Research Article / *Araştırma Makalesi*

Sinir Sistemi Cerrahisi Derg 2022;8(3):87-93
doi:10.54306/SSCD.2022.214

Effect of Valproic Acid on Vasospasm at Experimental Subarachnoidal Hemorrhage Model

Deneysel Subaraknoid Kanama Modelinde Valproik Asidin Serebral Vazospazm Üzerine Etkisi

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Atıf/Cite as: Çakır CÖ, Koçak A. Effect of valproic acid on vasospasm at experimental subarachnoidal hemorrhage model. J Nervous Sys Surgery 2022;8(3):87-93.

Geliş tarihi/Received: 09.10.2022 **Kabul tarihi/Accepted:** 24.11.2022 **Yayın tarihi/Publication date:** 21.12.2022

ABSTRACT

Background and Purpose: The main purpose of our study was to observe the changes occuring on arterial walls due to experimental SAH model and to investigate the effects of valproic acid on the basilar artery and brain tissues to prevent these changes and vasospasm.
Material and Method: We used 24 New Zeland rabbits. Animals were randomly divided into three groups as control (C), subarachnoidal hemorrhage (SAH) and valproic acid (VPA) groups. Cisterna magna puncture was done to all animals. SAH occured by giving non heparinised autologous blood except control group. 100 mg/kg of Valproic acid was given intra peritoneally to treatment group. All animals were sacrified after 48 hours. All experimental and surgical procedures were approved by İnönü University Animal Research Comittee.
Results: Our expectation was the arterial lumen area of SAH group will be smaller than control group. After statistical calculations we found that our expectation was similiar with our findings that the smallest artery lumen was seen in SAH group and the largest artery lumen was seen in control group. These differences were statistically significant.
Conclusion: Our findings showed that Valproic acid can prevent vasospasm by preventing arterial wall changes induced by SAH. It may be clinically beneficial at patients suffering from vasospasm due to SAH.

Keywords: Brain damage, Neuroprotective effect, Subarachnoid hemorrhage, Valproic acid, Vasospasm

ÖZ

Amaç: Çalışmamızın amacı deneysel subaraknoid kanamada arteryel damar duvarlarındaki değişiklikleri gözlemlemek ve bu değişiklikleri ve vazospazmı engellemek için valproik asidin baziller arter ve beyin dokusu üzerindeki etkilerini incelemektir.

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Materyal ve Metod: Çalışmamızda 24 adet Yeni Zelanda tavşanı kullanıldı. Denekler randomize olarak kontrol (K), subaraknoid kanama (SAK) ve valproik asid (VPA) olarak üç guruba bölündü.Tüm deneklere sisterna magna ponksiyonu yapıldı. Kontrol gurubu haricindekilere heparinize olmayan otolog kan verilerek subaraknoid kanama oluşturuldu. Tedavi gurubuna intra peritoneal 100 mg/kg valproik asid verildi. Tüm denekler 48 sanra sakrifiye edildiler. Tüm deneysel ve cerrahi prosedürler İnönü Üniversitesi Deney Hayvanları Araştırma Komitesi tarafından onaylandı.

Bulgular: Beklentimiz SAK grubunun arteriyel lümen alanının kontrol grubundan küçük olacağı yönündeydi. İstatistiksel hesaplamaların sonunda bulduğumuz sonuçların beklentimiz ile uyumlu olarak en küçük arter lümeninin SAK gurubunda ve en büyük arter lümeninin kontrol grubunda olduğunu gördük. Aradaki farklar istatistiksel olarak anlamlıydı.

Sonuç: Elde ettiğimiz sonuçlar valproik asidin SAK tarafından indüklenen arteryel duvar değişikliklerini önleyerek vazospazmı engelleyebileceğini göstermiştir. Valproik asid subaraknoid kanamaya bağlı gelişen vazospazmla mücadele eden hastalarda klinik olarak faydalı olabilecek bir ajandır.

Anahtar Kelimeler: Beyin hasarı, Nöroprotektif etki, Subaraknoid kanama, Valproik asid, Vazospazm

INTRODUCTION

Subarachnoidal hemorrhage (SAH) is one of the most important type of intracranial hemorrhage models with high mortality and morbidity rates. Also there are many factors causing SAH, rupture of intra cranial aneurysm is the most common reason. Vasospasm and cerebral ischemia due to this process occurring after SAH is one of the most important reasons of mortality and morbidity of SAH.

Results of many researches have shown that some excitator and inhibitor chemicals presenting after SAH play significant roles at cellular damage or neuronal protection [1-4]. Also recent studies have shown that there are many similarities between cerebral damaging and autoprotective mechanisms occurring after cerebral ischemia and epilepsy [5]. According to these demonstrations many researches have been done by using antiepileptic agents with the idea that the agents used to minimize the cerebral damage after epileptic seizure can also be used to minimize the ischemic damage.

Valproic acid (VPA) is used as an anti epileptic agent and it acts by several mechanisms. It acts as a sodium channel blocker and decreases the number of T type calcium channels on primary afferent neurons. And it is also observed that Gamma-amino-butyric-acid (GABA) level is increased in whole brain due to VPA. VPA also decreases glutamate secrection. Recent researchs have shown that VPA also increases anti-apoptotic-bcl family receptor numbers and by this way decreases the apoptotic cellular deaths [6].

There is an uncontrolled release of glutamate and aspartate after SAH. In seconds after neurons are sustained to glutamate. N-methyl-D-aspartate antagonist (NMDA) and Alfa-methyl-propionic acid (AMPA) receptors are activated. As a result; this causes entrance of sodium, calcium, and water through cells at damaged area. Increasing amount of intra cellular calcium causes the increase of oxidative stress. And VPA can prevent the changes on cerebral vessels due to ischemia by preventing this pathways.

Vasospasm generally starts after 3rd day of SAH and its severity increases between 4th and 12th days. It effects only intra dural cerebral arteries and the main effected vessels are the large brain stem arteries. Angiographic vasospasm can be demostrated with digital substraction angiography and it is more common although clinical vasospasm is less common, it causes cerebral ischemia and neurological deficit.

Many researchs have explained the clinical appereance of vasospasm with decrease of cerebral blood flow, disorder of cerebral micro circulation and micro embolies [7-9], and about in %50 of the patients the effects due to this process

is permanent. Unlike other types of ischemia vasospasm appears much later and is predictable. These facts a treatment period for this pathology.

Experimental SAH models of animals or biopsies of patients who have gone under angiographic vasospasm have shown some pathological changes at walls of effected vessels. Electron microscobic studies have shown many changes like vacuolisation of endothelial cells, disorder of interendothelial tight junctions, endothelial spillage and luminal micro thrombosis. Tunica intima has caused contraction of underlying media layers like internal elastic lamina [10,11]. Recent studies have shown edema, infiltration of polymorphic cells, formation of granulation tissue, fibroplasia due to migration and prolifaration of smooth cell muscles and intimal thickening due to collegenization.

The common qualification of vessels both at experimantal and human vasospastic process is thickened media layer, inflamatory and hypertrofic reactional changes at arteriel walls after SAH.Some myonecrosis at tunica media accompany to vasospasm. Cerebral arteries do not contain external elastic lamina but adventisia is generally thickened after SAH due to formation of granular tissue because of fibrin and erythrocytes [12].

Because cerebral blood flow decreases in spastic vessels, the most important factor to avoid from cerebral vasospasm is protection from hypotension to sustain cerebral perfusion. Systemic blood pressure must be kept in mild hypertensive (130-160 mm Hg). Surgical removal of the blood clot may be another method and also it is shown that some medical agents are protective in the process of vasospasm. These agents are high dose methyl prednisolone, vasodilator calcitonin releated peptid, hydroxil radical scavengers, papaverine, ET-1 inhibitors and calcium channel blockers. One of the action methods of valproic acid is blocking sodium channels and decreasing the number of T type calcium channels on primary afferent neurons. And also it is demonstrated that at high concantrations GABA levels are increased in whole brain and apoptotic cellular death is decreased by regulation of anti apoptotic bcl family [13].

MATERIAL and METHOD

We used 24 New Zeland rabbits each weigh between 2,4 and 3,3 kg. Animals were grouped into three as control (C), subarachnoidal hemorrhage (SAH) and valproic acid (VPA) groups.

GROUP I (Control: 6 rabbits): Cisterna magna was punctured without forming SAH in this group. They were sacrificed at the end of 48 hours.

GRUP II (SAH: 9 rabbits): Autologous blood is given after sisterma magna puncture to this group. All of the animals were sacrificed after 48 hours. %0.9 saline solution was given intra peritoenally to animals in this group.

GROUP III (SAH+VPA: 9 rabbits): Autologous blood is given after sisterna magna puncture. After formation of SAH 100 mg/kg intra peritoneal valproic acid is given for every 12 hours to these animals for 48 hours. (Depakin 400mg/4ml-Sanofi-Synthelobo)

Animals went under anesthesia before surgical procedure by giving 35 mg/kg Ketamin -hydrochloride (Ketalar %5 solution Parke Davis/ Eczacıbaşı, İstanbul), and xylazine (Rompun %2 solution, Bayer)

All animals were positioned at lateral position. The heads of animals were placed at hyperflexion

and sisterna magna puncture was performed via 23 gauge scalp vein set from atlanto occipital region. 2-3 cc cerebro spinal fluid was drained from animals. After CSF drainage, 2.5-3 cc non-heparinized autologous blood taken from ear artery was given into cisterna magna to all animals in SAH and valproik acid group to perform SAH at posterior circulation.

For occurence of clot formation around basillar artery, animals were positioned head down for 10 minutes. %0.9 saline solution was given to all animals at SAH group for every 12 hours for 48 hours.To all animals in VPA group 100 mg/kg valproic acid was given every 12 hours for 48 hours.All animals underwent ansthesia after 48 hours and thoracotomy was performed for each. By a catheter entering from left atrium to aorta . 1000 cc of %0.9 saline solution is given at 150 cm H2O pressure. Given saline solution is drained by exploring right atrium. We continued perfusion till solution gets clear. After this animals were sacrified by decapitation, brain and brainstem were extracted together.

Histo pathological researchs were studied at Erciyes University Veterinary Faculty Pathology Laboratory. The cerebrum of animals were put in %10 formaldehyde fixing solution for 5 days. Later tissues were passed through alcohol, methyl benzoate and benzol respectively and blocked with paraffin. 5µm sections obtained by microtome were stained with hematoxylin-eosin. Brain sections were observed with microscope (Olympos B X 51) and the sections of basillar artery were determined. Later with Leica DMD 108 model digital imaging systems the lumen diameters of basiller arteries of each group were measured from two opposing different points. The mean value of two measurements were defined as the single diameter of each basillar artery. Each vessel was was accepted as a circle by using ∏r2. Formula. Data was shown as mean values +/- SEM. Statistical analysis between groups were calculated by TUKEY multiple comparison method after One-Way-ANOVA and P<0,05 was accepted as statistically significant.

RESULTS

Macroscopically clot formation was observed around basillar arteries of SAH and SAH+VPA group but no clot formation was seen in control group (Figure 1, 2, 3).

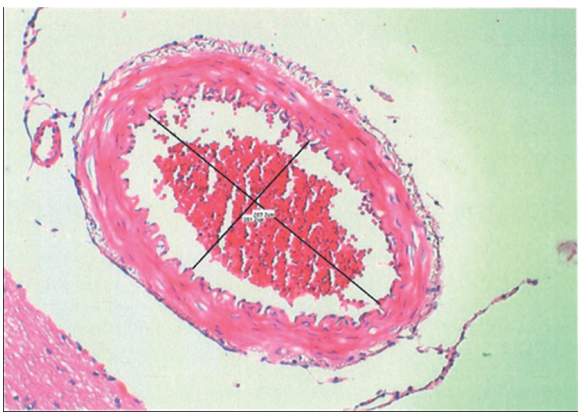

Figure 1. View of basillary artery of a control group rabbit.

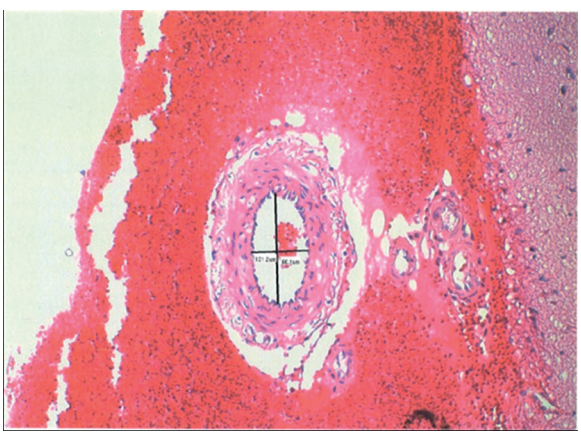

Figure 2. View of basillary artery of a SAH group rabbit.

Lumen surface areas of all animals in 3 groups were calculated and mean values were determined (Table 1). The mean value of Group C was 63171+638, Group SAH was 38350+3352, Group SAH+VPA was 43475+6060.

Table 1. Distribution of mean values of arterial lumen areas between groups.

	Control (n:6)	SAH (n:9)	SAH + Valproic acid (n:9)
	$\bar{X} \pm S\chi$	$\bar{X} \pm S\chi$	$\bar{X} \pm S\chi$
Arterial lumen area (μm^2)	63171±6386[a]	38350±3352[b]	43475±6060[b]

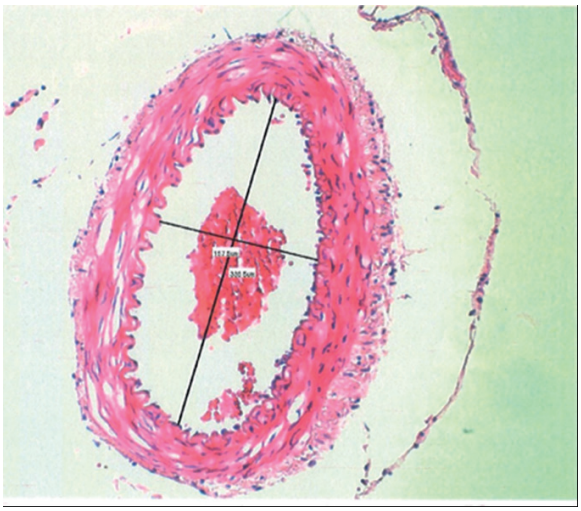

Figure 3. View of basillary artery of a treatment group rabbit.

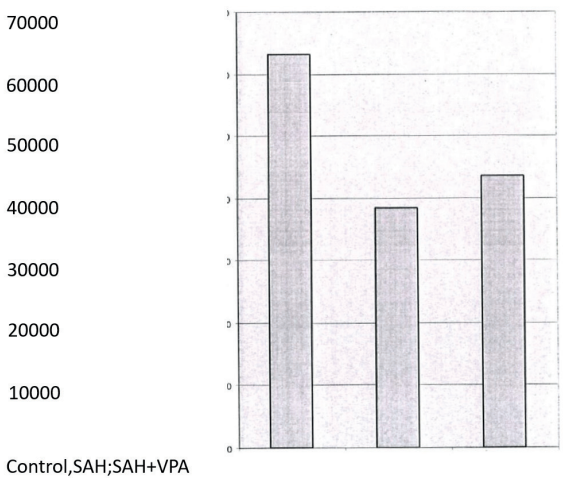

Figure 4. The graphical distribution of mean values of vessel lümen areas.

Differences between groups were statistically significant and it is shown appereantly on Figure 4 Statistical values of differences between grups are shown at Table 2.

Table 2. Statistical values of differences between groups.

Comparison of C-SAH	P-O.003
Comparison of C-SAH+VPA	P-O.008
Comparison of SAH-SAH+VPA	PzO.03

And we also studied immunohistochemical Terminal Deoxynücleotdyl Transferase dUTP Nick and labeling (TUNEL) for detection of nucleus fragmentation of DNA during apoptotic cell death in situ, by using apoptosis detection kit.

DISCUSSION

Symptomatic vasospasm occurs at about %20-40 of patients who has faced with SAH. Vasospasm generating after SAH is still a complicated and multifactorial process. It has a high mortality and morbidity rate due to the fact that it is the most difficult problem to over come; because we still could not understand its pathogenesis clearly. İschemic pathologies cause about %50 of early deaths of the patients who has survived from first SAH and aneurysm treatment. And the proper treatment for this process is still indeterminate [14-18].

H-H-H treatment (hypertension, hypervolemia, hemodilution) has been used as basic treatment method for SAH patients. Keeping away from hemoconcantration by hypervolemia is reasonable bu it is also intuitave. And also thypervolemia and hypertension may have side effects like pulmonary edema, cardiac and renal disfunction and increase of cerebral edema

Due to these factors; studies to understand and explain the pathogenesis of vasospasm which still keeps uncertanity and complicity are still going on being performed. Many treatment strategies are built and experienced acoording to the results of these researches. Understanding the pathogenesis of this process is indispensable.

Cerebral vasospasm is arterial narrowing due to blood clots in subarachnoidal space [19]. the most significant indicator for vasospasm is narrowinf basilar artery lümen and shortening of internal elsatic lamina. Also thickening of basilar arterial wall is accepted as another criteria of vasospasm.

As we mentioned before many treatment strategies targeting the factors which are playing role in pathogenesis of vasospasm have been tested and still going on to be tested. One of theses strategies is using antiepileptic agents. There are many similarities between the development of cerebral damage or auto protective mechanisms against these and epileptic seizures or protective effects of antiepileptic agents against these seizures. Due to this similarity it is thought that antiepileptic agents can minimize the damage after ischemia and many studies habe been done according to this idea by using several agents.

Antiepileptic agents are used against this process because of their multıble different acting ways but common property of them is they target the beginning or generation of seizures. While doing this they can also block toxic mechanisms which can cause neuronal damage releated with ischemia. For examole fenitoin, carbamazepine, topiromate like sodium channel blockers can prevent secrection of exitotoxic amin acids. Carbamazepine has also anti-inflammatory effects and this also improves neuronal protection.

Agents which are active on sodium channels like felbamate, gabapentin and levetiracetam can prevent calcium influv into ischemic cells and can reduce ischemic damage. They can do this by reducing glutamate secrection. Agents which can be activated by glutamate receptors like NMDA or AMPA can reduce exitotoxicity. These are some examples for neuro protective effects and their acting mechanisms for antiepileptic agents.

Valproic acid is one of these agents. It may act with several mechanisms. VPA may act by blocking sodium channels and it is also demonstrated that it can reduce calcium channels of T type on primary afferent neurons. At high concantrations of VPA GABA of wholw brain can decrease. It is also demonstrated that VPA can decrease apoptotic cellular death due to nincreasing of anti-apoptotic bcl receptor numbers. As aresult VPA can increase neuroprotective effect of GABA in brain while it decreases secrection of glutamate. According to all these factors and effects of VPA it is highly neuoroprotective agent.

CONCLUSION

The main purpose of our study was to observe the changes occuring on arterial walls due to experimental SAH model. Our expectation was the arterial lumen area of SAH group will be smaller than control group. After statistical calculations we found that our expectation was similiar with our findings that the smallest artery lumen was seen in SAH group and the largest artery lumen was seen in control group.

In our study we measured lumen area of basillar artery in SAH grup which is performed by sisterna magna puncture.

Conflict of interest: There is no conflict of interest in our study.
Funding: No financial support was received in our study.

Çıkar çatışması: Çalışmamızda herhangi bir çıkar çatışması bulunmamaktadır.
Finansal destek: Çalışmamızda finansal destek alınmamıştır.

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