# The Role of Intracranial Pressure and Subarachnoid Blood Clots in Early Brain Injury After Experimental Subarachnoid Hemorrhage in Rats

*Masato Naraoka, Toshio Fumoto, Yuchen Li, Takeshi Katagai, Hiroki Ohkuma*

OBJECTIVE: **Early brain injury after subarachnoid hemorrhage (SAH), which is considered a main factor leading to poor outcome, is believed to be caused by the increase of intracranial pressure (ICP) and/or the presence of subarachnoid blood clots (SBC) itself. The purpose of this study was to examine whether ICP or SBC is more important to neurologic deficit in the presence of apoptosis or edema.**

METHODS: **A total of 50 rats were allocated to 3 groups: an endovascular perforation SAH model (the SAH group), a cisterna magna saline injection model (the saline injection group), and a cisterna magna sham injection model (the sham injection group). Statistical analysis of correlations among the ICP, the grade of clot volume, neuronal apoptosis, brain water content (brain edema), and neurologic deficit was performed.**

RESULTS: **In the SAH group, each of increased ICP and clot volume was correlated with neuronal apoptosis and brain edema. In the saline injection group, increased ICP was associated with apoptosis, but it did not correlate with brain edema. Neuronal apoptosis ($r = 0.75$; $P < 0.01$) and brain edema ($r = 0.89$; $P < 0.01$) correlated independently with neurologic deficit in the SAH group.**

CONCLUSIONS: **The present study suggests that neuronal apoptosis is caused mainly by increased ICP, whereas brain edema is induced by SBC, and increased ICP could aggravate it in the presence of SBC. Brain edema could affect neurologic deficit, but apoptosis alone may be less influential. Not only ICP but also SBC seem important for brain damage in the acute stage of SAH.**

## INTRODUCTION

Delayed cerebral ischemia caused by narrowing of the major cerebral arteries after aneurysmal subarachnoid hemorrhage (SAH) had been considered an important cause of high mortality and morbidity.[1-4] However, recent studies have shown that the amelioration of angiographic vasospasm does not result in favorable outcome, and other factors are also considered to contribute to brain damage after SAH.[5,6] Early brain injury (EBI), which consists of pathogenic factors such as neuronal apoptosis,[6-9] blood-brain barrier disruption,[10,11] cerebral edema,[12,13] cerebral cortical spreading depolarization,[14,15] and microcirculatory disturbance,[16,17] occurs within the first 72 hours of the ictus and is believed to be a main cause of poor outcome.[6,14,18]

EBI is believed to be caused by the increase of intracranial pressure (ICP) and/or the presence of subarachnoid blood clots (SBC) itself.[6,8,19,20] In EBI, neuronal apoptosis and brain edema are main factors, and the relationship between ICP increase and neuronal apoptosis and/or brain edema have been investigated. However, whether an ICP increase or the presence of SBC is the more important causative factor for EBI has not been clarified. This lack of clarity is because these factors cannot be examined independently in experimental SAH models produced by the endovascular puncture method or the prechiasmatic cisterna

**Key words**
- Apoptosis
- Brain edema
- Early brain injury
- Intracranial pressure
- Subarachnoid hemorrhage

**Abbreviations and Acronyms**
**EBI**: Early brain injury
**ICP**: Intracranial pressure
**NeuN**: Neuronal nuclei
**SAH**: Subarachnoid hemorrhage
**SBC**: Subarachnoid blood clots
**TUNEL**: Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling

*Department of Neurosurgery, Hirosaki University School of Medicine and Hospital, Hirosaki, Japan*

*To whom correspondence should be addressed: Masato Naraoka M.D., Ph.D. [E-mail: nessun@hirosaki-u.ac.jp]*

*Citation: World Neurosurg. (2019) 129:e63-e72.*
*https://doi.org/10.1016/j.wneu.2019.05.009*

*Journal homepage: **www.journals.elsevier.com/world-neurosurgery***

*Available online: **www.sciencedirect.com***

*1878-8750/$ - see front matter* 

injection method. To develop a strategy for prevention or treatment of EBI, it is important to clarify how ICP increase and SBC induce neuronal apoptosis and cerebral edema.

To clarify the role of ICP increase and SBC in EBI, their influences on neuronal apoptosis and/or brain edema were investigated by comparing the results obtained in the endovascular perforation SAH model and the cisterna magna saline injection model. In addition, whether neuronal apoptosis or brain edema exerts the major influence on neurologic deficits was also analyzed.

## METHODS

All experimental protocols were planned according to the National Institutes of Health laboratory animal use guidelines and approved by the Animal Research Committee. All Sprague-Dawley rats (male, 350–450 g) were purchased from Central Laboratory for Experimental Animals Japan Inc. and were used in this study. All rats were maintained under standard conditions (temperature of 25°C ± 2°C, 12-hour light/dark cycle) and were given ad libitum access to food and water.

### Animal Experimental Design

A total of 50 rats were used and randomly divided into 3 groups: the SAH group ($n = 21$), in which SAH was produced by the endovascular perforation method[21]; the saline injection group ($n = 21$), in which saline was injected into the cisterna magna accompanied by an ICP increase equal to that of the SAH group[22]; and the sham injection group (only puncture into the cisterna magna without saline injection) ($n = 8$).

The reason we dared to choose the penetration arterial SAH model was for the relative advantages compared with the injection SAH model. We needed to investigate the neurologic deficit in the presence of apoptosis or edema at acute stage of SAH; however, some studies[13,23] have shown that any physiologic effects resulting from the presence of a ruptured subarachnoid artery were lost with the injection models. Because the hemorrhage emanates from the internal carotid artery bifurcation directly into the local subarachnoid space, the distribution as well as the mechanical and physiologic effects of the SBC more closely resemble true aneurysmal SAH.

Another group that showed SAH but without increased ICP would have been considered desirable as an addition to this study. We attempted to achieve that goal. However, our preliminary trial to make a model by injecting blood into the cisterna magna slowly resulted in failure; the attempt led to an ICP increased by 20–40 mm Hg despite the very slow injection. We abandoned the attempt and describe it later as a "limitation."

### SAH Model

Rats were anesthetized with 0.3 mg/kg of medetomidine, 4.0 mg/kg of midazolam, and 5.0 mg/kg of butorphanol via intraperitoneal injection. After a microsensor probe (SPR-671 Millar Micro-Tip, Millar, Houston, USA) was inserted into the left parietal cortex to a depth of 2 mm, the ICP was continuously monitored during the SAH by an ICP monitoring system (PL3508B35, Millar, Houston, USA), and digitally recorded using a PowerLab Unit (ADInstuments, Colorado, USA). The endovascular perforation SAH model has been described in detail.[21] For this procedure, the rat was placed supine, and the left carotid artery and its branches were explored. A tungsten wire (2.54 mm [0.10 in]) was inserted into the internal carotid artery stump and advanced around 22 mm. SAH was confirmed by an increase in ICP. Rectal temperature was maintained at 37°C ± 0.5°C and spontaneous breathing was observed under ICP monitoring for the following 60 minutes after the SAH procedure.

Twenty-four hours after SAH production, all animals had their neurologic deficits assessed with the modified Garcia score, as described later.[24] Twelve rats were then killed with perfusion-fixation under deep anesthesia. Perfusion-fixation was started with 100 mL of heparinized physiologic saline (5000 U/500 mL) at 37°C followed by fixation with 100 mL of 4% paraformaldehyde under a perfusion pressure of 73.5 mm Hg. Just after perfusion-fixation, the brain was removed, the SAH grade was quantified by a method described later, and the brain was stained immunohistochemically to assess neuronal apoptosis.

The other 9 rats were decapitated under deep anesthesia, the brain was immediately extracted, and the SAH grade of samples was quantified and used for the measurement of brain water content.

### Saline Injection and Sham Injection Model

The peak ICP set point in the saline injection group was divided into 3 ranges according to the distribution of the peak ICP in the SAH group; ICP >100 mmHg (n = 7), 100 > ICP >70 mmHg (n = 7), 70 > ICP >40 mmHg (n = 7).

ICP monitoring was carried out in the same manner as for the SAH model. A 27-G butterfly needle was percutaneously placed into the cisterna magna through the foramen magnum. After checking the backflow of cerebrospinal fluid, 100–300 μL of saline was injected into the cisterna magna within 10 seconds, so that the peak ICP was set in one of the 3 ranges outlined earlier. Rectal temperature was maintained at 37°C ± 0.5°C and spontaneous breathing was observed under ICP monitoring for the following 60 minutes after the procedure. The ICP probe was subsequently withdrawn, the burr hole was sealed with acrylic cement, and each animal was returned to its cage after recovery from the anesthesia. Twenty-four hours after the procedure, neurologic deficits were assessed for all animals and they were killed as with the SAH group. The sham group ($n = 8$) was subjected to the same puncture as the saline injection group, except that the saline was not injected. Four rats were killed with perfusion-fixation, and the other 4 rats were decapitated for the measurement of brain water content.

### Neurologic Deficit

Neurologic evaluation was performed at 24 hours after SAH production or saline injection using a modified Garcia Scale.[24] A total of 6 segments were tested: spontaneous activity, spontaneous movements of the 4 limbs, forelimb outstretching, and wire cage wall climbing, trunk touch reaction, and vibrissae touch response. These 6 tests were each scored from 0 to 3, and the total neurologic status was graded on a total score of 0–18.

### SAH Grade (Clot Volume)

The severity of clot volume was assessed using the SAH grading scale as previously described by Sugawara et al.[25] Rats were euthanized 24

hours after creation of SAH, the clot volume of basal cisterns was divided into 6 segments and each segment had a grade assessed from 0 to 3: grade 0, no hematoma; grade 1, minimal hematoma; grade 2, mediocre blood with visible arteries, and grade 3, hematoma covered all arteries. A total score was evaluated in a blinded manner ranging from 0 to 18 points through summing the score of each segment.

### Histology and Immunofluorescent Staining

Neuronal apoptosis was assessed by double-fluorescence staining with terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) and neuronal nuclei (NeuN) staining.[25] After perfusion-fixation, whole brains were quickly removed and fixed in 4% paraformaldehyde for 1 day, followed by 30% sucrose for an additional 3 days at 4°C. The brains were then frozen in OCT compound (SAKURA Finetek, Tokyo, Japan) and cut into 6-μm-thick coronal sections with a cryostat (Zeiss, Berlin, Germany), washed 3 times with phosphate-buffered saline, and anti-NeuN antibody was induced (ab150116, Abcam, Berlin, Germany) for 15 hours at 4°C. The sections were then washed 3 times with phosphate-buffered saline for 10 minutes and incubated with antimouse Gig TRITC (T5393, Sigma-Aldrich, Munich, Germany). In addition, TUNEL staining was performed using the In-Situ Cell Death Detection Kit with fluorescein (Roche, Basel, Switzerland) following the manufacturer's instructions. All immunohistologic analyses were performed on the basal cortex of the ipsilateral at puncture of the SAH model, left side. Neuronal apoptosis was identified by double staining with TUNEL and NeuN, and quantified by the apoptotic index. Six random photomicrographs (×400) of basal cortex in the left side were taken for counting the apoptotic index, which was expressed as the percentage of positive cells from each animals under a fluorescence microscope (KEYENCE, Osaka, Japan). Twenty-eight rats of each group (SAH group, $n = 12$; saline injection group, $n = 12$; sham injection group, $n = 4$) were prepared for staining, and counting was performed manually in a double-blinded manner. We performed immunostaining not only for the left basal cortex but also for the contralateral right basal cortex. However, in the SAH group, the apoptotic index decreased extremely at the contralateral basal cortex. For this reason, we analyzed only for the left basal cortex.

### Brain Water Content

As for the evaluation of brain edema, the brain water content was measured by the dry-weight method: [dry weight/total weight] × 100. After euthanasia, brain samples were quickly removed and the total weight was measured; the brain was then placed in a heated container at 105°C for 48 hours and the dry weight was measured to calculate the brain water content (%).

### Statistical Analysis

Statistical analysis was carried out using JMPpro 13 (SAS Institute Inc., Cary, North Carolina, USA). Data were presented as mean ± standard deviation. Statistical differences between neuronal apoptosis, brain edema, and neurologic deficits between each group were analyzed using the Mann-Whitney U test. Correlations were calculated using linear regression and the Spearman rank correlation coefficient. Differences were considered statistically significant if the $P$ value was $<0.05$.

## RESULTS

The mortality was 36% (12 of 33 rats) in the SAH group and 13% (3 of 24 rats) in the saline injection group. No rat died in the sham injection group. The remaining 50 rats in the SAH group ($n = 21$), the saline injection group ($n = 21$), and the sham injection group ($n = 8$) were examined and analyzed. The results of peak ICP value, neuronal apoptosis quantified by the apoptotic index, brain water content, and neurologic scores for both the SAH and the saline injection group are summarized in Table 1. There were significant differences in the brain water content and neurologic score between the SAH group and the saline injection group.

### ICP and SAH Grade

The time course for the peak ICP value in the SAH group and the ICP monitoring data for both groups are shown in Figure 1. There were no significant differences in the peak ICP values between the SAH group and the saline injection group (Table 1). The ICP values returned to baseline after 60 minutes in both groups; however, significant differences were observed up to 30 minutes (Table 2). SAH grade was positively correlated with the peak ICP value in the SAH group ($r = 0.98$; $P < 0.001$; Figure 2).

### ICP and Apoptosis

The findings of neuronal apoptosis by immunohistochemical staining and the relationship between the apoptotic index and the peak ICP value are shown in Figures 3 and 4. There was no significant difference in the apoptotic index between the SAH group and the saline injection group (Table 1). In the SAH group, the apoptotic index was positively correlated with the peak ICP value ($r = 0.90$; $P < 0.001$; Figure 3A). In the saline injection group, a positive correlation between the apoptotic

**Table 1.** Summary of Intracranial Pressure, Apoptosis, Brain Water Content, and Neurologic Score

| | Subarachnoid Hemorrhage | Saline Injection | Sham Injection | *P* Value |
|---|---|---|---|---|
| Peak intracranial pressure (mm Hg) | 91 ± 12.2 | 85 ± 12.7 | 18 ± 2.7 | 0.72 |
| Apoptotic index (%) | 5.3 ± 2.7 | 4.8 ± 2.3 | 0.8 ± 0.3 | 0.67 |
| Brain water content (%) | 79.1 ± 1.7 | 76.9 ± 0.13 | 75.9 ± 0.11 | 0.012* |
| Neurologic score | 16.0 ± 2.2 | 17.4 ± 0.45 | 17.7 ± 0.15 | <0.01* |

*Significant difference versus subarachnoid hemorrhage group with saline injection group.

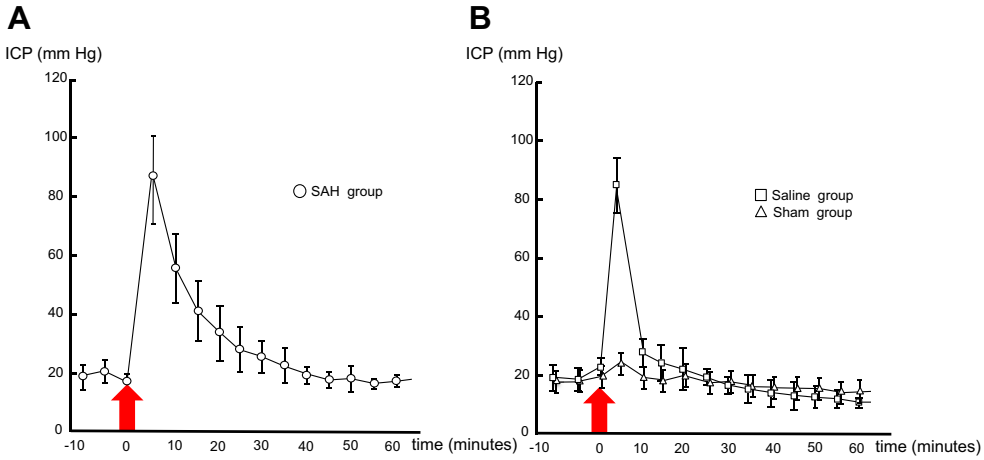


**Figure 1.** Intracranial pressure (ICP) monitoring of subarachnoid hemorrhage group (**A**) and saline injection group (**B**). Red *arrow*: puncture point.

index and the peak ICP value was seen, as was also the case with the SAH group ($r = 0.85$; $P < 0.01$; Figure 3B), although no neuronal apoptosis was seen in several rats that had peak ICP values <70 mm Hg.

### ICP and Brain Water Content (Brain Edema)

In the SAH group, the peak ICP value correlated positively with brain water content ($r = 0.84$; $P < 0.01$; Figure 5A). On the other hand, in the saline injection group, the brain water content was unchanged regardless of changes in the peak ICP value, and there was no correlation between the peak ICP value and brain water content ($r = 0.09$; $P < 0.01$; Figure 5B).

### SAH Grade and Apoptosis

In the SAH group, as the SAH grade increased, the apoptotic index also increased. And the SAH grade correlated positively with the apoptotic index ($r = 0.92$; $P < 0.01$; Figure 6A).

### SAH Grade and Brain Water Content (Brain Edema)

As the SAH grade increased, the brain water content also increased. The SAH grade also correlated positively with the brain water content ($r = 0.88$; $P < 0.01$; Figure 6B).

**Table 2.** Timeline of Intracranial Pressure Value

| | Baseline (mm Hg) | Peak (mm Hg) | 30 minutes (mm Hg) | 60 minutes (mm Hg) |
|---|---|---|---|---|
| Subarachnoid hemorrhage group | 20 ± 4.5 | 91 ± 12.2 | 32 ± 6.3 | 21 ± 4.2 |
| Saline injection group | 19 ± 5.2 | 85 ± 7.7 | 22 ± 3.8 | 17 ± 3.7 |
| Sham injection group | 18 ± 2.2 | 23 ± 3.7 | 18 ± 1.8 | 19 ± 2.7 |
| *P* value | 0.66 | 0.72 | 0.04* | 0.85 |

*Significant difference between SAH group and saline injection group.

### Apoptosis and Neurologic Score

Although there were similar appearances of neuronal apoptosis in both the SAH and the saline injection group (Figure 3), the apoptosis correlated negatively with the neurologic score only in the SAH group ($r = -0.75$; $P < 0.01$; Figure 7A). In the saline injection group, the neurologic score was stable regardless of the change in the apoptosis score, and no correlation was seen between the neurologic score and the apoptosis score ($r = -0.43$; $P = 0.16$; Figure 7B).

### Brain Water Content (Brain Edema) and Neurologic Score

As the brain water content increased, the neurologic score decreased in the SAH group. Therefore, the brain water content correlated negatively with the neurologic score ($r = 0.89$; $P < 0.01$; Figure 8A). Because almost no increased brain water content was seen in the saline injection group, there was no correlation between brain water content and the neurologic score in the saline injection group (Figure 8B).

## DISCUSSION

Many studies have indicated that the main factors in EBI are neuronal apoptosis and brain edema and that they are mainly caused by ICP increase and the presence of SBC.[6,8,19,20] However, few studies have attempted to show whether increased ICP or the presence of SBC is chiefly responsible for the occurrence of neuronal apoptosis and/or brain edema, which this study has attempted to clarify. In addition, whether neuronal apoptosis or brain edema mainly causes neurologic deterioration was also evaluated.

### Role of ICP Increase and Subarachnoid Hematoma on Neuronal Apoptosis

Several experimental studies have already shown a positive correlation between neuronal apoptosis and increased ICP.[9,26] However, the correlation between apoptosis and ICP cannot be determined, because these experiments were performed using an SAH model and the influence of SBC per se could not be ruled out. There have been no studies investigating the association between an ICP increase and neuronal apoptosis using the saline injection

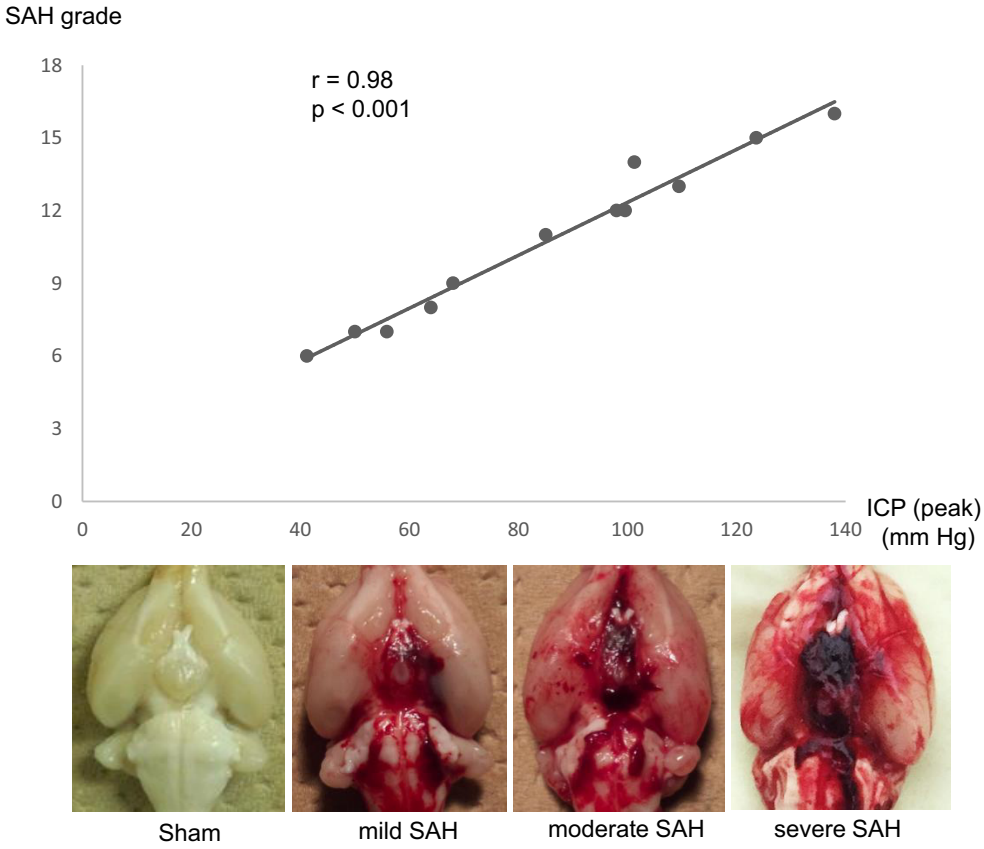


**Figure 2.** (**A**) The subarachnoid hemorrhage (SAH) grade correlates with the peak of intracranial pressure (ICP). (**B**) The SAH images of sham, mild, moderate, and severe SAH grade.

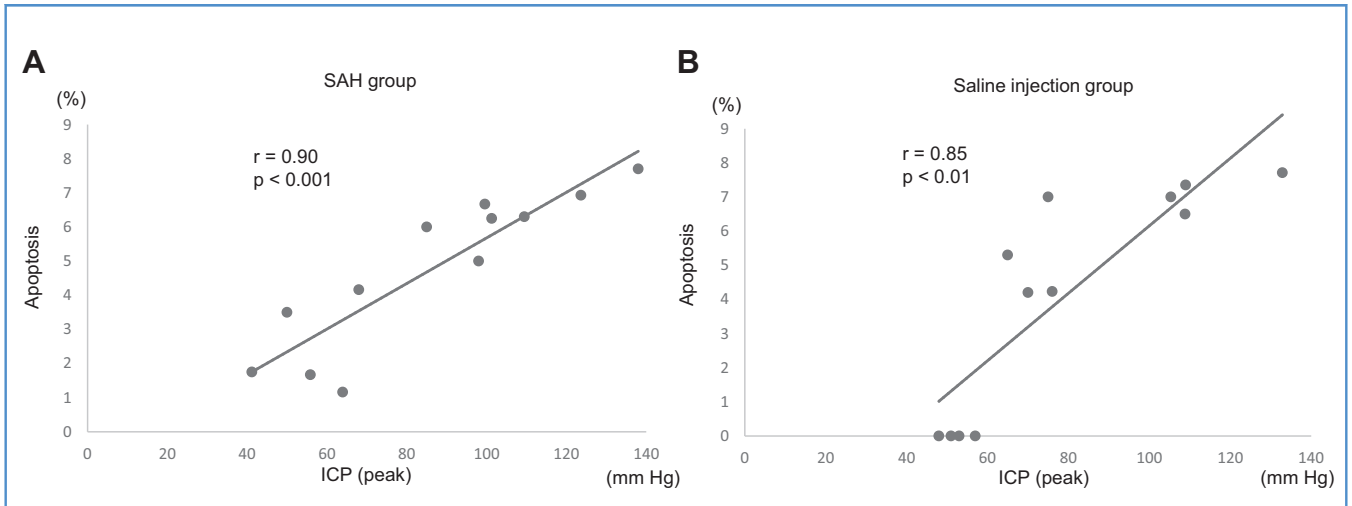


**Figure 3.** The apoptosis correlates with the peak of intracranial pressure (ICP) in the subarachnoid hemorrhage (SAH) group (**A**) and saline injection group (**B**).

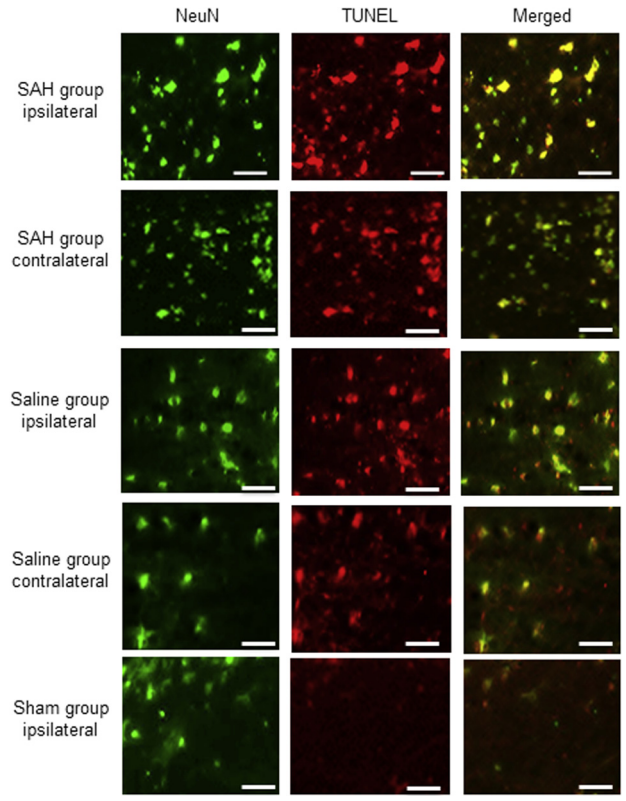


**Figure 4.** Neuronal cell apoptosis. Colocalization of terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) and neuronal nuclei (NeuN) were considered apoptotic cells. Scale bar = 100 μm. The positive cell levels were counted in 6 random fields of the relevant regions (×400). SAH, subarachnoid hemorrhage.

model. The present study showed that increased ICP itself could cause apoptosis, because neuronal apoptosis was observed to correlate with increased ICP not only in the SAH model but also in the saline-injected model.

On the other hand, some studies using an endovascular perforation or cisterna magna injection SAH model emphasized that neuronal apoptosis was induced by the existence of SBC.[13,27-29] However, ICP was not measured in these studies, and the influence of increased ICP cannot be denied, because increased ICP cannot be avoided in the production of SAH in any SAH model. To clarify the association between SBC and neuronal apoptosis, it would be desirable to carry out an investigation using an SAH model without increased ICP; however, this is difficult to produce.[30] Even in the present study, as a preliminary trial, we intended to produce an SBC model without increased ICP, but this failed.

In the present study, not only increased ICP ($r = 0.90$; $P < 0.001$; **Figure 3A**) but also the SAH grade ($r = 0.92$; $P < 0.01$; **Figure 6A**) correlated with the appearance of neuronal apoptosis. In addition, neuronal apoptosis appeared even at low ICP in the SAH group, whereas it was not observed at <60 mm Hg of ICP in the saline injection group. These results suggest that both ICP and SBC can be a cause of neuronal apoptosis. Although there was no difference in peak ICP values, it was suggested that the difference between the 2 groups in brain water contents may be caused by the difference in duration time of high ICP.

Increased ICP, which is accompanied by glucose and oxygen deficits in neurons and glia caused by decreased cerebral blood flow and hypoxic state, has been considered to cause neuronal apoptosis by initiating the death receptor pathway and/or the p53 pathway.[6,31-33] On the other hand, SBC augments cortical levels of reactive oxygen species and causes excessive oxidative stress,[28] and neuronal apoptosis can be induced via the PERK/eIF2α/CHOP pathway by oxidative stress.[34]

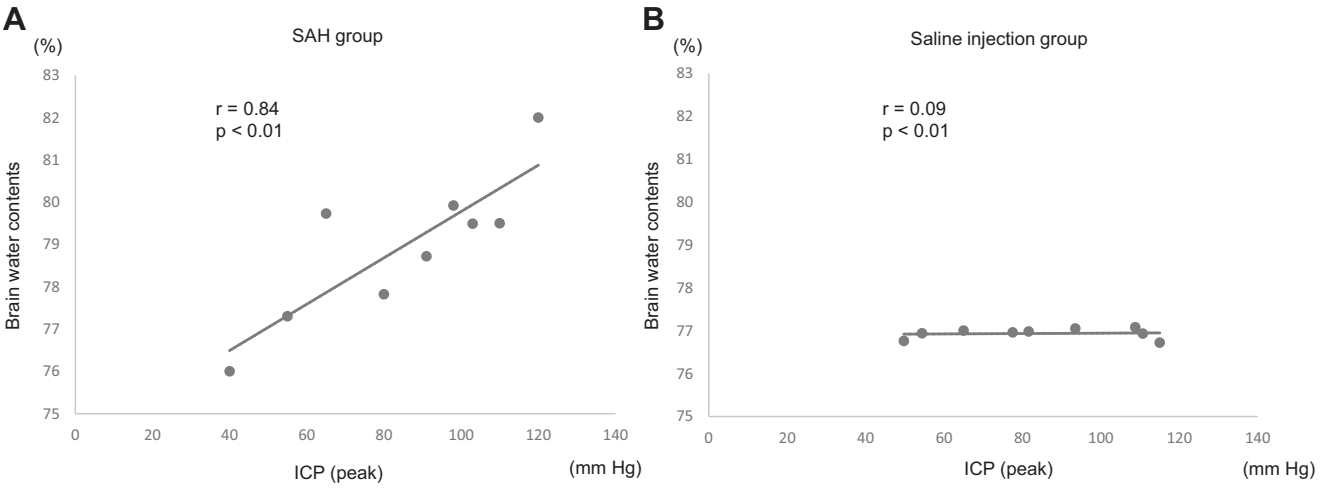


**Figure 5.** The brain water content correlates with the peak of intracranial pressure (ICP) in the subarachnoid hemorrhage group (**A**) and saline injection group (**B**).

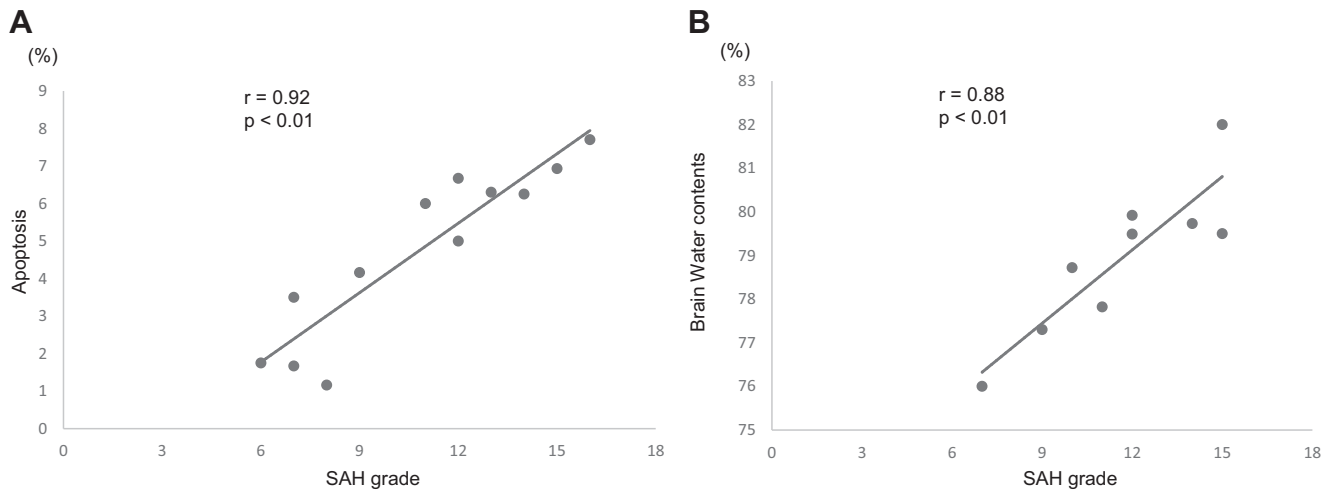


**Figure 6.** The subarachnoid hemorrhage (SAH) grade correlates with the apoptosis (**A**) and brain water content in the subarachnoid hemorrhage group (**B**).

## Role of ICP Increase and Subarachnoid Hematoma on Brain Edema

The association between increased ICP and brain edema has already been made clear in both endovascular perforation and prechiasmatic cisterna injection SAH models.[13,27,35] However, these studies did not mention the influence of SBC on brain edema, although these SAH models naturally replicate the existence of SBC.

It has already been shown that brain water content is not increased in the saline injection model if ICP is not increased.[27] In the present study, brain edema was not observed even under increased ICP in the saline injection model, which suggests that increased ICP is not a main cause of brain edema. On the other hand, brain edema in the SAH model was seen with the same range of ICP as the saline injection model, and a significant correlation was observed between the SAH grade and the degree of edema. Therefore, the main cause of brain edema could be attributable to the existence of SBC.

As for the mechanism by which SBC induce brain edema, inflammatory reaction products such as tumor necrosis factor α, interleukin 1β, and interleukin 6 and reactive oxygen species cascade products such as ZO-1 and MMP 9 are considered to be activated and upregulated by SBC and to cause blood-brain barrier collapse.[28,36,37]

## Influence of Neuronal Apoptosis and Brain Edema on Neurologic Deficits

It has not been decided whether neuronal apoptosis or brain edema is the more important cause of neurologic deficits and outcome, although such a determination would be significant to devising a strategy for the treatment of EBI.

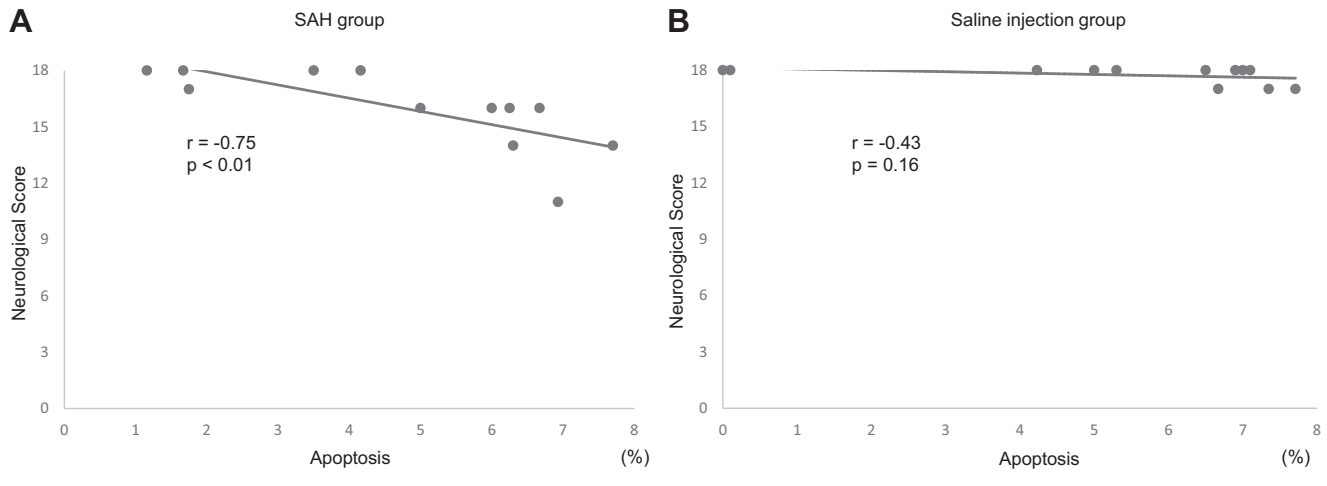


**Figure 7.** The neurologic score correlates with the apoptosis index in the subarachnoid hemorrhage group (**A**), and in the saline injection group (**B**).

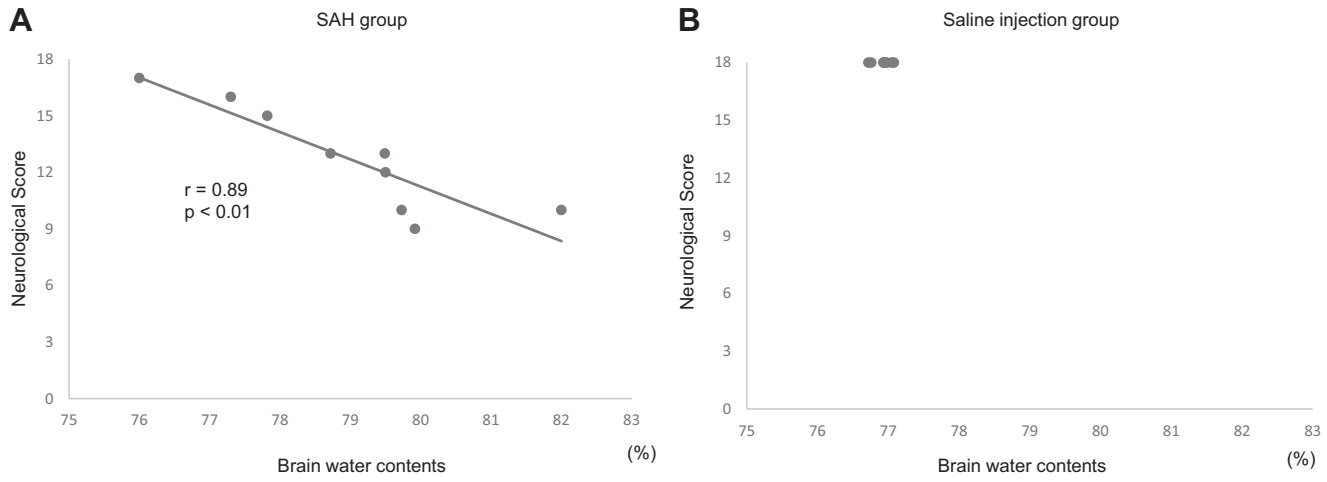


**Figure 8.** The neurologic score correlates with brain water content in the subarachnoid hemorrhage group (**A**) and in the saline injection group (**B**).

Some experimental studies indicated that neurologic deficits such as short working memory and sensorimotor function were seen in rats that showed neuronal apoptosis on histologic examination.[36,38,39] However, the correlation between the degree of neuronal apoptosis and the degree of neurologic deficits has not been examined. Thal et al.[40] reported that the neurologic deficits seen 24 hours after SAH production in the rat endovascular perforation model showed good correlation with increased brain water content, whereas remarkable neuronal apoptosis was not seen. Therefore, whether neuronal apoptosis or brain edema is the main factor causing neurologic impairment has not been clarified.

Several reports showed that the administration of antiapoptotic drugs improves symptoms of EBI with the amelioration of neuronal apoptosis. However, the amelioration of brain edema was also seen with the administration of antiapoptotic drugs in all of these studies.[41-45] Moreover, Park et al. showed that neuronal apoptosis was observed despite an improvement in neurologic deficit by antiapoptotic treatment.[46] In addition, a recent study indicated that serial improvement in neurologic deficits obtained by the administration of antiapoptotic drug was well correlated with serial improvement in brain edema, evaluated using 3. o-Tesla magnetic resonance imaging, in the rabbit endovascular perforation model, whereas it was not correlated with neuronal apoptosis.[47] Therefore, whether the improving effect of antiapoptotic drugs on neurologic deficits is based on amelioration of neuronal apoptosis or brain edema cannot be determined.

In the present study, in the SAH group, the correlation coefficient between neurologic deficits and brain water content was stronger than that between neurologic deficits and neuronal apoptosis. In the saline injection group, neuronal apoptosis showed no correlation with neurologic deficits.

## LIMITATIONS

To evaluate the causative influence of SBC alone on neuronal apoptosis and brain edema, it is desirable to perform an investigation using a model with SBC and without increased ICP. Before carrying out the present study, we tried to produce an SAH model without increased ICP by injecting blood slowly into the cisterna magna. However, it was impossible to produce that model. Therefore, the influence of SBC was derived by analyzing the difference between the results of the SAH group and the results of the saline injection group. However, the influence of SBC alone on apoptosis and brain edema remains a matter of speculation, and this should be clarified in the future after the appropriate model has been established.

In counting the apoptotic index, the counting of the positive cells was performed manually in a double-blinded manner. An average of 6 random photomicrographs of the basal cortex were taken from each animal at the acute stage of SAH; the examination site and time window were limited.

## CONCLUSIONS

At the acute stage of SAH, neuronal apoptosis itself on the basal cortex of the ipsilateral side of puncture in the endovascular SAH model does not seem to cause neurologic damage. For this reason, brain edema is considered a main cause of neurologic deficits, and its negative influence on neurologic status is enhanced by the coexistence of neuronal apoptosis. However, it cannot be determined whether brain edema alone causes neurologic deficit, because there has been no study using a model with brain edema only. The main cause of brain injury should be clarified in the future.

## ACKNOWLEDGMENTS

We thank Mark Inglin (University of Basel) for his editorial assistance.

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*Conflict of interest statement: This work was supported by JSPS KAKENHI grant number JP16K19993.*

*Received 13 January 2019; accepted 2 May 2019*

*Citation: World Neurosurg. (2019) 129:e63-e72.*
*https://doi.org/10.1016/j.wneu.2019.05.009*

*Journal homepage:* ***www.journals.elsevier.com/world-neurosurgery***

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