Effect of Decompressive Craniectomy on Outcome Following Subarachnoid Hemorrhage in Mice

Dominik Bühler, BSc; Sepiede Azghandi, BSc; Kathrin Schüller; Nikolaus Plesnila, MD, PhD

Background and Purpose—Elevated intracranial pressure (ICP) is a key feature of subarachnoid hemorrhage (SAH). Here, we examined the role of elevated ICP in the pathophysiology of SAH, and we investigated whether decreasing ICP by performing decompressive craniectomy (DC) can improve outcome.

Methods—SAH was induced in male C57BL/6 mice via endovascular Circle of Willis perforation in the following 4 groups: sham surgery, SAH, DC after SAH, and DC before SAH. DC was performed either 15 minutes before or after SAH induction. ICP, cerebral blood flow, heart rate, oxygen saturation, and end-tidal Pco2 were monitored for 45 minutes. After surgery, neurological function was evaluated daily for 7 days. After killing, hippocampal neurons, corpus callosum thickness, and ventricular volume were evaluated on paraformaldehyde-fixed coronal brain sections.

Results—Although DC reduced SAH-induced ICP, it yielded no beneficial effect with respect to posthemorrhagic hypoperfusion; moreover, DC increased the incidence of rebleeding, induced more severe neurological impairments, and caused higher mortality. Post SAH, mice that survived 7 days had no histopathologic differences, regardless of whether DC was performed.

Conclusions—Performing DC to reduce ICP either during or acutely after SAH resulted in more severe bleeding, a higher incidence of rebleeding, and poorer outcome. Thus, elevated post-hemorrhagic ICP plays an important role in controlling bleeding after SAH and should therefore not be reduced acutely. If DC is considered for treating a patient with SAH, the timing of decompression should take these effects into consideration. (Stroke. 2015;46:819-826. DOI: 10.1161/STROKEAHA.114.007703.)

Key Words: decompressive craniectomy ■ intracranial pressure ■ mouse model ■ subarachnoid hemorrhage

Subarachnoid hemorrhage (SAH) is a stroke subtype associated with high mortality and morbidity as a result of early and delayed ischemic processes. Nearly one quarter of all patients with SAH die shortly after hemorrhage because of elevated intracranial pressure (ICP) and the resulting global cerebral ischemia.1,2 Hospitalized patients further experience severe complications, including rebleeding, early brain injury, and delayed cerebral ischemia, which together contribute to a devastatingly high 1-month mortality rate of 50%.1,2

Early brain injury is the predominant cause of death after SAH and is characterized by elevated ICP, decreased cerebral blood flow (CBF), and global cerebral ischemia resulting in secondary injuries, including disruption of the blood–brain barrier, inflammation, and oxidative stress, which all cause neuronal cell death.3–5 In both animal and clinical studies, the severity of bleeding and the extent of decreased CBF correlated with neurological outcome6–8; however, how and to what extent the initial global ischemia or subsequent elevated ICP contribute to early brain injury remains poorly understood.3–5

A feasible way to reduce elevated ICP is decompressive craniectomy (DC), a technique that dates back more than a century.9 In recent years, both animal and clinical studies reported beneficial effects of DC for treating conditions, such as traumatic brain injury10,11 and malignant middle cerebral artery (MCA) infarction.12,13 With respect to SAH, however, relatively few studies have been published regarding the application of DC and its effect on outcome; moreover, the results published to date are contradictory and controversial.14–18

To address these questions, we performed DC to evaluate the role of SAH-induced elevated ICP and subsequent global ischemia and to investigate whether DC may serve as a therapeutic option for SAH using a standardized animal model.

Methods

In total, 41 male C57BL/6 mice (22–25 g; Charles River Laboratories, Sulzfeld, Germany) were used. All experiments were approved by the Government of Upper Bavaria (protocol number 55.2.1.54-2532-90-13) and were performed in accordance with standard ethical guidelines.

Experimental Design

Initially, 40 animals were assigned randomly to the following 4 experimental groups: sham surgery, SAH, DC after SAH, and DC before SAH (Figure 1). All assessments were performed by an
Decompressive Craniectomy
DC was performed either before or 15 minutes after SAH induction (Figure 1). To achieve a sufficient reduction in ICP, the skull bone was removed over both hemispheres (left, 8×4 mm; right, 4×4 mm; Figure 1) using a high-speed drill (Labset Uni-Drive N, Paggen, Starnberg, Germany) under continuous cooling with saline. Special attention was paid to leave the dura mater intact.

Endovascular Perforation Model for SAH Induction
The endovascular perforation model for inducing SAH was performed as previously described.19–21 In brief, the neck was opened by a midline incision, and the left common carotid artery was exposed. A 5-0 monofilament was introduced into the internal carotid artery via the external carotid artery and advanced toward the Circle of Willis. To induce SAH, the vessel wall was perforated close to the bifurcation between the anterior cerebral artery and the MCA; successful induction was confirmed by a sudden steep increase in ICP and a concomitant decrease in CBF. Then, the filament was withdrawn and the external carotid artery was ligated. In sham-operated animals, the monofilament was introduced into the internal carotid artery, but without vessel perforation. Rebleeding events resulted in an additional sudden increase in ICP above 25 mmHg.

Postoperative Care
At the end of surgery, the probes were removed and both the neck incision and the skin above the craniectomy were carefully closed. Anesthesia was terminated by a subcutaneous injection containing atipamezol (2.5 mg/kg), flumazenil (0.5 mg/kg), and naloxone (1.2 mg/kg). To prevent hypothermia, the animals were housed in an incubator with an ambient temperature of 30°C for 24 hours after surgery. For a period of 7 days, the mice were observed and received daily subcutaneous injections of carprofen (4 mg/kg) and 0.2 mL saline.

Neurological Evaluation and Body Weight
Daily neurological examinations were performed beginning 2 days before surgery until 7 days after surgery using a global SAH neuroscore (see Table 1 in the online-only Data Supplement; adapted from Bühlert et al.)21 Mice received a score ranging from 0 (no deficit) to 31 (severe neurological deficits). The tests were performed at approximately the same time each day (evening) to avoid any potential effect of circadian rhythm.

In addition, body weight was measured daily as a sensitive indicator of general well-being.

Tissue Harvesting and Histology
Seven days after surgery, the animals were euthanized under anesthesia by transcardial perfusion with 4% paraformaldehyde. The brains were harvested and postfixed in 4% paraformaldehyde for 24 hours. Coronal sections (4 μm) were stained with cresyl violet for subsequent histological analysis.19–21

Evaluation of Hydrocephalus and White Matter Damage
To measure hydrocephalus, 2 sections (100 μm apart, at Bregma +1 mm) were imaged, and the area of both ventricles, as well as total brain area, was measured using AxioVision software (Zeiss, Jena, Germany). Results are expressed as ventricle area divided by total brain area (ie, relative ventricle area).

To assess white matter damage, the most dorsal point (turning point) of the corpus callosum on both hemispheres (at Bregma +1 mm) was identified, and the perpendicular extent of the corpus callosum was measured. Results are expressed as the mean of 2 sections.

Quantification of Neuronal Damage
To quantify neuronal damage, sections between Bregma −1.6 mm and −2 mm were imaged. Regions of interest (0.3×0.2 mm²) were

Anesthesia and Monitoring
Mice were anesthetized and monitored as previously described.19–21 Anesthesia was induced using 4% isoflurane inhalation followed by an intraperitoneal injection of fentanyl (0.05 mg/kg), midazolam (5 mg/kg), and medetomidine (0.5 mg/kg); anesthesia was maintained by hourly injections containing one-third of the initial dose of anesthetics. After induction, mice were intubated and mechanically ventilated (Minivent, Hugo Sachs, March-Hugstetten, Germany) with a 70%/30% air/oxygen gas mixture, and end-tidal Pco2 was measured using microcapnometry (CI240, Columbus Instruments, Columbus, OH). A thermostatically regulated feedback-controlled heating pad (4 mg/kg SC).
selected in the CA1, CA2, and CA3 regions of the hippocampus, and viable pyramidal neurons were counted as previously described.\textsuperscript{19–21} Neurons were counted in 3 sections (at 50-μm intervals), and the results are expressed as the mean of these 3 sections.

\section*{Statistical Analysis}

Statistical analysis was performed using SigmaPlot 12.5 (Systat Software Inc., San Jose, CA) and Excel 2010 (Microsoft Corporation, Redmond, WA). The data were analyzed using either the Mann–Whitney test (for pairwise comparisons) or the Kruskal–Wallis 1-way analysis of variance on ranks followed by Dunn’s method as a post hoc test. Paired data were analyzed using the Wilcoxon signed-rank test. Mortality was analyzed using the LogRank test. Differences were considered statistically significant at $P < 0.05$. Except where indicated otherwise, all data are expressed as mean±SEM.

\section*{Results}

\subsection*{Physiological Monitoring}

SAH induction caused an immediate increase in ICP (from a baseline of 5 mm Hg to >50 mm Hg; $P < 0.001$ versus sham; Figure 2A). The 3 SAH groups did not differ significantly with respect to post-SAH ICP, even though 1 group of animals had a DC before SAH induction (58±3 mm Hg for SAH, 55±3 mm Hg for DC after SAH, and 51±5 mm Hg for DC before SAH; $P = 0.49$). After 15 minutes, ICP had decreased similarly among the 3 SAH groups (20±2 mm Hg for SAH, 18±1 mm Hg for DC after SAH, and 20±1 mm Hg for DC before SAH; $P = 0.63$). The time interval between 15 and 30 minutes after SAH was used to perform a craniectomy in the DC after SAH group, which led to a significant reduction in ICP (18±1 mm Hg at 15 minutes, 8±1 mm Hg at 30 minutes, and 10±1 mm Hg at 45 minutes; $P < 0.001$; Figure 2B). A similar time course was observed in the DC before SAH group (20±1 mm Hg at 15 minutes, 11±2 mm Hg at 30 minutes, and 11±2 mm Hg at 45 minutes; $P < 0.05$). Forty-five minutes after SAH, ICP was significantly lower in both the DC after SAH and the DC before SAH groups compared with the SAH group (17±2 mm Hg for SAH, 10±1 mm Hg for DC after SAH, and 11±2 mm Hg for DC before SAH; $P < 0.01$; Figure 2A and 2B).

SAH induction also caused a dramatic concomitant decrease in ipsilateral CBF to <20% of baseline ($P < 0.001$ versus sham; Figure 2C). All 3 SAH groups had a similar initial drop in CBF; however, 15 minutes after SAH, the DC

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Intraoperative monitoring of intracranial pressure (ICP) and cerebral blood flow (CBF). Continuous ICP and CBF recordings (A, C) revealed that subarachnoid hemorrhage (SAH) induced a strong increase in ICP (to ≈60 mm Hg), which led to transient global cerebral ischemia. ICP then decreased and CBF recovered. Both decompressive craniectomy (DC) groups had a significant reduction in ICP (B) but no improvement in CBF (D). Data from the DC before SAH group revealed that an open skull at the time of SAH induction does not prevent neither intracranial hypertension nor an hypoperfusion (A, C).}
\end{figure}
neuroscore values) than the mice in the SAH group (4±1 for sham, 8±2 for SAH, 12±2 for DC after SAH, 18±7 for DC before SAH; P<0.05; Figure 4A). This difference in scores remained significant 5 days after surgery.

Rebleeding
The mice in the DC before SAH group had a higher incidence of rebleeding (80%) compared with the SAH group (50%) and the DC after SAH group (40%; Figure 3A). In addition, these mice also showed a higher rate of rebleedings per animal (0.5±0.2 for SAH, 0.4±0.2 for DC after SAH, and 1.4±0.5 for DC before SAH; Figure 3B).

Neuroscore and Body Weight
Evaluation of daily neuroscore values revealed the most severe deficits on the first day after SAH induction; moreover, mice that underwent DC performed significantly poorer (ie, higher

Figure 3. Incidence of rebleeding. A, The mice in the decompressive craniectomy (DC) before subarachnoid hemorrhage (SAH) group had a higher incidence of rebleeding than the mice in the DC after SAH or SAH groups. B, Mice with DC before SAH had a higher rate of rebleedings per animal.

Figure 4. Functional outcome. A, All groups with subarachnoid hemorrhage (SAH) had a significantly worse neuroscore (best score, 0; worst score, 31; see Table I in the online-only Data Supplement) on the first day after surgery, and all groups recovered gradually. The mice with decompressive craniectomy (DC) had significantly worse neuroscores in the first 5 days compared with the SAH group. B, All groups had a considerable decrease in body weight in the first 3 days after surgery. The mice in the DC before SAH group and the DC after SAH group had significantly more weight loss and recovered more slowly than the Sham and SAH group. C, All mice with DC before SAH died within the first 7 days after hemorrhage.
A loss in body weight could be seen in all 4 study groups; however, the mice in the 2 groups that underwent DC had the largest decrease (Figure 4B). In addition, these 2 groups recovered their body weight significantly more slowly than the sham and SAH groups.

Mortality
We also observed a significant difference in mortality rate between study groups, showing higher mortality for mice with DC (P<0.001; Figure 4C). In the DC before SAH group, no mouse survived until day 7, and some animals developed massive ventricular protrusions that resulted in the rupture of the dura mater and prolapsed brain parenchyma through the craniectomy (see Figure IV in the online-only Data Supplement). Therefore, because of ethical considerations and regulatory issues, only 5 animals were used in this group. In addition, because all 5 mice in this group died before the 7-day end point, no histomorphometry or histopathology data were available for this group.

Histomorphometry
Seven days after surgery, ventricle size was determined in coronal sections as a measure of hydrocephalus (1.2%±0.1% for sham, 2.5%±0.3% for SAH; P<0.001; Figure 5A). Performing DC after SAH had no significant effect on relative ventricle area (2.5%±0.3% for SAH, 2.3%±0.3% for DC after SAH).

The corpus callosum was significantly thinner in the ipsilateral hemisphere compared with the contralateral hemisphere in both the SAH and DC after SAH groups (Figure 5B). This difference between the ipsilateral and contralateral hemispheres in the SAH groups was also reflected by a significant decrease in the ipsilateral/contralateral thickness ratio (1.00±0.01 for sham, 0.88±0.01 for SAH, and 0.91±0.04 for DC after SAH; P<0.01; Figure 5C). There was no difference between the SAH group and the DC after SAH group.

Histopathology
There was a significant loss of viable pyramidal neurons in all regions of interest 7 days after SAH (Figure 6). DC had no significant effect on neuronal survival, but there was a tendency toward increased neuronal loss in all 4 regions studied in the ipsilateral hippocampus.

Discussion
Performing DC—either before or after SAH induction—reduced ICP significantly; however, the mice in the DC groups experienced a higher incidence of rebleeding, poorer functional outcome, and increased mortality. In comparing...
the SAH group with the DC after SAH group, we were surprised to find that the CBF values were not affected by DC, despite the DC-induced reduction in ICP. This finding suggests that within minutes after SAH, cerebral hypoperfusion is not dependent on ICP, but is dependent on spasms in cerebral microcirculation, which supports recent findings by our group and others.22,23

The experiments in this study were performed in intubated and ventilated mice, with all relevant physiological parameters monitored. This experimental set-up enabled us to detect, control, and therefore exclude any potential systemic artifacts in our animals. A second advantage of our experimental approach is our use of an SAH model in which hemorrhage is induced by vascular perforation; only this method revealed that DC caused rebleeding that led to a high mortality rate and poor functional outcome. Using a different SAH model (eg, injecting a predetermined volume of blood) would not have revealed these results because in these models rebleeding does not occur.

One goal of our study—specifically, to clarify the role of early ICP-induced global cerebral ischemia in brain damage after SAH—could not be achieved. The primary reason why we did not reach this goal was that both end points required for determining such a correlation were not reached. First, the mice maximally decompressed before SAH developed post-hemorrhagic intracranial hypertension that was on par with mice that were not decompressed—indicating more severe hemorrhage leading to a larger hematoma in decompressed mice; second, none of these animals survived to the end of the 7-day observation period. Nevertheless, rather than answering our initial question, these experiments revealed 2 other important points. First, in addition to its detrimental effect (in terms of rapidly inducing global ischemia), the initial peak in ICP after SAH has also a beneficial aspect because it helps stop bleeding from the injured vessel and helps prevent rebleeding. These findings support the concept of a brain tamponade24 in which high ICP stops post-SAH bleeding, and these results are consistent with previous findings from our laboratory, which show that preventing rebleeding is highly beneficial after experimental SAH.25 The second important point revealed by our results is that reduced ICP does not improve CBF. This ICP-independent reduction in CBF might reflect the early onset of microvasospasm22,23 and may account for the similar or worse pathophysiological findings in our study—in accordance with early brain injury.

Because our animal model recapitulates the predominant secondary pathologies after SAH, we were able to investigate further effects of DC on functional outcome. Performing established neurological examinations26 revealed that DC is associated with aggravated functional outcome, which is also reflected by the more affected body weight changes. In addition to hydrocephalus formation (as a result of reduced reabsorption of cerebrospinal fluid),3 several studies have reported hippocampal damage as a sensitive marker for neuronal damage in this model.19,26 In our study, the mice in the DC after SAH group did not have significantly worse damage than the SAH group, but they showed a tendency toward more neuronal cell death in the ipsilateral hippocampus.

White matter injury associated with SAH is a relatively poorly investigated field that has only recently begun to be addressed in more detail.27,28 In our study, we found histological changes 7 days after SAH, and comparison of corpus callosum thickness of both hemispheres indicated white matter thinning ipsilateral to the SAH induction site. Similar observations were reported recently in a rat model.27 One possible explanation for this lateralization effect may be the presence of a more pronounced vasospasm near the initial bleeding site.29

Many studies have investigated the role of DC in traumatic brain injury10,11 and malignant MCA infarction,12,13 and the
beneficial effects of DC in malignant MCA infarction have led to the recommendation of including DC in current treatment guidelines. However, only a few case reports describe the application of DC in SAH patients, and the results of these reports are controversial because of different time points and indications for DC. For example, in patients with intractable intracranial hypertension or signs of brain swelling, DC can have a beneficial effect on functional outcome, whereas DC has no benefit in cases of delayed cerebral ischemia. However, when looking in further detail at long-term quality of life rather than survival rate, the positive effects seem to be diminished by a reduced overall quality of life in craniectomy-ized patients, which is consistent with our results, despite the early, clinically not relevant time points of DC in the current study. Therefore, randomized controlled clinical trials are urgently needed to clarify the indication and consequences of DC in SAH patients.

In summary, although performing DC either before or after SAH effectively reduced ICP, it did not induce an overall beneficial effect. In contrast, decompression before SAH resulted in a higher incidence of rebleeding and a higher mortality rate. Therefore, because posthemorrhagic ICP elevation seems to play an important role in the cessation of bleeding, it should not be reduced directly after SAH. Mechanistically, our data suggest that the hypoperfusion observed directly after SAH is not caused by elevated ICP, but is likely caused by microcirculatory dysfunction. This hypothesis is based on the finding that DC did not affect posthemorrhagic hypoperfusion, despite reducing ICP. Taken together, our results reveal that DC does not have a net beneficial effect when performed early after SAH. Therefore, clinical evaluations should pay particular attention to both the degree of bleeding and the timing of decompression.

Acknowledgments
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Disclosures
None.

References


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Dominik Bühler, BSc¹; Sepiede Azghandi, BSc¹; Kathrin Schüller¹;
Nikolaus Plesnila, MD, PhD¹ ²

¹ Laboratory of Experimental Stroke Research, Institute for Stroke and Dementia Research,
University of Munich Medical Center, Munich, Germany
² Munich Cluster for Systems Neurology (Synergy), Munich, Germany

Address for correspondence:
Prof. Nikolaus Plesnila
Institute for Stroke and Dementia Research (ISD)
Max-Lebsche-Platz 30, 81377 Munich, Germany
Phone: +49 (0)89 4400 46 219
Fax: +49 (0)89 4400 46113
E-mail: nikolaus.plesnila@med.uni-muenchen.de
### SUPPLEMENTAL TABLES

**Table I.** Neuroscore for SAH (adapted from Bühler et al.¹)

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<th>Task</th>
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<td></td>
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<td></td>
<td>No movements (comatose)</td>
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<tr>
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<tr>
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<td></td>
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</tr>
<tr>
<td></td>
<td>Absent</td>
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<tr>
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<td></td>
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<tr>
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<td><strong>Beam walk (3cm, 2cm, 1cm)</strong></td>
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¹ Front paws reaching ground when lifted by tail
SUPPLEMENTAL FIGURES

FIGURE I

Figure I. Effects of DC in sham-operated animals on physiological and functional outcome. During surgery, there were no major alterations in ICP (A) or CBF (B). DC had also no influence on neurological outcome (C) or body weight recovery (D) after sham-surgery.
**Figure II.** Histological evaluation of DC in sham-operated animals seven days after surgery. DC had no influence on the number of hippocampal pyramidal neurons (A). There were also no alterations in corpus callosum thickness (B) or in relative ventricle area (C).
**Figure III.** Physiological monitoring during surgery. A, Cushing response to elevated ICP resulted in a drop in heart rate. The oxygen saturation (B), respiratory rate (C), as well as the end-tidal CO₂ (D) were in comparable ranges between the groups and constant during the whole surgery.
Figure IV. Craniectomy sites and outcome after SAH induction. Top left, Craniectomy sites and probe positions for physiological monitoring are indicated. Top right, Craniectomy (white dashed line) above both hemispheres without opening the dura mater. The skull above the superior sagittal sinus was left intact. Bottom left, In animals with DC after SAH there was a slight protrusion of brain parenchyma through the craniectomy due to increased ICP (white dashed line). Bottom right, In the DC before SAH group the dura mater could sometimes not withstand the high ICP and ruptured (red dashed line). ICP = intracranial pressure; LDF = laser Doppler flowmeter
SUPPLEMENTAL REFERENCES