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ORIGINAL ARTICLE

The Effect of the ACE Inhibitor Lisinopril on Cerebral Vasospasm After Experimental Subarachnoid Hemorrhage

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Abstract

Introduction: The aim of this study was to compare the efficacy of the ACE (Angiotensin-Converting Enzyme) inhibitor lisinopril on basilar artery vasospasm in an experimental subarachnoid hemorrhage (SAH) model in rats.

Methods: A total of 32 Wistar albino rats were divided into four groups: Group I (n=8), the control group; Group II (n=8), the vasospasm group; Group III (n=8), the vasospasm group treated with 5 mg/kg/day for 7 days starting within the first 24 hours; and Group IV (n=8), the vasospasm group treated with 10 mg/kg/day for 7 days starting within the first 24 hours. After 7 days, the basilar artery was excised and examined histopathologically under a light microscope.

Results: The study found that 10 mg/kg/day lisinopril significantly prevented vasospasm following SAH. The mean vessel wall thickness was lowest in the lisinopril 10 mg group and highest in the SAH group, with a statistically significant difference. In Group III, a dose of 5 mg/kg/day of lisinopril reduced wall thickness, while in Group IV, a dose of 10 mg/kg/day was more effective. Group III had a greater decrease in lumen area compared to Group II, but not as much as Group IV. Comparing the vessel lumen thickness of Group II with Group I, there was a significant decrease in Group IV. Although not as much as in Group IV, the lumen diameter increased in Group III compared to Group II. Group IV had an increase in lumen diameter similar to that of Group I.

Discussion and Conclusion: The study findings suggest that intraperitoneal administration of lisinopril at a dose of 10 mg/kg/day can prevent morphologic vasospasm after experimental vasospasm. However, the dose of 5 mg/kg/day of lisinopril is less effective than the 10 mg/kg/day dose.

Keywords: ACE inhibitor; basilar artery; lisinopril; subarachnoid hemorrhage; vasospasm.

Subarachnoid hemorrhage (SAH) is a severe type of stroke with high morbidity and mortality rates.[1] It is typically caused by trauma. Non-traumatic causes of SAH include aneurysms and arteriovenous malformations (AVMs) (80%), unknown causes (10-15%), vascular anomalies, tumors, infections, systemic diseases, and spinal diseases (5-10%).[2]

Approximately two hours after the occurrence of SAH, the first cellular reactions in the meninges are observed, which are caused by erythrocytes and their degradation products. Polymorphonuclear leukocytes first appear in the arachnoid membrane, followed by lymphocytes and then macrophage exudation. During the cellular repair process, erythrocyte and fibrin aggregation, hemosiderin accumulation, and phagocytosis are noted. As connective tissue organizes, fibroblasts synthesize reticulum fibers.[3]

The leading cause of morbidity and mortality after fixing a ruptured aneurysm is cerebral vasospasm. Patients are most at risk of cerebral vasospasm between days 4 and 14 after

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hemorrhage.[4] It is crucial to begin vasospasm treatment as soon as possible after controlling the subarachnoid hemorrhage, even though the risk of vasospasm starts on day 1.[5]

Despite numerous clinical and experimental investigations, the pathophysiology of cerebral vasospasm after SAH remains unclear. In addition to the numerous pathophysiological effects that occur following SAH, the renin-angiotensin system is also activated. This results in an increase in both renin and angiotensin levels in the plasma, with angiotensin subsequently causing contraction and hypertrophy in vascular smooth muscles.[6]

Angiotensin II (AII) has a dual effect on the vasculature. While it causes contraction of small vessels, it has the opposite effect on larger vessels, increasing phosphorylation of myosin light chain in vascular smooth muscle cells in pathological inflammatory states.[7]

Angiotensin-converting enzyme (ACE) inhibitors have been demonstrated to increase the release of arachidonic acid (by triggering prostaglandin) by blocking the formation of angiotensin. Furthermore, they have been shown to decrease kinin and plasmin in the vascular wall by blocking the contractile effect in smooth muscle cells and to prevent vascular damage by inhibiting neo-intima formation.[8]

This study investigates the effect of lisinopril, a long-acting ACE-II inhibitor, on vasospasm in the basilar arteries of rats with an experimental SAH model. Lisinopril is believed to suppress the vascular response by inhibiting the conversion of angiotensin I to active AII.[9]

Materials and Methods

The surgical experimental section of the study was conducted at the Basic Sciences Laboratory of Istanbul University Cerrahpaşa Medical Faculty, Istanbul University, following the protocol of the Animal Protection Association. The study used randomly selected Wistar Albino rats with an average weight of 200-250 grams. Experimental SAH was induced using the model of Barry et al.,[10] and histopathological examination of the basilar artery was performed after cerebral vasospasm.

Four groups, each consisting of eight rats, were randomly selected from a total of 32 rats. The groups were as follows:

- Group I (n=8, control): Suboccipital craniectomy and C1 laminectomy were performed using microsurgical techniques. After dura incision, the basilar arteries were dissected using microdissection.
- Group II (n=8, vasospasm): 0.5 ml of fresh non-heparinized blood taken from the ventral tail artery of vasospasm group rats was injected into the cisterna magna after CSF drainage. The basilar artery was dissected using the same surgical procedure (Fig. 1).
- Group III (n=8, vasospasm+5 mg/kg/day lisinopril): Lisinopril was administered intraperitoneally at 5 mg/kg/day for 7 days, starting 24 hours after the induction of experimental vasospasm.
- Group IV (n=8, vasospasm+10 mg/kg/day lisinopril): Lisinopril was administered intraperitoneally at 10 mg/kg/day for 7 days, starting 24 hours after the induction of experimental vasospasm.

After 7 days, the rats were anesthetized and sacrificed. The basilar artery was then exposed without disrupting the brain's integrity. For histopathological examination, the artery was fixed in a 10% formalin solution, washed with alcohol for dehydration, and then passed through xylene and paraffin. After 24 hours, it was cut to a thickness of 5 micrometers and stained with Hematoxylin-Eosin. The preparations underwent examination using an Olympus BX7 microscope at ×40 and ×100 magnification. The resulting images were transferred to a computer for histomorphometric analysis. The lumen areas and wall

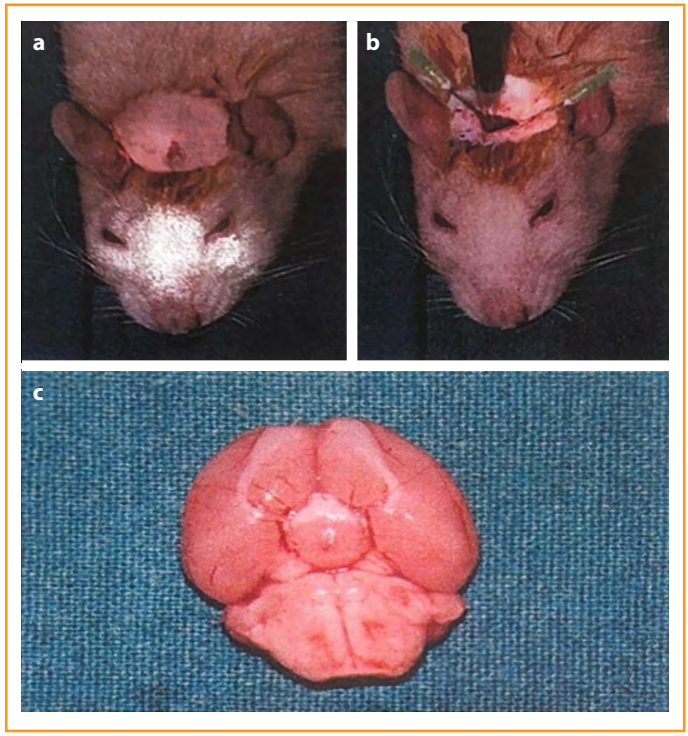

Figure 1. A vasospasm model was created after SAH by injecting 0.5 ml of fresh non-heparinized blood from the ventral tail artery of rats into the cisterna magna following CSF drainage. Total brain tissue was removed after the experiment.

thicknesses of the basilar artery were measured using an image analysis system. These values were then calculated as unit values over squares.[11]

Statistical Analysis

The data were analyzed using the NPar test and Mann-Whitney U tests. The NPar test was employed to compare the distribution of non-normal parameters between groups. A significance level of $p<0.01$ was used to determine statistical significance. The results showed that $p<0.01$ was statistically significant.

Results

Histopathologic Findings

The preparations prepared from all sections of the basilar artery were examined under a light microscope. No degeneration or proliferation was detected in the endothelial cells arranged in a single row in Group I (control group). The invagination of the lamina elastica interna was normal. The medial smooth muscle cells appeared normal in size and were arranged side by side. Loose connective tissue cells were observed in the vascular adventitia, while macrophages, polymorphonuclear leukocytes, and erythrocytes were not present (Fig. 2a).

In Group II (vasospasm group), endothelial cell rupture, edema, proliferation into the lumen, thickening of the lamina elastica interna, and increased invagination were observed. In the adventitia, chromatin condensation in medial smooth muscle cell nuclei, shortening and thickening of cell length, necrosis, macrophages, and erythrocytes were present. Grossly, a significant decrease in lamina distance and a significant thickening of the vessel wall were observed (Fig. 2b).

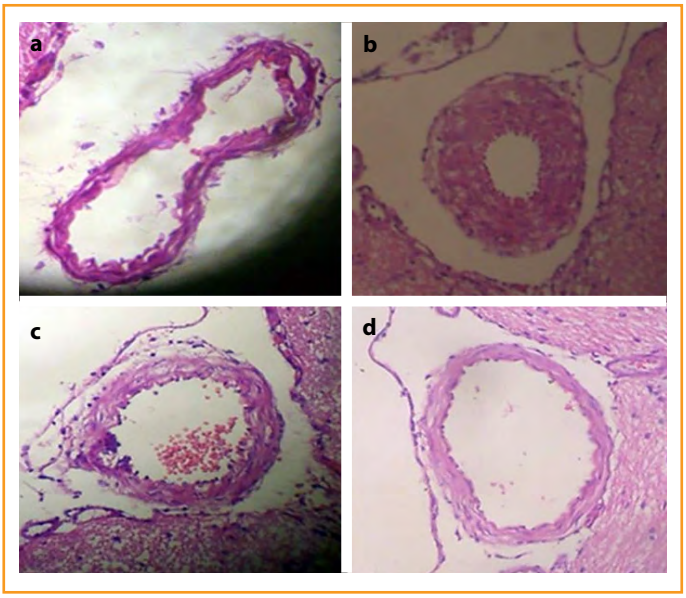

Figure 2. Shows H&E X200 images of the control group **(a)**, vasospasm group **(b)**, 5mg/kg/day lisinopril **(c)**, and 10mg/kg/day lisinopril group **(d)** under a light microscope.

In Group III (5 mg/kg/day lisinopril; vasospasm), endothelial cells appeared edematous in some areas, with more frequent lamina elastica invaginations than in Group I but fewer than in Group II. A decrease in chromatin content in the medial smooth muscle cells was observed, and although not as severe as in Group II, shortening and thickening of the smooth muscle cells were present. Erythrocytes and macrophages were found in the adventitia, which remained loose (Fig. 2c).

In Group IV (10 mg/kg/day lisinopril; vasospasm), no proliferation or edema was detected in endothelial cells, as in Group I. The thickening and invagination of the internal elastic lamina had significantly decreased, the size of the smooth muscle cells was normal, and no edema was observed (Fig. 2d).

The analysis results indicate a significant difference in mean lumen area and vessel wall thickness (Table 1).

The minimum mean vessel wall thickness was observed in the group treated with 10 mg of lisinopril, while the maximum was observed in the SAH group. Treatment with

Table 1. Lumen diameters and wall thicknesses in groups

Group	Lumen	Wall Thickness
Control		
Mean	1047,.0	27.63
N	8	8
SD	78.513	2.326
Vasospasm		
Mean	211.25	66.00
N	8	8
SD	35.632	5.952
5mg lisinopril		
Mean	458.75	38.00
N	8	8
SD	58.661	4.276
10 mg lisinopril		
Mean	1145.00	27.75
N	8	8
SD	63.019	2.252
Total		
Mean	715.63	39.84
N	32	32
SD	402.532	16.377

SD: Standard Deviation.

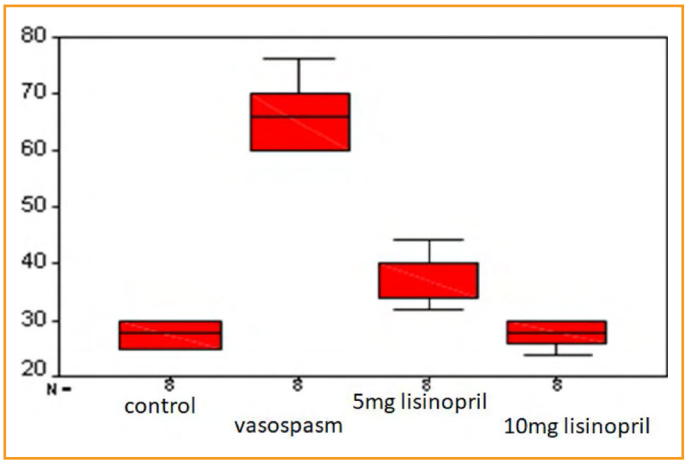

Figure 3. Shows the mean vessel wall thicknesses in the groups.

5 mg of lisinopril resulted in a decrease in wall thickness, but 10 mg was found to be more effective (Figs. 3 and 4) ($p<0.01$). The difference in wall thickness between Group I and Group III was significant.

The vasospasm group had a thicker vessel wall than the other groups, as revealed by the lumen area examination. When comparing Group II and Group IV in terms of wall thickness, a significant decrease was observed in Group IV ($p<0.01$). Group III showed a decrease in wall thickness compared to Group II, but not as much as Group IV ($p<0.01$). Group III exhibited a reduction in lumen area compared to Group II, but not to the same extent as Group IV. A significant decrease in vessel lumen thickness was observed when comparing Group II to Group I. When comparing Group I and Group IV, a significant difference was observed ($p<0.01$).

Discussion

Cerebral vasospasm typically starts between days 3-5 after SAH and reaches its peak on days 7-8.[12] It is the primary cause of morbidity and mortality in SAH. The etiology of vasospasm is multifactorial and may include prolonged contraction of smooth muscle, narrowing of the lumen due to structural changes in the vessel wall, inflammation, denervation hypersensitivity, endothelial dysfunction, and immunologic reactions. Angiotensin II production in the cerebral artery wall and the vasoconstrictor effect of endothelin secreted from the vascular endothelium may contribute to the pathogenesis of cerebral vasospasm.[13]

According to reports, Angiotensin II (AII) causes smooth muscle cell contraction, thickens the basement membrane, stimulates endothelial cells to activate hypertrophy and proliferation, increases protein synthesis and cell mass in arterial smooth muscle cells, and enhances the

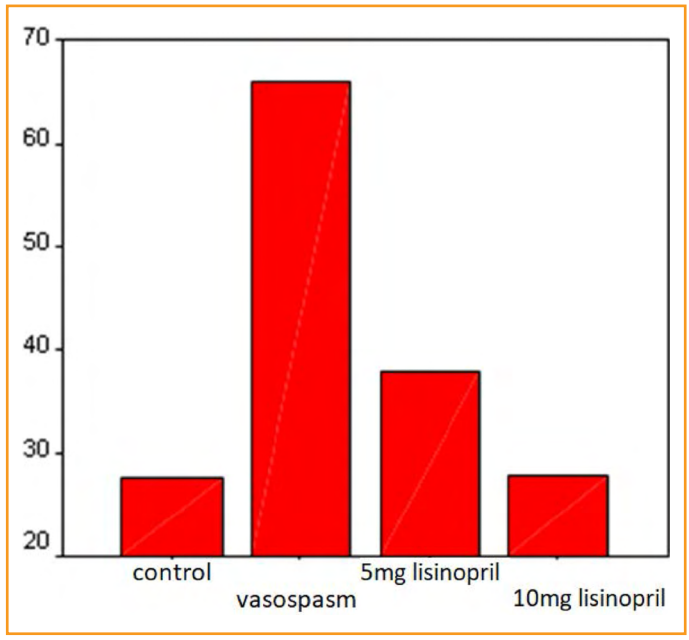

Figure 4. Shows the mean vessel wall thicknesses in the groups.

effect on smooth muscles by stimulating epidermal and platelet-derived growth factors released from platelets.[14]

AII is a potent oxidant that converts nitric oxide (NO) to peroxynitrite. It has been suggested that increased systemic arterial pressure in response to increased intracranial pressure after SAH stimulates activation of the Renin-Angiotensin System (RAS), leading to an increase in renin and angiotensin levels.[6] RAS also activates the sympathetic nervous system, which in turn increases renin release from the kidney, further increasing angiotensin activity. RAS has a negative effect on vessels by disrupting the rhythm of NO and stimulating the accumulation of LDL. Local ACE in tissues exacerbates vascular damage by decreasing bradykinin, which increases NO, and by suppressing kinin-nitric oxide-dependent vasodilation. This damage can be counteracted by ACE inhibitors, which stabilize endothelial function, decrease vascular reactivity, and increase vasodilator prostaglandin synthesis.[15]

AII mediates harmful effects on the brain, such as hypertension, inflammation, increased oxidative stress, blood-brain barrier disruption, and neurotoxicity.[16] According to reports, endothelial cells in the arterial wall, without vasospasm, are arranged in a single layer on the internal elastic lamina. These cells form tight junctions with each other and with the basement membrane. Chromatin condensation is absent, and normal heterochromatin is present in all cells. The cytoplasm does not exhibit budding or vacuolization.[17]

In vasospasm after SAH, morphologic changes occur in

almost every layer of the vessel wall. Folds of the lamina elastica are formed, tight junctions between endothelial cells are separated, and the remaining endothelial cells bud into the lumen. Apoptosis-like changes occur in endothelial cells (chromatin aggregation, budding, cytoplasm condensation, cytoplasm vacuolization), fluid accumulates in the subendothelial space, and necrosis of smooth muscle cells is observed. The lamina elastica disintegrates, the intima swells, lymphocytes, plasma cells, mast cells, macrophages, and connective tissue increase in the adventitia, followed by progressive thickening of the intima due to the accumulation of smooth muscle cells.[18]

Gavras et al.[19] showed that ACE inhibitor treatment reversed angiographic spasm of the basilar artery of dogs 72 hours after experimental SAH. Treatment with ACE inhibitors has been shown to reduce hypertrophy in the media layer of arteries and prevent myointimal proliferation due to vascular damage.[15] Similar to this study, ACE inhibitors are thought to suppress the vascular response to injury in Group IV, which was treated with 10 mg lisinopril.[15]

There are studies showing that lisinopril, an ACE inhibitor, prevents vasospasm due to its effects, such as decreasing vasoconstrictor prostaglandins, increasing vasodilator prostaglandins, decreasing aldosterone release by inhibiting AII formation, and reducing the tendency to thrombosis and atherosclerosis.[20]

Our study investigated the effect of lisinopril, a long-acting ACE inhibitor, on chronic vasospasm after SAH at two different doses. The results showed that lisinopril prevented morphologic changes in arteries due to endothelial damage after SAH, indicating its potential benefits. The study found that intraperitoneal administration of lisinopril at a dose of 10 mg/kg/day prevented morphologic vasospasm and eliminated morphologic changes in brain vessels caused by endothelial damage. The effects were less pronounced at a dose of 5 mg/kg/day and were not as effective as the 10 mg/kg/day dose, indicating a dose-dependent effect. Further studies are needed to determine the optimal dose range of lisinopril and its effect on chronic morphologic vasospasm. Furthermore, as molecular biology and genetic technology continue to advance, the investigation of the role of ACE inhibitors in cerebrovascular events may warrant inclusion in the vasospasm protocol.

Conclusions

In this experimental study, it was found that lisinopril, an ACE inhibitor, prevented the morphologic changes in arteries secondary to endothelial damage after SAH in a dose-dependent manner. The intraperitoneal administration of lisinopril at a dose of 10 mg/kg/day clearly prevented chronic morphologic vasospasm, while a dose of 5 mg/kg/day reduced vasospasm but was not as effective as the 10 mg/kg/day dose.

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Conflict of Interest: The authors declare that there is no conflict of interest.

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References

1. Nieuwkamp DJ, Setz LE, Algra A, Linn FH, de Rooij NK, Rinkel GJ. Changes in case fatality of aneurysmal subarachnoid haemorrhage over time, according to age, sex, and region: A meta-analysis. Lancet Neurol 2009;8:635–42. [CrossRef]
2. Rossi J, Hermier M, Eker OF, Berthezene Y, Bani-Sadr A. Etiologies of spontaneous acute intracerebral hemorrhage: A pictorial review. Clin Imaging 2023;95:10–23. [CrossRef]
3. Chen Y, Galea I, Macdonald RL, Wong GKC, Zhang JH. Rethinking the initial changes in subarachnoid haemorrhage: Focusing on real-time metabolism during early brain injury. EBioMedicine 2022;83:104223. [CrossRef]
4. Rumalla K, Lin M, Ding L, Gaddis M, Giannotta SL, Attenello FJ, et al. Risk factors for cerebral vasospasm in aneurysmal subarachnoid hemorrhage: A population-based study of 8346 patients. World Neurosurg 2021;145:e233–41. [CrossRef]
5. Ditz C, Machner B, Schacht H, Neumann A, Schramm P, Tronnier VM, et al. Effects of post-interventional antiplatelet therapy on angiographic vasospasm, delayed cerebral ischemia, and clinical outcome after aneurysmal subarachnoid hemorrhage: A single-center experience. Neurosurg Rev 2021;44:2899–912.
6. Fassot C, Lambert G, Gaudet-Lambert E, Friberg P, Elghozi JL. Beneficial effect of renin-angiotensin system for maintaining blood pressure control following subarachnoid haemorrhage. Brain Res Bull 1999;50:127–32. [CrossRef]
7. Cavarape A, Bauer J, Bartoli E, Endlich K, Parekh N. Effects of angiotensin II, arginine vasopressin and tromboxane A2 in renal vascular bed: Role of rho-kinase. Nephrol Dial Transplant 2003;18:1764–9. [CrossRef]
8. Xu L, Schüler R, Xu C, Seebeck N, Markova M, Murahovschi V, et al. Arachidonic acid inhibits the production of angiotensin-

converting enzyme in human primary adipocytes via a NF-κB-dependent pathway. Ann Transl Med 2020;8:1652. [CrossRef]
9. Farhy RD, Carretero OA, Ho KL, Scicli AG. Role of kinins and nitric oxide in the effects of angiotensin converting enzyme inhibitors on neointima formation. Circ Res 1993;72:1202–10.
10. Barry KJ, Gogjian MA, Stein BM. Small animal model for investigation of subarachnoid hemorrhage and cerebral vasospasm. Stroke 1979;10:538–41. [CrossRef]
11. Tierney TS, Clatterbuck RE, Lawson C, Thai QA, Rhines LD, Tamargo RJ. Prevention and reversal of experimental posthemorrhagic vasospasm by the periadventitial administration of nitric oxide from a controlled-release polymer. Neurosurgery 2001;49:945–51. [CrossRef]
12. Bauer AM, Rasmussen PA. Treatment of intracranial vasospasm following subarachnoid hemorrhage. Front Neurol 2014;5:72.
13. Tanabe Y, Sakata K, Yamada H, Ito T, Takada M. Cerebral vasospasm and ultrastructural changes in cerebral arterial wall. An experimental study. J Neurosurg 1978;49:229–38. [CrossRef]
14. Goa KL, Haria M, Wilde MI. Lisinopril. A review of its pharmacology and use in the management of the complications of diabetes mellitus. Drugs 1997;53:1081–105.
15. Iplikcioglu AC, Bayar MA, Sav A, Erbengi T. Angiotensin-converting enzyme inhibitor cilazapril prevents chronic morphologic vasospasm in rat. Surg Neurol 1994;41:294–8.
16. Farag E, Sessler DI, Ebrahim Z, Kurz A, Morgan J, Ahuja S, et al. The renin angiotensin system and the brain: New developments. J Clin Neurosci 2017;46:1–8. [CrossRef]
17. Zubkov AY, Ogihara K, Tumu P, Patlolla A, Lewis AI, Parent AD, et al. Mitogen-activated protein kinase mediation of hemolysate-induced contraction in rabbit basilar artery. J Neurosurg 1999;90:1091–7.[CrossRef]
18. Nakagomi T, Kassell NF, Hongo K, Sasaki T. Pharmacological reversibility of experimental cerebral vasospasm. Neurosurgery 1990;27:582–6. [CrossRef]
19. Gavras H, Andrews P, Papadakis N. Reversal of experimental delayed cerebral vasospasm by angiotensin-converting enzyme inhibition. J Neurosurg 1981;55:884–8. [CrossRef]
20. Vaughan DE. The renin-angiotensin system and fibrinolysis. Am J Cardiol 1997;79:12–6. [CrossRef]