appearance. An examination of the cerebellum tissue in all groups revealed Purkinje cells (arrows) of normal histological appearance in the control (Figure 4A) and sertraline (Figure 4D) groups. Noticeable degenerate Purkinje cells (Figure 4B) were observed in the SAH group and a significant decrease in the degenerate Purkinje cells (Figure 4C) in the SAH + sertraline group. Before the subjects were sacrificed, blood samples were collected from all of them to check serum TBARS, GSH, SOD, CAT, GPX, TNF- α and levels. In the SAH + sertraline group, there was a statistically significant decrease in TBARS (Figure 5A), TNF-α (Figure 5F) and IL-1β (Figure 5E) levels and a significant increase in GSH (Figure 5B), SOD (Figure 5C) and CAT (Figure 5D) levels as compared to the SAH group. In this study, conducted in light of all this information, sertraline was found to reduce the acute inflammation parameters and increase antioxidant parameters occurring after a SAH. Moreover, histological examinations showed that it reduced the degenerated Purkinje cells and mononuclear cell infiltration, containing and reducing cerebral tissue damage.The results show

that sertraline reduced the adverse effects following a SAH.

Conclusion

CVS is indicated as the most important cause of mortality and morbidity after a SAH. The amount of blood at the subarachnoid space and the severity of vasospasm are interrelated. As a result of the obtained results, a decrease in mononuclear cell infiltration, vascular congestion, and neuron degeneration was detected in the treatment group. Results of TBARS and immunological parameters, TNF-α and IL-1β, from the biochemical analysis are consistent with the results of histopathological examination. A significant increase was detected in CAT, TBARS, GSH, and GPX values. However, more research should be done on this subject. Analyses of other SSRI compounds from the same family as sertraline and more tests on VEGF expression, which is thought to play an important role in cerebral oxygenation homeostasis, can largely contribute to the knowledge and understanding of this process.

Conflict of interests

No benefits in any form have been or will be received from a commercial party related directly or indirectly to the subject of this manuscript.

Financial Disclosure

The advisor of the dissertation was the second author (MNÖ). It was conducted as part of a project which was supported by the Scientific Research Projects Unit of Malatya Inonu University, Project No.: 2016-44.

Ethical approval

In our study, 40 adult male rats of the Spraque-Dawley breed, weighing between 250–350 g and not used in any previous study were used with the permission obtained from the İnönü University, experimental animals ethics committee on January 15, 2016, no. 2016/A-04.

References

1. Yasargil MG, Fox JL. The microsurgical approach to intracranial aneurysms. Surg Neurol. 1975;3:7-14.
2. Kassell NF, Shaffrey ME, Shaffrey CL. Cerebral vasospasm following aneurysmal subarachnoid hemorrhage. In: Apuzzo MLJ, ed, Brain surgery: Complication avoidance and management. 1. New York: Churchill Livingstone, 1993;847-56.
3. Liu-DeRyke X, Rhoney DH. Cerebral vasospasm after aneurysmal subarachnoid hemorrhage: An overview of pharmacologic management. Pharmacotherapy. 2006;26:182-203.
4. Göker B, Akçakaya MO, Hamamcıoğlu MK, et al. Serebral vazospazm: Klinik izlem ve tedavi [Cerebral vasospasm: Clinical monitoring and treatment]. Türk Nöroşir Derg. 2018;28:119-23.
5. Duan W, Peng Q, Masuda N, et al. Sertraline slows disease progression and increases neurogenesis in N171-82Q mouse model of Huntington's disease. Neurobiol Dis. 2008;30:312-22.
6. Kumar P, Kumar A. Possible neuroprotective effect of Withania somnifera root extract against 3-nitropropionic acid-induced behavioral, biochemical, and mitochondrial dysfunction in an animal model of Huntington's disease. J Med Food. 2009;12:591-600.
7. Hiemke C, Härtter S. Pharmacokinetics of selective serotonin reuptake inhibitors. Pharmacol Ther. 2000;85:11-28.
8. Huzarska M, Zieliński M, Herman ZS. Repeated treatment with antidepressants enhances dopamine D1 receptor gene expression in the rat brain. Eur J Pharmacol. 2006;532:208-13.
9. Gill JS, Jamwal S, Kumar P, et al. Sertraline and venlafaxine improves motor performance and neurobehavioral deficit in quinolinic acid induced Huntington's like symptoms in rats: Possible neurotransmitters modulation. Pharmacol Rep. 2017;69:306-13.
10. 1Siepmann T, Penzlin AI, Kepplinger J, et al. Selective serotonin reuptake inhibitors to improve outcome in acute ischemic stroke: Possible mechanisms and clinical evidence. Brain Behav. 2015;5:e00373.
11. Young JB, Singh TD, Rabinstein AA, et al. SSRI/SNRI use is not associated with increased risk of delayed cerebral ischemia after aSAH. Neurocrit Care. 2016;24:197-201.
12. Shin TK, Kang MS, Lee HY, et al. Fluoxetine and sertraline attenuate postischemic brain injury in mice. Korean J Physiol Pharmacol. 2009;13:257-63.
13. Richardson BP. Serotonin and nociception. Ann N Y Acad Sci. 1990;600:511-9.
14. Abd-Elsameea AA, Moustaf AA, Mohamed AM. Modulation of the oxidative stress by metformin in the cerebrum of rats exposed to global cerebral ischemia and ischemia/reperfusion. Eur Rev Med Pharmacol Sci. 2014;18:2387-92.
15. Kumar P, Kumar A. Possible role of sertraline against 3-nitropropionic acid induced behavioral, oxidative stress and mitochondrial dysfunctions in rat brain. Prog Neuropsychopharmacology Biol Psychiatry. 2009;33:100-8.