Turk Neurosurg 34(1):148-159, 2024

Original Investigation

DOI: 10.5137/1019-5149.JTN.43427-23.2

Received: 05.02.2023
Accepted: 29.05.2023

Published Online: 29.12.2023

Dexamethasone Addition Impairs the Therapeutic Effects of Nimodipine for Subarachnoid Hemorrhage: An Experimental Animal Study

Selin BOZDAG[1], Hasan Kamil SUCU[2], Zekiye Sultan ALTUN[3], Aslı Kahraman AKKALP[4], Osman YILMAZ[5], Demet CELIKKAYA[6]

[1]Kastamonu Training and Research Hospital, Department of Neurosurgery, Kastamonu, Türkiye
[2]Izmir Katip Celebi University Ataturk Training and Research Hospital, Department of Neurosurgery, Izmir, Türkiye
[3]Dokuz Eylul University Faculty of Medicine, Department of Oncology, Izmir, Türkiye
[4]Izmir Katip Celebi University Ataturk Training and Research Hospital, Department of Pathology, Izmir, Türkiye
[5]Dokuz Eylul University Faculty of Medicine, Department of Laboratory Animal Science, Izmir, Türkiye
[6]University of Health Sciences, Izmir Tepecik Training and Research Hospital, Department of Invertentional Neuroradiology, Izmir, Türkiye

Corresponding author: Selin BOZDAG ✉ selin.bzdg@gmail.com

ABSTRACT

AIM: To evaluate the effects of the combination of nimodipine and dexamethasone in subarachnoid hemorrhage (SAH).

MATERIAL and METHODS: In this study, 35 female adult Wistar Albino rats were randomly assigned to four groups: Sham (n=8), SAH with no treatment (n=9), SAH with nimodipine (n=9, oral gavage, 12 mg/kg, BID) treatment, and SAH with combined therapy with nimodipine and dexamethasone (n=9, intraperitoneally, 1mg/kg, BID). The "cisterna magna double injection of autologous blood" model was used. The animals were euthanized 5 days after the first injection.

RESULTS: Of the total, five rats died before euthanasia. The SAH+Nontreatment group showed the worst score in neurological examinations, and the most severe histopathological findings were noted in terms of vasospasm. The SAH+Nimodipine group showed the best neurological score and the closest histopathological results to those of the Sham group, whereas adding dexamethasone to nimodipine treatment (the SAH+Nimodipine+Dexamethasone group) worsened the neurological and histopathological outcomes.

CONCLUSION: We thus concluded that the therapeutic effects of nimodipine were impaired when combined with dexamethasone. We thus hypothesized that dexamethasone possibly induces the CYP3A4-enzyme that metabolizes nimodipine. However, it should be noted that our results are based on laboratory findings obtained on a small sample, therefore further studies with drug–drug interaction on a larger sample size through CYP3A4-enzyme and clinical confirmation are warranted.

KEYWORDS: Delayed Cerebral Ischemia, Dexamethasone, Nimodipine, Subarachnoid Hemorrhage, Vasospasm

ABBREVIATIONS: SAH: Subarachnoid hemorrhage, **DCI:** Delayed cerebral ischemia, **FDA:** Food and Drug Administration, **IL-1β:** Interleukin-1β, **IL-6:** Interleukin-6, **TNF-α:** Tumor necrosis factor-alpha, **mGNS:** The modified Garcia's neurological score, **CYP3A4:** Cytochrome-P450-3A4

Selin BOZDAG : 0000-0002-3355-8954
Hasan Kamil SUCU : 0000-0002-2795-9049
Zekiye Sultan ALTUN : 0000-0002-1558-4534
Aslı Kahraman AKKALP : 0000-0001-5781-8506
Osman YILMAZ : 0000-0001-7817-7576
Demet CELIKKAYA : 0000-0002-3390-8678

INTRODUCTION

Although significant progress has been made in diagnosis and treatment areas, subarachnoid hemorrhage (SAH) continues to be associated with high mortality and morbidity rates. More than 50% of the surviving patients develop deficits that required lifelong care, which keep them from returning to work and decrease their quality of life (5,28,33,60,70). Since SAH affects the younger population, unlike ischemic stroke (48), it has more significant social and economic impacts on the patients, their families, and the society at large. The most feared complication of SAH is delayed cerebral ischemia (DCI), which occurs in ≈30% of the patients who survive the initial hemorrhage, making it the leading cause of prolonged intensive care admission, poor functional outcome, and death (17,29,45,47). While the primary injury from the initial bleeding cannot be changed, the secondary damage due to DCI might be a target to improve the outcomes (24).

Recently, it was shown that the interaction of multiple pathological pathways such as vasospasm, microvascular dysfunction, microthromboembolism, and cortical spreading depolarization led to DCI (9,12,32,38,39,56,64,67). The current DCI rescue therapy protocols are mainly ineffective and expensive (7). Therefore, the essential treatment for DCI is prevention. Several pharmacological agents (such as magnesium, clazosentan, antiplatelet drugs, and statins) have been evaluated and failed to prove any beneficial effect in DCI. Presently, nimodipine is the only drug shown to reduce the risk of poor neurological outcomes, which is generally recommended in the guidelines and approved by the Food and Drug Administration (FDA) for DCI prophylaxis (3,8,15,16,41,65,68,76). The rationale for using calcium antagonists to prevent secondary ischemia was initially based on blocking L-type calcium channels, thereby preventing calcium influx into vascular smooth muscle cells and reducing the incidence of cerebral vasospasm (5). However, unlike other drugs, nimodipine has been demonstrated to provide beneficial effects without any angiographic evidence of cerebral vasodilation (5). This has led scientists to theorize that nimodipine slightly attenuates vasospasm, inhibits cortical spreading depolarization, reduces microthrombosis, and infers a neuroprotective effect that protects nerve cells from early morphological and functional damages (18,34,66,69).

In SAH patients who underwent microsurgical clipping, dexamethasone is widely used for various purposes, including postcraniotomy cerebral edema and severe headaches that are attributable to meningeal inflammation (chemical meningitis) (10,22,24,42). However, there is no level 1 evidence, and the decision to use dexamethasone is at the discretion of the attending neurosurgeon or neurointensivist. Furthermore, there is a strong evidence that inflammation contributes to poor outcomes in SAH. Different inflammatory mediators, including interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor alfa (TNF-α), correlate with or even cause vasospasm and DCI (14,24,51). These immune mediators have been discussed as potentially promising targets. Many of these immune mediators have been proven to be influenced by glucocorticoids. In line with these reports, inflammation has gained focus in the recent research efforts toward preventing DCI, and studies have suggested a beneficial effect of dexamethasone in SAH (24).

To the best of our knowledge, the effect of the addition of dexamethasone in alters nimodipine's effect on DCI has never been studied so far. Therefore, we evaluated the impact of adding dexamethasone to nimodipine therapy in a rat SAH model.

MATERIAL and METHODS

The study was conducted under the CAMARADES and ARRIVE guidelines (31,57), The experimental protocols were approved by the Local Ethics Committee on Animal Research of Dokuz Eylul University (Date: January 13, 2021; No: 01/2021). Healthy female adult Wistar Albino rats weighing 250–400 g were included in the study. The sample size was calculated as 32 for analysis of variance (ANOVA) testing using G*Power (version 3.1.9.7) and increased to 35 because the mortality rate of this model was 6%–8% in the literature (72). The animals were assigned to the following 4 groups: Sham, SAH+Non-treatment, and SAH+Nimodipine, SAH+Nimodipine+Dexamethasone, which were created by blocked randomization (Figure 1 shows the experimental design). *Ad libitum* feeding, 25°C temperature, and 12-h light/dark cycle, light intensity in the range of 130–300 lux, and a background noise of around 50 dB were provided to the animals.

The Induction of SAH

Intraperitoneal administration of 50–60 mg/kg ketamine (Ketalar, Pfizer) and 5–10 mg/kg xylazine (Rompun, Bayer) was used for anesthesia. This combination had no gross effect on the cerebral blood flow (72). Thirty minutes before the surgical procedure, 40 mg/kg cefazolin (Cezol, Deva) was administered intraperitoneally to the animals.

The nonheparinized arterial blood was collected through a percutaneous arterial puncture using a 26-G insulin syringe after ascertaining conditions for vasodilation of the tail ventral artery. The operating table was an inclined plate that allowed the animal's head to be flexed without blocking their airway (Concorde-like position). Next, the neck muscles were dissected along the midline after a skin incision was created between the inion and C1. The caudal margin of the occipital bone and the atlantooccipital membrane were exposed sequentially to avoid any unnecessary dissection. Craniectomy was not performed. Under the operating microscope, the cisterna magna was punctured with a 26-G insulin injector at an angle of 60 degrees. The bevel tip was positioned through the ventral face of the occipital bone, and the needle was inserted into the cisterna magna only as far as its cut end. Clear CSF (0.15 mL) was slowly aspirated, and the same amount of nonheparinized autologous blood was injected slowly (0.05 mL of blood every 30 s) to avoid acute intracranial hyper-pressure. The injector was retained inside the animal for 30 seconds to prevent the backflow of blood. Then, a thin gelatin sponge was placed on the membrane. The animals were then brought in a head-down position and retained as such for approximately 20 min to ensure an optimal subarachnoid distribution of the

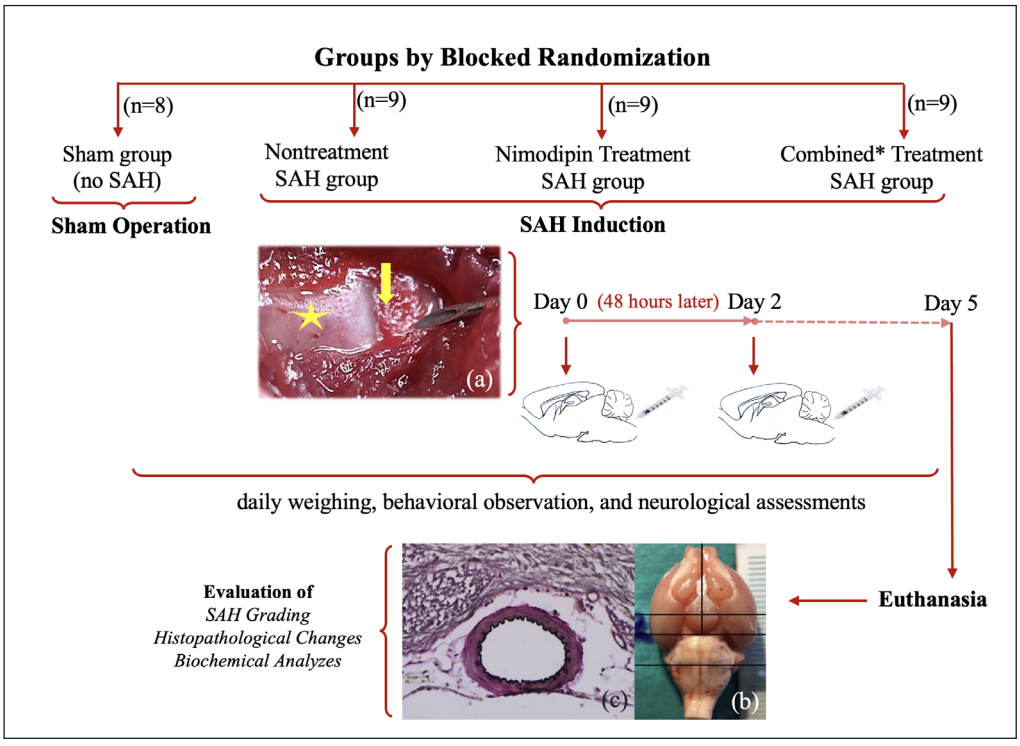

Figure 1: The diagram illustrating the experimental design. **a)** SAH induction. After the caudal margin of the occipital bone and the atlantooccipital membrane were exposed, the cisterna magna was punctured with a 26-G injector under the operating microscope. Star: Occipital bone, Arrow: Atlantooccipital membrane. **b)** After the brains were removed, the brain base was photographed with a high-resolution digital camera for SAH grading, before placing them in a formalin solution. **c)** Evaluation of histopathological changes and biochemical analysis. *combined therapy with nimodipine and dexamethasone.

administered autologous blood. During this procedure and the postoperative recovery period, a heating pad was used to maintain the animals' body temperature at about 37°C.

Forty-eight hours after the first injection, the same procedure was repeated. However, considering that the intracranial pressures of the animals may have increased, the process was performed with 0.1 mL CSF and blood.

The same technique was used for the Sham group, except that saline was injected into the cisterna magna instead of blood.

Drug Administration

The rat therapeutic dose for nimodipine is not established by the literature. Therefore, allometric scaling was used for nimodipine dose conversion between animals and humans (24,43). The experimental animal dose of nimodipine was determined to be 24 mg/kg/day. The desired blood nimodipine concentration was such that the drug level did not fall below 7 ng/mL (2). With reference to the time-concentration curves (36) in the rat pharmacokinetic and pharmacodynamic studies, nimodipine was administered at 12 mg/kg twice daily via oral gavage in the SAH+Nimodipine and SAH+Nimodipine+-Dexamethasone groups.

Dexamethasone dose in the rats is also not standardized in the literature. Therefore, the most commonly used amount with proven beneficial effects was selected in the cerebral edema experiments for the rats (62). In the SAH+Nimodipine+Dexamethasone group, dexamethasone was administered at 1 mg/kg dose, twice daily, intraperitoneally in addition to nimodipine.

Postoperative Follow-up and Neurological Scores

The follow-up of animals included daily weighing, behavioral observation, and neurological assessments. The animals developing unpredictable diseases (such as epilepsy) or showing weight loss >15% were excluded from the experiment. Neurobehavioral assessment was evaluated based on the modified Garcia's neurological score (mGNS) (59). This scale consists of six tests (i.e., spontaneous activity, spontaneous movement of all four limbs, foresaw outstretching, climbing, body proprioception and response to whisker stimulation, and cylinder test); each test is scored between 0 and 3. The maximum score is 18. The higher scores indicate better functionality. Motor functions were evaluated in detail with the cylinder test (55).

Euthanasia and SAH Grading

The animals were euthanized 5 days after the first injection. Perfusing and fixing the brain through the vascular system and removing the brain from the skull technique were performed (49). A 16-G cannula was inserted into the left ventricle toward the ascending aorta after thoracotomy. The descending aorta and inferior vena cava were clamped to prevent the infusion from reaching the lower half of the animal and to reduce the solution and time required for adequate perfusion. After an incision in the right atrium, the animals were perfused at physiological pressure with phosphate-buffered saline followed by 10% formalin administration. The perfusion process was considered to a complete success when clear fluid began to flow out from the cut in the right atrium and the rat eyes showed whitening.

After the brains were removed, the brain base was photographed with a high-resolution digital camera before placing them in a formalin solution. The photographs were taken to include Willis's polygon and the basilar artery. SAH in the basal cisterns was graded as described in the literature (50). The basal cisterns were divided into six sections in the photographs. Each section was assigned a score of 0–3 depending on the amount of subarachnoid blood clots. The scores obtained from the 6 sections were then summed (range: 0–18 points).

The Evaluation of Histopathological Changes

The brain was cut into approximately 3–4 mm coronal blocks and immersed in 10% formalin fixative solution at least 10–15 times of the tissue volume at 4°C for 24 h. (49). For the histopathological examinations, routine sample preparation (dehydration → transparency → paraffinization) was performed, and 5-μ-thick sections were prepared. For diameter and wall thickness measurements, transverse sections of the basilar arteries were evaluated in hematoxylin- and eosin-stained preparations under light microscopy, as described by Sabri and Macdonald (52). Corrugation of the internal elastic lamina due to vasospasm was demonstrated by Verhoeff-van Gieson staining (11). We applied our scoring system to evaluate elastic lamina corrugation because there was no scaling system used in the literature. The findings were categorized as "absent=0, mild=1–2, moderate=3, and severe=4 or more" according to the number of corrugations at a wall distance of 50 μm at x100 magnification. Morphological changes such as degenerated cells, neuronal shrinkage, vacuolization, and hyperchromatism was evaluated in Toluidine Blue and H&E-stained preparations (52).

Biochemical Analysis

The serum IL-1β, IL-6, and TNF-α levels were measured by using ELISA kits (Sunredbio, China). In addition, the serum Na, K, and Ca levels were examined with the VETSCAN analyzer (Zoetis, US). The CSF samples were obtained from the cisterna magna before euthanasia, and the glutamate levels in CSF associated with neuronal damage were studied by using ELISA kits (Sunredbio, China).

Each group of evaluations (i.e., neurological–[SB, HKS, OS, DC]*, microscopic–[SB, AKA]*, and biochemical–[SB, ZSA]*, **the initials of the researchers' names*) was performed by separate investigators in the blinded fashion.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics (Version 29). Statistical differences between the groups were evaluated by ANOVA and Post-Hoc tests if the data were normally distributed and necessary, and with the Kruskal–Wallis test if it did not fit the normal distribution or involved categorical data. For repeated measurements, each group was evaluated within itself using the Bonferroni correction method, Paired t-test, and Wilcoxon test. The results were considered to be significant at $p<0.05$.

RESULTS

Mortality and Complications

The mortality rate was 14.3% (n=5), and the group distribution is shown in Table I. One animal in the Sham group was euthanized 3 days after the first injection because of developing status epilepticus and showing a weight loss > 15% and was not included in the data analysis, but included in the mortality calculation. A ventral mesencephalon hematoma was identified in its postmortem study (Figure 2). One animal died immediately after blood injection into the cisterna magna, and three animals died in the days following SAH induction. Severe SAH was detected in two animals and significant hydrocephalus in one (Figure 2). No significant difference was noted in the mortality rates between the groups (Table I). One animal (dropout animal) showed signs of pain (such as the accumulation of porphyria around the eye) and two animals that completed the study (euthanized 5 days after the first injection) showed minimal necrosis in the distal tail. There was no difference in the preoperative mean weights between the groups. After operations, all animals showed drowsiness and decreased appetite, with significant weight loss in all groups (Table I). Preeuthanasia weights were different between the Sham group and the SAH+Nimodipine+Dexamethasone group ($p=0.027$), albeit no significant differences were identified in other post-hoc test comparisons.

Neurological Scores

The neurological status of each group in the preoperative and preeuthanasia periods was compared within themselves; the Sham group and SAH+Nimodipine group displayed no neurological deterioration, whereas, in the SAH+Nontreatment group and SAH+Nimodipine+Dexamethasone group, the neurological status was significantly deteriorated. Table I shows the detailed results for all groups.

When the preeuthanasia neurological examination findings of the groups were compared, the SAH+Nimodipine group was found to be indistinguishable from the Sham group. In contrast, the SAH+Nimodipine+Dexamethasone group was indifferent to the SAH group that did not receive any treatment. On the other hand, there was a significant difference between the SAH+Nimodipine and SAH+Nimodipine+Dexamethasone groups in favor of the SAH+Nimodipine group. Table II shows the results for all groups.

SAH Grades

The median SAH grade was 10 (range: 4–14). There was no significant difference between the groups regarding SAH grading (Table I).

Histological Evaluations of Basilar Artery and Brain Parenchyma

All histological examination parameters (diameters/ wall thickness/ corrugation scores of basal laminae) were significantly different between the Sham group and both SAH+Nontreatment and SAH+Nimodipine+Dexamethasone groups. In addition, there was a significant difference between the SAH+Nimodipine group and both SAH+Nontreatment and SAH+N-

Table I: Summary of Parameters in Each Group

	Sham	SAH + Nontreatment	SAH + Nimodipine	SAH + Combination Therapy*	The test comparing groups p value
Number of Animals	8	9	9	9	
Mortality & Follow-up					
Animal Loss (n) / Mortality (%)	1 (13)		1 (11)	3 (33)	Kruskal-Wallis; 0.247
Causes of Mortality	Euthanized (3. Day)	-	Sudden Death (During SAH induction)	2 Severe SAH, 1 Hydrocephalus (1., 2., 4. days respectively)	
Mean Body Weight ± SD					One-way ANOVA;
Pre-operative (g)	277.86 ± 12.2	275.56 ± 11.02	278.50 ± 10.30	280.0 ± 23.5	0.944
Pre-euthanasia (g)	268.14 ± 14.61	249.22 ± 9.78	257.13 ± 15.97	244.33 ± 14.67	0.019
p value (Paired t-test)	0.001	0.001	0.002	0.001	-
Mean Garcia Neurologic Score					Kruskal – Wallis;
Pre-operative	18	18	18	18	1
Pre-euthanasia	18	15	17.5	14.5	0.001
p value (Wilcoxon test)	1	0.011	0.066	0.028	-
Mean Cylinder Test (%)					Kruskal – Wallis;
Pre-operative	50	50	50	50	1
Pre-euthanasia	50	30	50	30	0.000
p value (Wilcoxon test)	1	0.007	0.157	0.026	-
Morphological & Biochemical studies					
SAH Grade	-	10 (min 4; max 13)	8 (min 5; max 14)	8 (min 7; max 12)	Kruskal-Wallis; 0.834
Mean Diameter of BA ± SD (μm)	347.6 ± 71.92	213.1 ± 31.0	372.2 ± 80.0	277.6 ± 64.2	One Way ANOVA; 0.001
Wall thickness of BA± SD (μm)	24.7 ± 4.15	36.4 ± 4.90	26.7 ± 4.50	36.2 ± 8.75	One Way ANOVA; 0.001
Score of Elastic Lamina Corrugation	1 (min 1; max 2)	3.5 (min 1; max 4)	1 (min 1; max 2)	3 (min 1; max 4)	Kruskal – Wallis; 0.001
Degenerated neuronal cells†	-	+	-	-	N/A
Serum IL-1 (pg/mL)	20.04 ± 13.18	102.82 ± 15.40	47.62 ± 19.81	61.18 ± 26.21	One Way ANOVA; 0.000
Serum IL-6 (pg/mL)	34.64 (min 19.56; max 57.89)	55.27 (min 25.09; max 82.79)	31.15 (min 24.17; max 44.63)	49.98 (min 26.91; max 94.01)	Kruskal – Wallis; 0.209
Serum TNF (ng/mL)	18.29 (min 9.57; max 39.65)	65.70 (min 38.29; max 140.23)	33,70 (min 9.46; max 78.68)	125.52 (min 83.88; max 144.53)	Kruskal – Wallis; 0.001
Serum Na (mmol/L)	147.57 ± 7.07	149,56 ± 4,83	148.17 ± 5.60	145.33 ± 1.86	One Way ANOVA; 0.560
Serum K (mmol/L)	7.70 (min 6.70; max 8.50)	7.00 (min 5.40; max 8.70)	7.38 (min 6.00; max 8.5)	7,63 (min 6.70; max 8.50)	Kruskal – Wallis; 0.185
Serum Ca (mg/dL)	11.87 ± 1.02	10.91 ± 1.12	11.59 ± 1.72	10.97 ± 0.68	One Way ANOVA; 0.368
CSF Glutamat (μmol/L)	25.84 ± 6.62	173.27 ± 42.69	110.03 ± 25.87	183.99 ± 46.38	One Way ANOVA; 0.000
CSF could not be obtained (n)	-	3	1	2	

Combination therapy includes nimopidine and dexamethason treatments as mentioned in the text. † Degenerated neuronal cells were observed in some of the animals in the nontreatment group (Figure 2).* ***SD:* *Standard Deviation, Mean Cylinder Test* ***(%):*** *Median percentage of touch of the neglected side (if any) to the inner surface of the cylinder.* ***BA:*** *Basilar Artery,* ***NA:*** *Not Applicable.*

imodipine+Dexamethasone groups. However, there was no difference between the SAH+Nimodipine group and the Sham group or between the SAH+Nimodipine+Dexamethasone group and the SAH+Nontreatment group in any of the histological examination parameters (Tables I and II).

Degenerated neuronal cells were observed in some animals in the SAH+Nontreatment group (Figure 2). In addition, thrombosis was detected in the posterior communicating artery of one animal in the SAH+Nontreatment group (Figure 2).

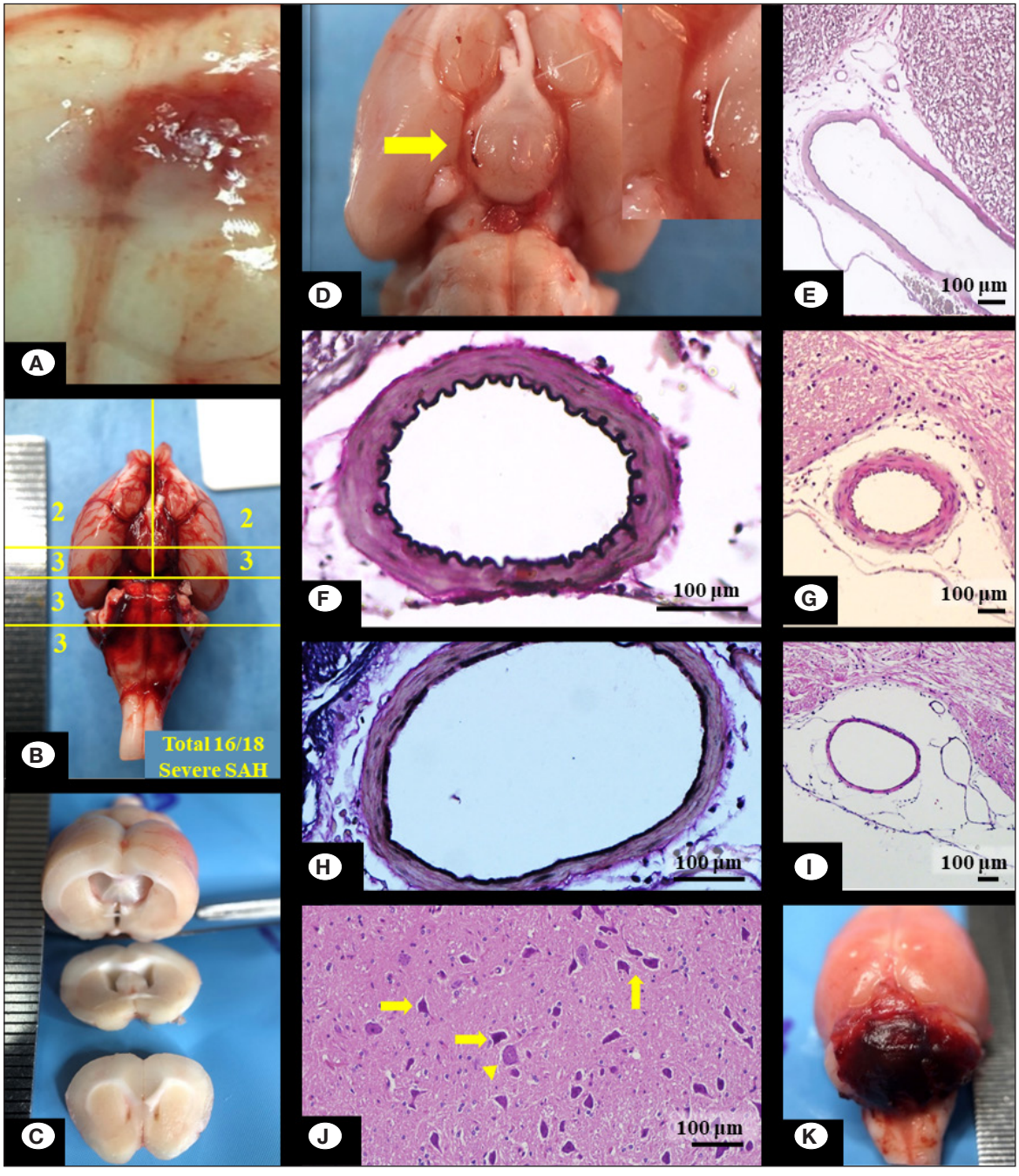

Figure 2: The diagram illustrating the experimental results. Part I: Examples of major complications causing mortality: **A)** mesencephalon hematoma, **B)** severe SAH, and **C)** significant hydrocephalus. Part II: Histological Evaluations of the Basilar Artery and Brain Parenchyma. **D)** Thrombosis was observed in the posterior communicating artery of one animal, which was noticeable at a high magnification of the brain base photograph obtained with a high-resolution camera. **E)** Tortuosity of the basilar artery. **F, G)** Preparations of the SAH group. Internal elastic lamina corrugation of the basilar artery. **H, I)** Preparations of the Sham group. **J)** Neuronal degeneration findings. Arrows indicate basophilic necrotic cells. Arrowhead indicates chromatolysis. **K)** The clot fills the cisterna magna.

Biochemical Analysis

The serum TNF-α levels were significantly higher in the SAH+Nontreatment and SAH+Nimodipine+Dexamethasone groups, but not in the SAH+Nimodipine group when compared with the Sham group. The serum IL-1β levels were higher in all SAH groups when compared to that in the Sham group. In addition, it was significantly higher in the nontreatment group than in the SAH+Nimodipine group. No statistically significant difference was detected on comparing the SAH+Nontreatment group with the SAH+Nimodipine+Dexamethasone group. The CSF glutamate levels were significantly lower in the Sham group than in all SAH groups. In the SAH+Nimodipine group, glutamate was significantly lower than in the SAH+Nontreatment and SAH+Combination therapy groups. No statistically significant difference was detected on comparing the SAH+Nontreatment group with the SAH+Nimodipine+Dexamethasone groups. No difference was detected between the groups in terms of their IL-6, Na, K, and Ca values in the serum biochemical tests. Detailed results of all biochemical analysis are shown in Tables I and II.

DISCUSSION

We applied the model described by Yang et al. that includes slow injection and reducing the amount in the second injection (72). The desired SAH grades and low mortality rates in our study probably reflects the advantages of using this model. The first aspect was to ensure that the stay of blood in the subarachnoid space was more extended with the double injection. Because the blood in the subarachnoid space is cleared faster in rats than in humans as the CSF turnover is approximately three times in the former (13,23). In addition, blood leakage from a large puncture region is a common issue in injection models for SAH induction. In this model, craniectomy or complete exposure of the atlantooccipital membrane is avoided, and only a tiny region is exposed to identify the boundary of the occipital bone and the atlantooccipital membrane, which helps to prevent leaks and retains the blood longer in the desired area for SAH induction. This step has been shown to significantly reduce the physical and psychological burden of surgery on the animal, resulting in less tissue destruction and minimizing the trauma. The second aspect is that, not giving a large amount of blood at a time did not cause brain stem compression or death (72). The overall mortality rate in previous double-injection models has been as high as nearly 50% (25-27,63). Although it is not as low as in the study of Yang et al., who described the new modified model that we used, our mortality rate of 14% is quite low when compared to that in the past series. The difference in the mortality rate between ours and Yang's series may be attributed to difference in the laboratory conditions and the surgeons hand experience. In some studies, the mortality rate was not specified

Table II: The p Values When the Neurological, Histological and Biochemical Parameters of the Groups were Compared Using Post Hoc Tests

	Neurological Scores (Garcia Neurological Score / Cylinder Test)			
	Sham	**SAH + Nontreatment**	**SAH + Nimodipine**	**SAH + Combination Therapy***
Sham	NA	0.001/0.000	0.273/0.792	0.022/0.023
SAH + Nontreatment	0.001/0.000	NA	0.031/0.001	0.998/0.929
SAH + Nimodipine	0.273/0.792	0.031/0.001	NA	0.049/0.033
SAH + Combination Therapy*	0.022/0.023	0.998/0.929	0.049/0.033	NA
	Histological Evaluations (diameters / wall thickness / corrugation of basal lamina of basilar arteries)			
Sham	NA	0.005/0.000/0.002	0.999/1.000/1.000	0.031/0.006/0.046
SAH + Nontreatment	0.005/0.000/0.002	NA	0.001/0.001/0.003	0.730/1.000/1.000
SAH + Nimodipine	0.999/1.000/1.000	0.001/0.001/0.003	NA	0.040/0.003/0.019
SAH + Combination Therapy*	0.031/0.006/0.046	0.730/1.000/1.000	0.040/0.003/0.019	NA
	Biochemical Analyzes (TNF-α / serum IL-1β / CSF Glutamat)			
Sham	NA	0.010/0.000/0.001	0.432/0.032/0.008	0.001/0.039/0.001
SAH + Nontreatment	0.010/0.000/0.001	NA	0.152/0.000/0.020	0.006/0.37/0.927
SAH + Nimodipine	0.432/0.032/0.008	0.152/0.000/0.020	NA	0.001/0.721/0.007
SAH + Combination Therapy*	0.001/0.039/0.001	0.006/0.37/0.927	0.001/0.721/0.007	NA

Combination therapy includes nimopidine and dexamethason treatments as mentioned in the text.* ***Purple:* *NA;* ***Green:*** *All p values <0.05;* ***Red:*** *All p values >0.05;* ***Yellow:*** *p value either <0.05 or >0.05.* ***NA:*** *Not Applicable,* ***SAH:*** *Subarachnoid hemorrhage.*

(36,53), while, in others, it was reported as having no mortality. (19) This result is inconsistent with the ordinary course of SAH, which has a well-known mortality rate. Apart from the sudden death and severe SAH considered as the cause of death, we described two fatal complications (1 ventral mesencephalon hematoma + status epilepticus and one hydrocephalus) that have never been mentioned in the literature so far to the best of our knowledge (61).

Another distinguishing feature of our study is the detailed neurological follow-up in addition to the histopathological evaluations. Other than vasospasm, pathways involved in the pathophysiology of DCI are ignored in most studies performed with histopathological evaluation alone (6,53,73). In contrast, the literature suggests that DCI can develop even without vasospasm. Therefore, the crucial role of angiographic vasospasm is being questioned now. Furthermore, it has been shown that DCI, the primary prognostic determinant in surviving patients, is a multifactorial process (9,12,32,38-40,45,56,64,67). The neurological status is DCI's primary indicator and critical in the SAH follow-up. Unfortunately, most studies excluded neurological follow-up after SAH induction (6,53,73). In a few studies, neurological follow-up has been mentioned, but no reproducible scoring system was used (23). In our study, the neurologic score of the SAH+Nimodipine group did not worsen after the operation. As expected, nimodipine treatment significantly prevented poor neurological outcomes. However, in the SAH+Nimodipine+Dexamethasone group, the outcomes did not improve with the addition of dexamethasone's anti-inflammatory effect to the treatment; surprisingly, worse results were observed when compared to those for the SAH+Nimodipine group (Table I). No significant difference was detected between the results for the SAH+Nontreatment and SAH+Nimodipine+Dexamethasone groups (Table II).

The wall thickness and diameter of the basilar artery in the Sham and nontreatment SAH groups were compatible with most literature reports (20,23,37). Our histopathological results also supported neurological findings. While significant signs of vasospasm were observed in the untreated group, no statistically significant difference was recorded between the results of the SAH+Nimodipine group and the Sham group. The corrugation of the elastic lamina is one of the vasospasms most important histopathological features. Most studies have not evaluated corrugation or evaluated it without any reproducible objective criteria (73). We performed Verhoeff-van Gieson staining to evaluate elastic lamina corrugation for the first time, to the best of our knowledge, in this context. We also used our developed reproducible scoring system. The corrugation results were correlated with the diameter and wall thickness measurements. Significantly less corrugation of the elastic lamina was observed in the SAH+Nimodipine group than in the other SAH groups. Similar to the neurological evaluation results, no significant histopathological difference was recorded between the SAH+Nontreatment and SAH+Nimodipine+Dexamethasone groups.

Excess glutamate overactivated receptors, causing intracellular Ca^{2+} overload and leading to excitotoxicity associated with ischemic injury. In experimental studies, it has been shown that glutamate elevation in the cerebral interstitial fluid predicts ischemia (54). Consistent with the literature reports, the CSF glutamate levels were significantly higher in all SAH groups in the present study. In addition, the CSF glutamate levels were significantly higher in the SAH+Nontreatment (p=0.008) and SAH+Nimodipine+Dexamethasone (p=0.001) groups, but not in the SAH+Nimodipine group (p=0.927) when compared to the Sham group.

It has been shown that IL-1β was induced during the early brain injury period of SAH, resulting in the formation of tight junctions of the blood–brain barrier to open, and it has been argued that IL-1β may be a therapeutic target in this treatment approach (4,21). In our study, the serum IL-1β levels conformed to those reported by Sozen et al. (58).

TNF-α is a critical cytokine involved in initiating inflammatory responses and is thought to play the central role in the generation of oxidative stress and in the apoptosis of endothelial cells commonly observed in SAH (71). In our study, consistent with the literature, a significant increase was observed in the nontreatment SAH group when compared with the Sham group. However, the combination group showed the highest TNF-α level unexpectedly ($p<0.05$), which can be possibly explained by Zeng et al.'s report who examined the relationship between dexamethasone and TNF-α on alveolar macrophages and noted that a high dexamethasone level may be an inducer for TNF-α (75).

The current rescue therapy protocols for DCI in the guidelines aim to prevent vasospasm and ischemia secondary to vasospasm. It is based on the principle of avoiding hypotension, hypovolemia, and anemia. These treatments include intravenous crystalloid and colloid fluid administration, inotropic support, blood transfusions, electrolyte replacements, intensive care follow-up, mechanical ventilation, brain-protecting sedation, intraarterial drug administration, balloon angioplasty, and decompressive craniectomy. However, these treatments have only a slight effect on DCI and are quite expensive. Chou reported that the cost of these treatments is an average of 40,000$ per patient (7). Although the goal of therapeutic strategies in SAH patients is to prevent DCI and improve the clinical outcomes, past studies have, until recently, been designed to treat vasospasm with ET-1 receptor antagonists. However, nimodipine has shown additional neuroprotective properties after its introduction into clinical practice. Therefore, nimodipine could also have abrogated these pathological processes besides the vasodilator effect (15). In our study, the positive impact of nimodipine was demonstrated not only on vasospasm in the basilar artery but also on the clinical outcomes.

Dexamethasone is a steroidal anti-inflammatory agent widely used to treat different conditions, particularly in patients undergoing craniotomy (5), including the management of postcraniotomy cerebral edema and the treatment of severe headaches attributable to meningeal inflammation (22). However, there is an ongoing debate about its clinical significance in SAH. Despite strong evidence that inflammation contributes to poor outcomes, making it a potentially promising target, our study does not suggest a beneficial effect of the addition of dexamethasone in SAH. Our study, in fact, showed that when

dexamethasone is added to nimodipine, worse results are obtained compared to that than when nimodipine is used alone. We believe that the mechanism behind this result may involve a drug–drug interaction via the CYP3A4 enzyme considering an ongoing debate about dexamethasone's potential as an inducer of CYP3A4 (1). CYP3A subfamily enzymes metabolizes ~30% of almost all clinically used drugs (74). On the other hand, nimodipine undergoes extensive presystemic elimination, wherein the CYP3A4 (cytochrome-P450-3A4) enzyme plays a primary role (36,77). Unfortunately, to overcome presystemic elimination, the IV-form usage of nimodipine is not preferred in DCI prophylaxis because it causes severe hypotension. The plasma concentration and efficacy of nimodipine were significantly reduced when coadministered with strong CYP3A4 inducers. Therefore, some inserts for nimodipine (e.g., Bayer, Canada, 2011 version) mention that the concomitant use of strong inducers of CYP3A4 (e.g., phenytoin) is contraindicated. However, moderate or weak inducers of CYP3A4 can reduce the efficacy of nimodipine. Therefore, it is recommended that patients using the medium or weak inducers of CYP3A4 be closely monitored for the lack of effectiveness. The dose of nimodipine should be increased if deemed necessary. However, there are no specific warnings for the usage of dexamethasone in inserts for nimodipine.

The inability to provide clinical translation of experimental SAH studies has kept clinical needs unmet (46). One of the most likely reasons for this situation is the lack of standardization in experimental studies (40). To ensure strict standardization, each parameter was selected for a specific reason in the present study design, as specified below:

- Adult age: The adult age group was selected because young rats are prone to asymptomatic vasospasm, which is quickly reversible, (44) while older ones have less response to treatment (35,44). In fact, this situation is the same for humans. A study by Lanzino et al. in 1996 reported that although the incidence of asymptomatic vasospasm is lower in the elderly than in younger patients, symptomatic vasospasm increases with age (35).
- Female rat: Some compounds metabolized by CYP3A enzymes in humans can be metabolized by CYP2C enzymes in male rats (30). Therefore, male rats may not be suitable for studying drugs based on CYP3A enzyme metabolism. It has also been shown that the CYP3A content in liver microsomes of the dexamethasone-treated female rats increases similar to that in human liver microsomes (30). Since the drugs used in our study are affected by CYP3A4 metabolism, only female rats were used.

Limitations

While SAH was not graded differently between the groups in our animal model, the neurological functions and histological findings for the SAH+Nimodipine group were close to those of the Sham group. However, they deteriorated in the SAH+Nimodipine+Dexamethasone group, similar to that in the SAH+Nontreatment group. Our hypothesis to explain these results is that CYP3A4 is induced by dexamethasone. Unfortunately, we could not measure the nimodipine serum levels and CYP metabolism to prove this point.

The lack of the inclusion of a group with dexamethasone but without nimodipine limits some of our findings. As there is no nimodipine-free application in the SAH treatment routine generally, we did not consider it necessary to add a dexamethasone group without nimodipine to the present experimental design.

Thirty-two animals were needed based on their power calculation, but the study ended up with 30 due to dropouts and mortality. Also, three of the rats (3/9) in the combined treatment group died. We may have added more rats, but could not.

CSF could not be obtained from five animals before euthanasia. However, the postmortem studies of these animals revealed that the blood clot partly occupied the cisterna magna (Figure 2). The distribution of these 5 animals among the groups is shown in Table I.

CONCLUSION

Unlike all other treatment trials for DCI, nimodipine exhibits neuroprotective effects and is effective against vasospasm. Consequently, it is the only treatment option that improves clinical outcomes after SAH. Our findings suggest that adding dexamethasone, which potentially targets inflammation, reduces the effects of nimodipine on DCI. Thus, it seems that dexamethasone possibly induces the CYP3A4 enzyme that metabolizes nimodipine. Therefore, dexamethasone should not be preferred for patients receiving nimodipine therapy for SAH. However, if dexamethasone treatment is necessary, these patients should be monitored more closely regarding the efficacy, and the nimodipine dose should be increased. However, please note that our results are based on laboratory findings from a small series that required drug–drug interaction studies through CYP3A4 enzyme metabolism and clinical confirmation.

ACKNOWLEDGEMENTS

The Turkish Neurosurgical Society provided financial support for this study (a research grant) (No: 2021/1; Date: 04/02/2021.

AUTHORSHIP CONTRIBUTION

Study conception and design: SB, DC
Data collection: SB, HKS, ZSA, AKA, OY, DC
Analysis and interpretation of results: SB, HKS
Draft manuscript preparation: SB
Critical revision of the article: HKS
Other (study supervision, fundings, materials, etc...): HKS
All authors (SB, HKS, ZSA, AKA, OY, DC) reviewed the results and approved the final version of the manuscript.

REFERENCES

1. Al Rihani SB, Deodhar M, Dow P, Turgeon J, Michaud V: Is dexamethasone a substrate, an inducer, or a substrate-inducer of CYP3As? Arch Phar & Pharmacol Res 2(5):000546, 2020
2. Allen GS, Ahn HS, Preziosi TJ, Battye R, Boone SC, Boone SC, Chou SN, Kelly DL, Weir BK, Crabbe RA, Lavik PJ, Rosenbloom SB, Dorsey FC, Ingram CR, Mellits DE, Bertsch LA, Boisvert DP, Hundley MB, Johnson RK, Strom JA, Transou CR: Cerebral arterial spasm-a controlled trial of nimodipine in patients with subarachnoid hemorrhage. N Engl J Med 308:619-624, 1983
3. Asano T, Sano K: Pathogenetic role of no-reflow phenomenon in experimental subarachnoid hemorrhage in dogs. J Neurosurg 46:454-466, 1977
4. Aydin HE, Bektur NE, Ozbek Z, Oner S, Baycu C, Kilic FS: Comparison of the effects and mechanism of the curcumin with different drugs in experimental vasospasm after subarachnoid hemorrhage. Turk Neurosurg 27:884-893, 2017
5. Bederson JB, Connolly ES Jr, Batjer HH, Dacey RG, Dion JE, Diringer MN, Duldner JE Jr, Harbaugh RE, Patel AB, Rosenwasser RH: Guidelines for the management of aneurysmal subarachnoid hemorrhage: A statement for healthcare professionals from a special writing group of the Stroke Council, American Heart Association. Stroke 40:994-1025, 2009
6. Bilginer B, Onal MB, Narin F, Soylemezoglu F, Ziyal IM, Ozgen T: The effects of intravenous cilostazol and nimodipine on cerebral vasospasm after subarachnoid hemorrhage in an experimental rabbit model. Turk Neurosurg 19:374-379, 2009
7. Chou CH, Reed SD, Allsbrook JS, Steele JL, Schulman KA, Alexander MJ: Costs of vasospasm in patients with aneurysmal subarachnoid hemorrhage. Neurosurgery 67:345-351; discussion 351-352, 2010
8. Connolly ES, Jr., Rabinstein AA, Carhuapoma JR, Derdeyn CP, Dion J, Higashida RT, Hoh BL, Kirkness CJ, Naidech AM, Ogilvy CS, Patel AB, Thompson BG, Vespa P: Guidelines for the management of aneurysmal subarachnoid hemorrhage: A guideline for healthcare professionals from the American Heart Association/american Stroke Association. Stroke 43:1711-1737, 2012
9. Cossu G, Messerer M, Oddo M, Daniel RT: To look beyond vasospasm in aneurysmal subarachnoid haemorrhage. Biomed Res Int 2014:628597, 2014
10. Czorlich P, Sauvigny T, Ricklefs F, Abboud T, Nierhaus A, Vettorazzi E, Reuter DA, Regelsberger J, Westphal M, Schmidt NO: Impact of dexamethasone in patients with aneurysmal subarachnoid haemorrhage. Eur J Neurol 24:645-651, 2017
11. Dai D, Ding YH, Kadirvel R, Lewis DA, Kallmes DF: Experience with microaneurysm formation at the basilar terminus in the rabbit elastase aneurysm model. AJNR Am J Neuroradiol 31:300-303, 2010
12. Dankbaar JW, Rijsdijk M, van der Schaaf IC, Velthuis BK, Wermer MJ, Rinkel GJ: Relationship between vasospasm, cerebral perfusion, and delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage. Neuroradiology 51:813-819, 2009
13. de Lange EC: Utility of CSF in translational neuroscience. J Pharmacokinet Pharmacodyn 40:315-326, 2013
14. de Oliveira Manoel AL, Macdonald RL: Neuroinflammation as a target for intervention in subarachnoid hemorrhage. Front Neurol 9:292, 2018
15. Dorhout Mees SM, Rinkel GJ, Feigin VL, Algra A, van den Bergh WM, Vermeulen M, van Gijn J: Calcium antagonists for aneurysmal subarachnoid haemorrhage. Cochrane Database Syst Rev 2007:Cd000277, 2007
16. Dorhout Mees SM, van den Bergh WM, Algra A, Rinkel GJ: Antiplatelet therapy for aneurysmal subarachnoid haemorrhage. Cochrane Database Syst Rev 2007:Cd006184, 2007
17. Dorsch NW, King MT: A review of cerebral vasospasm in aneurysmal subarachnoid haemorrhage Part I: Incidence and effects. J Clin Neurosci 1:19-26, 1994
18. Dreier JP: The role of spreading depression, spreading depolarization and spreading ischemia in neurological disease. Nat Med 17:439-447, 2011
19. Dudhani RV, Kyle M, Dedeo C, Riordan M, Deshaies EM: A low mortality rat model to assess delayed cerebral vasospasm after experimental subarachnoid hemorrhage. J Vis Exp:e4157, 2013
20. El Amki M, Dubois M, Lefevre-Scelles A, Magne N, Roussel M, Clavier T, Guichet PO, Gérardin E, Compère V, Castel H: Long-lasting cerebral vasospasm, microthrombosis, apoptosis and paravascular alterations associated with neurological deficits in a mouse model of subarachnoid hemorrhage. Mol Neurobiol 55:2763-2779, 2018
21. Geraghty JR, Testai FD: Delayed cerebral ischemia after subarachnoid hemorrhage: Beyond vasospasm and towards a multifactorial pathophysiology. Curr Atheroscler Rep 19:50, 2017
22. Greenberg MS: Handbook of Neurosurgery, 9th ed. New York: Thieme, 2020:1232
23. Gules I, Satoh M, Clower BR, Nanda A, Zhang JH: Comparison of three rat models of cerebral vasospasm. Am J Physiol Heart Circ Physiol 283:H2551-2559, 2002
24. Güresir E, Lampmann T, Bele S, Czabanka M, Czorlich P, Gempt J, Goldbrunner R, Hurth H, Hermann E, Jabbarli R, Krauthausen M, König R, Lindner D, Malinova V, Meixensberger J, Mielke D, Németh R, Darkwah Oppong M, Pala A, Prinz V, Rashidi A, Roder C, Sandalcioglu IE, Sauvigny T, Schebesch KM, Timmer M, Vajkoczy P, Wessels L, Wild F, Wilhelm C, Wostrack M, Vatter H, Coch C: Fight INflammation to Improve outcome after aneurysmal Subarachnoid HEmorRhage (FINISHER) trial: Study protocol for a randomized controlled trial. Int J Stroke 18(2):174749302210935, 2022
25. Güresir E, Raabe A, Jaiimsin A, Dias S, Raab P, Seifert V, Vatter H: Histological evidence of delayed ischemic brain tissue damage in the rat double-hemorrhage model. J Neurol Sci 293:18-22, 2010
26. Güresir E, Vasiliadis N, Dias S, Raab P, Seifert V, Vatter H: The effect of common carotid artery occlusion on delayed brain tissue damage in the rat double subarachnoid hemorrhage model. Acta Neurochir (Wien) 154:11-19, 2012
27. Güresir E, Vasiliadis N, Konczalla J, Raab P, Hattingen E, Seifert V, Vatter H: Erythropoietin prevents delayed hemodynamic dysfunction after subarachnoid hemorrhage in a randomized controlled experimental setting. J Neurol Sci 332:128-135, 2013

28. Hanalioglu S, Sahin B, Sayyahmelli S, Ozaydin B, Erginoglu U, Aycan A, Baskaya MK: The role of microsurgery for poor-grade aneurysmal subarachnoid hemorrhages in the endovascular era. Acta Neurochir (Wien) 164:781-793, 2022
29. Hop JW, Rinkel GJ, Algra A, van Gijn J: Case-fatality rates and functional outcome after subarachnoid hemorrhage: A systematic review. Stroke 28:660-664, 1997
30. Kanazu T, Yamaguchi Y, Okamura N, Baba T, Koike M: Model for the drug-drug interaction responsible for CYP3A enzyme inhibition. II: Establishment and evaluation of dexamethasone-pretreated female rats. Xenobiotica 34:403-413, 2004
31. Kilkenny C, Browne WJ, Cuthi I, Emerson M, Altman DG: Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. Vet Clin Pathol 41:27-31, 2012
32. Kramer A, Fletcher J: Do endothelin-receptor antagonists prevent delayed neurological deficits and poor outcomes after aneurysmal subarachnoid hemorrhage?: A meta-analysis. Stroke 40:3403-3406, 2009
33. Kreiter KT, Rosengart AJ, Claassen J, Fitzsimmons BF, Peery S, Du YE, Connolly ES, Mayer SA: Depressed mood and quality of life after subarachnoid hemorrhage. J Neurol Sci 335:64-71, 2013
34. Langham J, Goldfrad C, Teasdale G, Shaw D, Rowan K: Calcium channel blockers for acute traumatic brain injury. Cochrane Database Syst Rev 2003(4):Cd000565, 2003
35. Lanzino G, Kassell NF, Germanson TP, Kongable GL, Truskowski LL, Torner JC, Jane JA: Age and outcome after aneurysmal subarachnoid hemorrhage: Why do older patients fare worse? J Neurosurg 85:410-418, 1996
36. Lee CK, Choi JS, Choi DH: Effects of pravastatin on the pharmacokinetic parameters of nimodipine after oral and intravenous administration in rats: Possible role of CYP3A4 inhibition by pravastatin. Indian J Pharmacol 44:624-628, 2012
37. Lee JY, Huang DL, Keep R, Sagher O: Characterization of an improved double hemorrhage rat model for the study of delayed cerebral vasospasm. J Neurosci Methods 168:358-366, 2008
38. Macdonald RL: Clazosentan: An endothelin receptor antagonist for treatment of vasospasm after subarachnoid hemorrhage. Expert Opin Investig Drugs 17:1761-1767, 2008
39. Macdonald RL: Delayed neurological deterioration after subarachnoid haemorrhage. Nat Rev Neurol 10:44-58, 2014
40. Macdonald RL: Vasospasm: My first 25 years-what worked? What didn't? What next? Acta Neurochir Suppl 120:1-10, 2015
41. Macdonald RL, Higashida RT, Keller E, Mayer SA, Molyneux A, Raabe A, Vajkoczy P, Wanke I, Bach D, Frey A, Marr A, Roux S, Kassell N: Clazosentan, an endothelin receptor antagonist, in patients with aneurysmal subarachnoid haemorrhage undergoing surgical clipping: A randomised, double-blind, placebo-controlled phase 3 trial (CONSCIOUS-2). Lancet Neurol 10:618-625, 2011
42. Mohney N, Williamson CA, Rothman E, Ball R, Sheehan KM, Pandey AS, Fletcher JJ, Jacobs TL, Thompson BG, Rajajee V: A propensity score analysis of the impact of dexamethasone use on delayed cerebral ischemia and poor functional outcomes after subarachnoid hemorrhage. World Neurosurg 109:e655-e661, 2018
43. Nair AB, Jacob S: A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm 7:27-31, 2016
44. Nakajima M, Date I, Takahashi K, Ninomiya Y, Asari S, Ohmoto T: Effects of aging on cerebral vasospasm after subarachnoid hemorrhage in rabbits. Stroke 32:620-628, 2001
45. Oberman DZ, Rabelo NN, Pipek LZ, Telles J, Barbat NC, Coelho A, Yoshikawa MH, Barbosa GB, Teixeira MJ, Figueiredo EG: Prospective analysis of cerebral edema admission and clinical outcome in ruptured intracranial aneurysm. Turk Neurosurg 33:610-617, 2023
46. Oka F, Chung DY, Suzuki M, Ayata C: Delayed cerebral ischemia after subarachnoid hemorrhage: experimental-clinical disconnect and the unmet need. Neurocrit Care 32:238-251, 2020
47. Pegoli M, Mandrekar J, Rabinstein AA, Lanzino G: Predictors of excellent functional outcome in aneurysmal subarachnoid hemorrhage. J Neurosurg 122:414-418, 2015
48. Rincon F, Rossenwasser RH, Dumont A: The epidemiology of admissions of nontraumatic subarachnoid hemorrhage in the United States. Neurosurgery 73:217-222; discussion 212-213, 2013
49. JC RA, Sugawara T, Jadhav V, Zhang JH: Rodent surgical procedures and tissue collection. In: Chen J, Xu ZC, Xu XM, Zhang JH (ed), Animal Models of Acute Neurological Injuries. New Jersey: Humana Press, 2009
50. JC RA, Sugawara T, Zhang JH: Aneurysmal subarachnoid hemorrhage grading in animal models. In: Chen J, Xu XM, Xu ZC, Zhang JH (ed), Animal Models of Acute Neurological Injuries II (Springer Protocols Handbooks), vol 1. New Jersey: Humana Press Totowa, 2012:321-330
51. Saand AR, Yu F, Chen J, Chou SH: Systemic inflammation in hemorrhagic strokes - A novel neurological sign and therapeutic target? J Cereb Blood Flow Metab 39:959-988, 2019
52. Sabri M. MRL: Vasospasm: Measurement of diameter, perimeter, and wall thickness. In: Chen J, Xu XM, Xu ZC, Zhang JH (ed), Animal Models of Acute Neurological Injuries II. (Springer Protocols Handbooks), vol 1. New Jersey: Humana Press Totowa, 2012: 473-479
53. Sahin MH, Akyuz E, Kadioglu HH: The effects of necrostatin-1 on cerebral vasospasm-induced subarachnoid hemorrhage. Turk Neurosurg 32(6):923-929, 2022
54. Sarrafzadeh A, Haux D, Sakowitz O, Benndorf G, Herzog H, Kuechler I, Unterberg A: Acute focal neurological deficits in aneurysmal subarachnoid hemorrhage: Relation of clinical course, CT findings, and metabolite abnormalities monitored with bedside microdialysis. Stroke 34:1382-1388, 2003
55. Schaar KL, Brenneman MM, Savitz SI: Functional assessments in the rodent stroke model. Exp Transl Stroke Med 2:13, 2010
56. Sehba FA, Pluta RM, Zhang JH: Metamorphosis of subarachnoid hemorrhage research: From delayed vasospasm to early brain injury. Mol Neurobiol 43:27-40, 2011
57. Sena E, van der Worp HB, Howells D, Macleod M: How can we improve the pre-clinical development of drugs for stroke? Trends Neurosci 30:433-439, 2007

58. Sozen T, Tsuchiyama R, Hasegawa Y, Suzuki H, Jadhav V, Nishizawa S, Zhang JH: Role of interleukin-1beta in early brain injury after subarachnoid hemorrhage in mice. Stroke 40:2519-2525, 2009
59. Suzuki HZJ: Neurobehavioral assessments of subarachnoid hemorrhage. In: Chen J, Xu XM, Xu ZC, Zhang JH (ed), Animal Models of Acute Neurological Injuries II. Springer Protocols Handbooks, vol 1. New Jersey: Humana Press Totowa, 2012
60. Taufique Z, May T, Meyers E, Falo C, Mayer SA, Agarwal S, Park S, Connolly ES, Claassen J, Schmidt JM: Predictors of poor quality of life 1 year after subarachnoid hemorrhage. Neurosurgery 78:256-264, 2016
61. Turk C, Camlar M, Diniz G, Arslan FD, Oren MM, Ozer F: Effects of lutein on brain damage and vasospasm in an experimental subarachnoid hemorrhage model. World Neurosurg 143:e450-e455, 2020
62. Vachon P, Moreau JP: Low doses of dexamethasone decrease brain water content of collagenase-induced cerebral hematoma. Can J Vet Res 67:157-159, 2003
63. Vatter H, Weidauer S, Konczalla J, Dettmann E, Zimmermann M, Raabe A, Preibisch C, Zanella FE, Seifert V: Time course in the development of cerebral vasospasm after experimental subarachnoid hemorrhage: Clinical and neuroradiological assessment of the rat double hemorrhage model. Neurosurgery 58:1190-1197, 2006
64. Vergouwen MD, Ilodigwe D, Macdonald RL: Cerebral infarction after subarachnoid hemorrhage contributes to poor outcome by vasospasm-dependent and -independent effects. Stroke 42:924-929, 2011
65. Vergouwen MD, Meijers JC, Geskus RB, Coert BA, Horn J, Stroes ES, van der Poll T, Vermeulen M, Roos YB: Biologic effects of simvastatin in patients with aneurysmal subarachnoid hemorrhage: A double-blind, placebo-controlled randomized trial. J Cereb Blood Flow Metab 29:1444-1453, 2009
66. Vergouwen MD, Vermeulen M, Coert BA, Stroes ES, Roos YB: Microthrombosis after aneurysmal subarachnoid hemorrhage: An additional explanation for delayed cerebral ischemia. J Cereb Blood Flow Metab 28:1761-1770, 2008
67. Vergouwen MD, Vermeulen M, van Gijn J, Rinkel GJ, Wijdicks EF, Muizelaar JP, Mendelow AD, Juvela S, Yonas H, Terbrugge KG, Macdonald RL, Diringer MN, Broderick JP, Dreier JP, Roos YB: Definition of delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage as an outcome event in clinical trials and observational studies: Proposal of a multidisciplinary research group. Stroke 41:2391-2395, 2010
68. Vollmer DG, Hongo K, Ogawa H, Tsukahara T, Kassell NF: A study of the effectiveness of the iron-chelating agent deferoxamine as vasospasm prophylaxis in a rabbit model of subarachnoid hemorrhage. Neurosurgery 28:27-32, 1991
69. Wellman GC, Koide M: Impact of subarachnoid hemorrhage on parenchymal arteriolar function. Acta Neurochir Suppl 115:173-177, 2013
70. Wong GK, Lam SW, Ngai K, Wong A, Siu D, Poon WS, Mok V, Cognitive Dysfunction after Aneurysmal Subarachnoid Hemorrhage I: Cognitive domain deficits in patients with aneurysmal subarachnoid haemorrhage at 1 year. J Neurol Neurosurg Psychiatry 84:1054-1058, 2013
71. Wu W, Guan Y, Zhao G, Fu XJ, Guo TZ, Liu YT, Ren XL, Wang W, Liu HR, Li YQ: Elevated IL-6 and TNF-α levels in cerebrospinal fluid of subarachnoid hemorrhage patients. Mol Neurobiol 53:3277-3285, 2016
72. Yang L, Lai WT, Wu YS, Zhang JA, Zhou XH, Yan J, Fang C, Zeng EM, Tang B, Peng CL, Zhao Y, Hong T: Simple and efficient rat model for studying delayed cerebral ischemia after subarachnoid hemorrhage. J Neurosci Methods 304:146-153, 2018
73. Yilmaz C, Cansever T, Kircelli A, Ozen OI, Aydemir F, Akar A, Caner H: The effects of proanthocyanidin on vasospasm after experimental subarachnoidal hemorrhage in rats. Turk Neurosurg 28:667-674, 2018
74. Zanger UM, Schwab M: Cytochrome P450 enzymes in drug metabolism: Regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacol Ther 138:103-141, 2013
75. Zeng S, Qiao H, Lv XW, Fan D, Liu T, Xie D: High-dose dexamethasone induced LPS-stimulated rat alveolar macrophages apoptosis. Drug Des Devel Ther 11:3097-3104, 2017
76. Zhao XD, Zhou YT, Zhang X, Zhuang Z, Shi JX: A meta analysis of treating subarachnoid hemorrhage with magnesium sulfate. J Clin Neurosci 16:1394-1397, 2009
77. Zhou SF: Drugs behave as substrates, inhibitors and inducers of human cytochrome P450 3A4. Curr Drug Metab 9:310-322, 2008