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Rolle der intrakraniellen Hypertension für den sekundären Hirnschaden nach Subarachnoidalblutung

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München, den 17.07.2017

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Abkürzungsverzeichnis

BBB	Blut-Hirn-Schranke (engl. *blood-brain barrier*)
CBF	Zerebrale Durchblutung (engl. *cerebral blood flow*)
CPP	Zerebraler Perfusionsdruck (engl. *cerebral perfusion pressure*)
CSF	Liquor cerebrospinalis (engl. *cerebrospinal fluid*)
CT	Computertomographie
DC	Dekompressive Kraniektomie (engl. *decompressive craniectomy*)
EBI	Früher Hirnschaden (engl. *early brain injury*)
GCS	Glasgow Coma Scale
ICP	Intrakranieller Druck (engl. *intracranial pressure*)
MRT	Magnetresonanztomographie
ROS	Reaktive Sauerstoffspezies (engl. *reactive oxygen species*)
SAB / SAH	Subarachnoidalblutung (engl. *subarachnoid hemorrhage*)
SAS	Subarachnoidalraum (engl. *subarachnoid space*)
WFNS	World Federation of Neurosurgical Societies

Studientitel

CONSCIOUS-2	**C**lazosentan to **O**vercome **N**eurological i**SC**hemia and **I**nfarct **O**cc**U**rring after **S**ubarachnoid hemorrhage - Phase 2
DECRA	**DEC**ompressive **CRA**niectomy Trial
RESCUEicp	**R**andomised **E**valuation of **S**urgery with **C**raniectomy for **U**ncontrollable **E**levation of **I**ntra-**C**ranial **P**ressure

Publikationsliste

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Weitere Publikationen im Rahmen der Arbeit

A Murine Model of Subarachnoid Hemorrhage

Kathrin Schüller, **Dominik Bühler**, Nikolaus Plesnila
Journal of Visualized Experiments, November 2013, Issue 81, e50845

Are we barking up the wrong vessels?
The cerebral microcirculation after subarachnoid hemorrhage

Nicole Terpolilli, Christian Brem, **Dominik Bühler**, Nikolaus Plesnila
Stroke, October 2015, Volume 46, Issue 10, pp 3014-3019

Konferenzbeiträge

Vorträge

Involvement of NADPH oxidase after SAH

Dominik Bühler, Nikolaus Plesnila
Advisory Board Meeting of the Institute for Stroke and Dementia Research, University of Munich Medical Center (München, 06. August 2013)

Bedeutung der NADPH-Oxidase nach experimenteller Subarachnoidalblutung (SAB) bei der Maus

Dominik Bühler, Nikolaus Plesnila
Statusseminar des Promotionsstudiums "Molekulare Medizin" und "Systembiologische Medizin" im Rahmen des Förderprogramm für Forschung und Lehre der LMU München (Herrsching, 9. - 11. Mai 2014)

Decompressive Craniectomy after Subarachnoid Hemorrhage – experimental results in mice

Dominik Bühler, Sepiede Azghandi, Kathrin Schüller, Nikolaus Plesnila
40. Jahrestagung der Sektion Intrakranieller Druck, Hirndurchblutung und Hydrozephalus der Deutschen Gesellschaft für Neurochirurgie (München, 07. - 08. November 2014)

Poster

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Dominik Bühler, Sepiede Azghandi, Kathrin Schüller, Nikolaus Plesnila
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Bestätigung der Ko-Autoren

Die Autoren leisteten folgende Arbeitsanteile zu den Publikationen.

Protocol for the Induction of Subarachnoid Hemorrhage in Mice by Perforation of the Circle of Willis with an Endovascular Filament

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Schreiben des Manuskripts:	DB, KS und NP

Effect of Decompressive Craniectomy on Outcome Following Subarachnoid Hemorrhage in Mice

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Datenakquisition:	DB und SA
Datenanalyse:	DB und KS
Schreiben des Manuskripts:	DB und NP

Hiermit bestätige ich die obigen Angaben zu den Arbeitsanteilen aller Ko-Autoren.

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Dominik Bühler

München, den 17.07.2017

Prof. Dr. med. Nikolaus Plesnila

1 Einleitung

Die Folgen einer Schlaganfallerkrankung sind die häufigsten Ursachen für bleibende neurologische Defizite in westlichen Industrieländern.[1] Darüber hinaus sind Schlaganfälle nach kardiovaskulären und onkologischen Erkrankungen die dritthäufigste Todesursache weltweit.[2] Neben einer ischämischen Mangelversorgung des Gehirns als Hauptursache, sind in 15% der Fälle zerebrale Blutungen Auslöser für das Auftreten eines Schlaganfalls.[3,4] Unter den verschiedenen Ursachen einer zerebralen Blutung hat die Subarachnoidalblutung (SAB) eine der schlechtesten Prognosen.[5] Bei einer SAB kommt es durch Ruptur eines zerebralen Aneurysmas zum Austritt von Blut in den äußeren Liquorraum und in der Folge zu einem dauerhaft erhöhten intrakraniellen Druck (ICP, engl. *intracranial pressure*) und einer gestörten zerebralen Durchblutung (CBF, engl. *cerebral blood flow*), die zu einer ischämischen Schädigung des Gehirns führen.[6] Allerdings sind die zugrundeliegenden Pathomechanismen dieser Durchblutungsstörung noch nicht vollständig verstanden, weshalb bis heute keine kausalen Therapien zur Prävention und Reduktion von Spätkomplikationen nach SAB zur Verfügung stehen.[6]

1.1 Epidemiologie und Ätiologie

Die Subarachnoidalblutung weist eine jährliche Inzidenz von 6-10 Personen pro 100.000 Einwohner auf.[5,7] Somit ist die SAB ursächlich für 3-4% aller Schlaganfallpatienten.[5,7] Da jedoch der Altersgipfel mit Mitte 50 deutlich unter dem des ischämischen Schlaganfalls liegt, sind überwiegend berufstätige Personen betroffen.[8,9] Daher sind die kumulativen sozio-ökonomische Ausgaben für Patienten nach überlebter SAB in etwa vergleichbar mit denen für Patienten nach ischämischen Schlaganfällen - obwohl diese eine zwanzigfach höhere Prävalenz aufweisen.[8,10]

In 85% der Fälle ist eine spontane Ruptur eines zerebralen Aneurysmas - einer dünnwandigen Ausstülpung der Gefäßwand - Ursache für eine SAB.[5] Solche Aneurysmen können angeboren sein oder sich im Laufe des Lebens ausbilden. Risikofaktoren hierfür sind eine familiäre Disposition, Hypertonie, Rauchen oder eine Bindegewebsschwäche.[7] Typische Lokalisationen befinden sich in der Nähe des Circulus arteriosus Willisii, einem arteriellen Gefäßring an der Gehirnbasis bestehend aus Anastomosen der großen hirnversorgenden Arterien.[11] Zu den selteneren Ursachen einer SAB zählen traumatische Ereignisse, arterio-venöse Malformationen oder Vaskulitiden.[12]

1.2 Klinisches Bild

Bei einer Subarachnoidalblutung kommt es zum Austritt von Blut in den Subarachnoidalraum, d.h. in den äußeren Liquorraum, und somit zum direkten Kontakt von Blutbestandteilen mit den Meningen, u.a. der Dura mater (Abbildung 1). Dies verursacht eine Reizung der Dura, welche von 80% der Patienten als ein plötzlich einsetzender Vernichtungskopfschmerz empfunden wird (Abbildung 1).[7,13] Außerdem bewirkt der Masseneffekt des Hämatoms einen Anstieg des intrakraniellen Drucks (ICP), welcher zu einer Abnahme des zerebralen Perfusionsdrucks und zu einer Minderperfusion des Gehirns führt.[6] Etwa jeder zweite SAB Patient erleidet initial einen kurzfristigen Bewusstseinsverlust und 14% sind beim Eintreffen in der Klinik noch komatös.[13,14] Bis zu 21% aller SAB Patienten versterben bereits innerhalb der ersten 24 Stunden, 12% sogar bevor sie medizinisch versorgt werden.[15,16] Während des ersten Monats verstirbt ein weiteres Drittel der Patienten an den Spätfolgen der SAB oder auf Grund von Rezidivblutungen.[15,16] Dadurch ergibt sich eine hohe 30-Tages-Mortalität von etwa 44%, welche sich in den letzten 30 Jahren trotz großer Fortschritte der Notfall- und Intensivmedizin nur langsam verbessern ließ.[15,17]

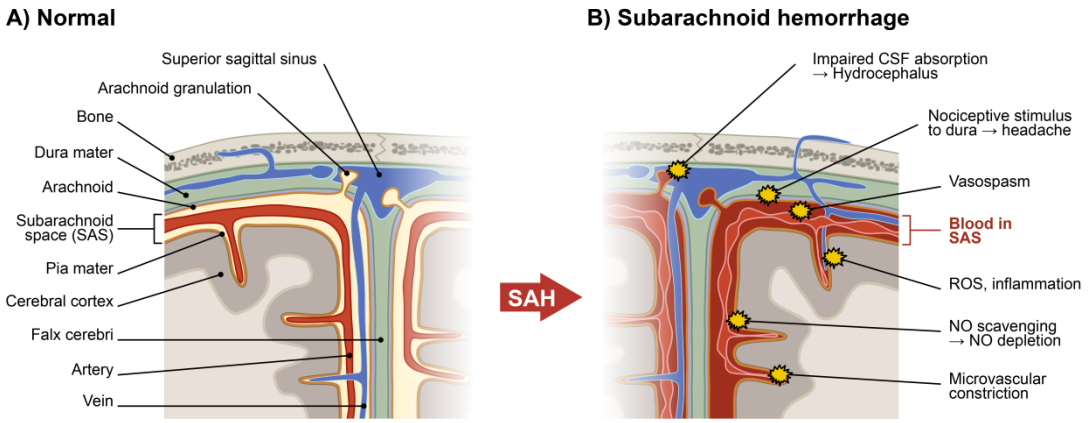

Abbildung 1. Pathologien nach Subarachnoidalblutung. Diese schematische Darstellung für den Bereich des Interhemisphärenspalts zeigt die Verteilung des extravasierten Blutes im Subarachnoidalraum. Darüber hinaus sind verschiedene Pathologien dargestellt, die sich infolge einer Subarachnoidalblutung entwickeln. (Abbildung publiziert in Terpolilli et al.[6])
CSF = Liquor cerebrospinalis; NO = Stickstoffmonoxid; ROS = Reaktive Sauerstoffspezies; SAH = Subarachnoidalblutung; SAS = Subarachnoidalraum

Wird das initiale Blutungsereignis überlebt, leiden auf Grund des erhöhten ICPs in der Akutphase 77% der Patienten unter Übelkeit und Erbrechen, bei 35% kommt es zur Ausbildung eines Meningismus und bei 9% lassen sich Defizite der Hirnnerven nachweisen.[13,14,18] Bis zu 26% der Patienten erleiden fokale oder generalisierte epileptische Anfälle.[7,13] Weitere Befunde sind Einblutungen in den Glaskörper (Terson-Syndrom, ca. jeder vierte Patient) und kardiale Komplikationen bis hin zum Herzstillstand (ca. 4% aller Patienten).[7,19]

Rezidivblutungen sind eine weitere gefürchtete Komplikation.[5] Innerhalb der ersten 24 Stunden erleiden knapp 14% aller Patienten eine Rezidivblutung, welche mit einer höheren Mortalitätsrate und einem schlechteren klinischen Outcome verbunden ist.[13,20] Außerdem entwickelt sich bei 53% der Patienten mit SAB auf Basis einer verminderten Liquorrückresorption an den Meningen ein akuter Hydrozephalus innerhalb der ersten drei Tage nach der Blutung (Abbildung 1).[21]

In dem Zeitraum zwischen 4 bis 14 Tagen nach einer SAB besteht ein hohes Risiko für die Ausbildung von Spasmen der großen hirnversorgenden Gefäße (Abbildung 1), die zu einer zerebralen Minderperfusion und ischämischen Hirnschädigungen führen.[22] Bei etwa 34% der Patienten tritt solch eine verzögerte zerebrale Ischämie auf, welche sich in Form von fokal-neurologischen Ausfällen oder reduziertem Bewusstsein äußert.[22]

Für die initiale Beurteilung einer SAB wurden mehrere Klassifikationen entwickelt, welche in der Notfalldiagnostik schnell zu erheben sind und dabei gut mit dem weiteren Krankheitsverlauf korrelieren.[5] Eine immer noch weit verbreitete Einteilung wurde 1968 von Hunt und Hess eingeführt und umfasst 5 Schweregrade (Tabelle 1).[23] 1988 wurde von der World Federation of Neurological Surgeons (WFNS) eine neue Einteilung vorgeschlagen, die ebenfalls in 5 Grade abgestuft ist, sich aber mehr am Glasgow Coma Scale (GCS) orientiert (Tabelle 1).[24]

Tabelle 1. Klinische Schweregradklassifikation nach Hunt und Hess[23] bzw. WFNS (World Federation of Neurological Surgeons)[24]

Grad	Hunt und Hess	WFNS
I	Asymptomatisch oder leichter Kopfschmerz und leichter Meningismus	GCS 15 ohne fokales Defizit
II	Mäßige bis starke Kopfschmerzen, Meningismus, evtl. Hirnnervenausfälle	GCS 13-14 ohne fokales Defizit
III	Somnolenz, Verwirrtheit oder leichte fokal-neurologische Ausfälle	GCS 13-14 mit fokalem Defizit
IV	Sopor, mäßige bis schwere fokal-neurologische Ausfälle, evtl. Streckphänomene und vegetative Störungen	GCS 7-12 ohne/mit fokalem Defizit
V	Tiefes Koma, Zeichen der Einklemmung	GCS <7 ohne/mit fokalem Defizit

In der SAB Diagnostik nimmt die Computertomographie eine zentrale Rolle ein, da die Sensitivität innerhalb der ersten drei Tage bei nahezu 100% liegt.[13] Außerdem lässt sich auf den CT-Aufnahmen das Blutungsausmaß sowie das Vorhandensein von intraparenchymatösen Hämatomen beurteilen, welche zum einen das Auftreten von Vasospasmen begünstigen und zum anderen das spätere neurologische Outcome der Patienten verschlechtern.[13,25] Darüber hinaus ist die Bestimmung der Blutungsquelle wichtig bei der Therapieplanung, da je nach Lokalisation und Konfiguration des Aneurysmas ein bestimmtes operatives Verfahren besser geeignet ist.[13,26] Auf die Unterschiede der möglichen Therapieverfahren wird im Kapitel 1.5 eingegangen. Im späteren Krankheitsverlauf kann mittels nicht-invasiver transkranieller Dopplersonographie die zerebrale Durchblutung kontrolliert und die Ausbildung von Vasospasmen überwacht werden.[27]

1.3 Pathophysiologie

Die pathophysiologischen Vorgänge, die nach einer Subarachnoidalblutung auftreten, sind komplex und bis heute nur unzureichend verstanden.[6] Bis vor kurzem hatten sich die Erforschung der Pathomechanismen und die Erarbeitung potenzieller Therapiestrategien vornehmlich auf die verzögerten ischämisch-neurologischen Defizite auf Grund von Vasospasmen der großen Arterien konzentriert, welche 4 bis 14 Tage nach SAB auftreten.[28] Jedoch konnte bisher keine Studie, die bei den verzögerten Vasospasmen ansetzt, den Krankheitsverlauf wesentlich verbessern.[28] Jüngstes Beispiel hierfür ist die CONSCIOUS-2 Studie, eine randomisierte, multizentrische, internationale Phase III Studie, bei der Clazosentan, ein Antagonist am vasokonstriktorisch wirkenden Endothelin-1-Rezeptor, zur Prophylaxe des späten Vasopasmus eingesetzt wurde.[29] Die Hemmung des Endothelin-1-Rezeptors verhinderte diesen späten Vasospasmus nach SAB, verbesserte aber weder das Überleben noch das neurologische Outcome der Patienten.

Daraus wurde gefolgert, dass der späte Vasospasmus keine kausale Rolle für die Entwicklung des sekundären Hirnschadens nach SAB spielt und daher andere Mechanismen involviert sein müssen. So wurden vor allem in experimentellen Studien der letzten Jahre verstärkt die Akutphase nach SAB und die früh auftretenden Prozesse innerhalb der ersten 48 Stunden untersucht, wie z.B. eine intrakranielle Hypertonie, eine Störung der Mikrozirkulation oder die Ausbildung eines Hirnödems.[6,30] Diese Vorgänge werden unter dem Begriff „Early Brain Injury“ (EBI, *früher Hirnschaden*) zusammengefasst und sind mitverantwortlich für den Untergang von Hirnparenchym.[30,31] Aus den bisher gewonnen Einsichten in diese Prozesse lassen sich Zusammenhänge zwischen den einzelnen Pathologien ableiten (Abbildung 2).[6]

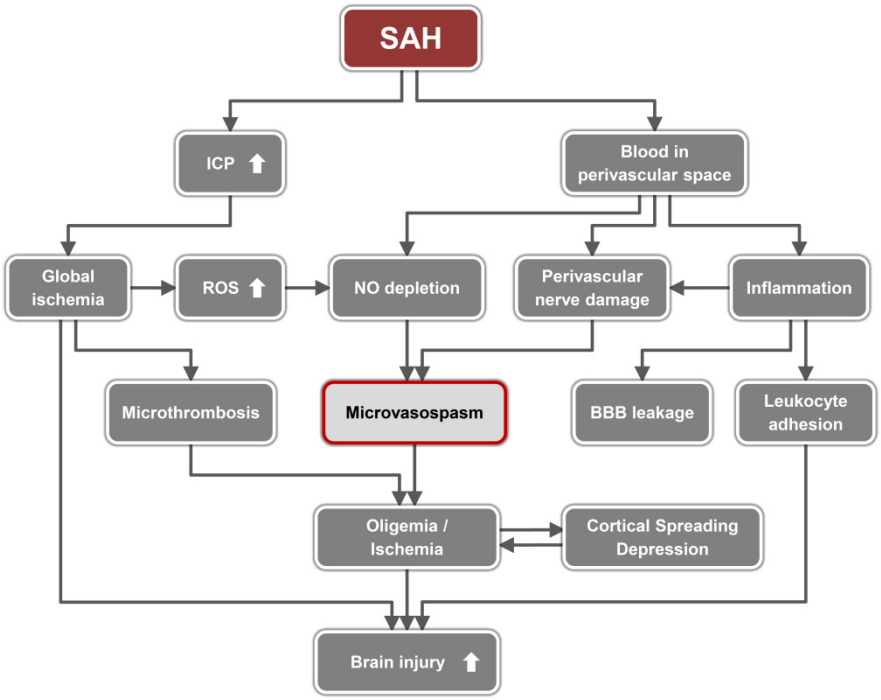

Abbildung 2. Pathophysiologie bei SAB. Die pathophysiologischen Prozesse nach SAB bilden ein komplexes Netzwerk und sind bis heute nicht völlig verstanden. Die unterschiedlichen Prozesse verstärken sich teils gegenseitig und führen am Ende über Vasospasmen zu ischämischen Schäden des Gehirns. (Abbildung publiziert in Terpolilli et al.[6]) *BBB = Blut-Hirn-Schranke; ICP = Intrakranieller Druck; NO = Stickstoffmonoxid; ROS = Reaktive Sauerstoffspezies; SAH = Subarachnoidalblutung*

Diese pathophysiologischen Prozesse beeinflussen sich gegenseitig (Abbildung 2) und stellen potenzielle Ansatzpunkte für neue Therapiestrategien dar, um frühzeitig zu intervenieren und somit positiv auf die spätere neurologische Funktion des Patienten zu wirken.[6] Im Folgenden wird auf die wichtigsten Zusammenhänge eingegangen, die für das Verständnis der vorliegenden kumulativen Dissertation relevant sind.

Intrakranieller Druck (ICP) und zerebrale Durchblutung (CBF)

Bei einer SAB kommt es zur Ruptur einer großen intrakraniellen Arterie und dadurch zu einem raschen Austritt von Blut in den Subarachnoidalraum (Abbildung 1). Der Masseneffekt des Hämatoms und die Verbindung des arteriellen Hochdrucksystems mit dem intrakraniellen Raum bewirkt einen vorübergehenden massiven Anstieg des

intrakraniellen Drucks (ICP) bis hin zu Werten des systolischen Blutdrucks.[32] Dies hat ein Absinken des zerebralen Perfusionsdrucks (CPP, engl. *cerebral perfusion pressure*) zur Folge, der sich aus der Differenz des mittleren arteriellen Drucks und des intrakraniellen Drucks ergibt.[28] Der CPP kann in den ersten Minuten nach SAB so tief absinken, dass es zu einer globalen Ischämie des Gehirns kommt (Abbildung 2), die innerhalb von 3 bis 5 Minuten bereits erste bleibende neuronale Schädigungen verursachen kann.[5,28,33] Daher spielt die intrakranielle Hypertonie in der Akutphase der SAB eine entscheidende Rolle für die Entwicklung des EBI.[28,34]

Allerdings führt die intrakranielle Hypertonie mittels Kompression auch zum Sistieren der Blutung und die einsetzende Hämostase bewirkt einen provisorischen Verschluss der Blutungsquelle.[32] Somit besteht keine Verbindung mehr zwischen dem intrakraniellen Raum und dem arteriellen Hochdrucksystems, sodass der ICP wieder absinkt.[35,36] Dies geschieht auf Grund kompensatorischer Volumenänderungen wie der Reduktion des intrakraniellen intravasalen Blutvolumens gemäß der Monro-Kellie-Doktrin, nach der die Summe der intrakraniellen Komponenten - Parenchym, Blut und Liquor - konstant bleiben muss, um einer Druckerhöhung entgegenzuwirken.[35,36]

Innerhalb von 5 bis 10 Minuten stabilisiert sich der ICP, aber infolge des Masseneffekts des Hämatoms bleiben die ICP Werte verglichen mit dem physiologischen Zustand erhöht.[5,32] Im Rahmen einer systemischen Reaktion des vegetativen Nervensystems auf die intrakranielle Hypertension kommt es zu einer reaktiven Blutdruckerhöhung.[37] Dieser Vorgang ist als Cushing Reflex bekannt.[37] Jedoch bleibt trotz der reflektorischen Blutdrucksteigerung der CPP erniedrigt, sodass sich die zerebrale Durchblutung (CBF) nicht vollständig normalisiert.[38]

Diese experimentellen Ergebnisse bestätigten sich im klinischen Alltag, sodass eine frühe zerebrale Minderperfusion nach SAB mittels CT oder MRT nachweisbar ist.[34,39] Der Grad der Minderperfusion hat sich dabei als guter Prädiktor für das spätere funktionelle Outcome des Patienten herausgestellt.[34,39] Allerdings lässt sich die zerebrale Minderperfusion nicht alleine durch die intrakranielle Hypertonie erklären, sondern es muss zusätzlich auch Veränderungen im zerebralen Gefäßsystem geben, welche zu einer Reduktion des CBF beitragen (Abbildung 2).[6,38]

Vasospasmus

Eine Reduktion der zerebralen Durchblutung kann auch durch Engstellen im Gefäßsystem bewirkt werden. Eine gefürchtete Spätkomplikation nach SAB ist ein Vasospasmus der großen intrakraniellen Arterien, der sich bei 34% der Patienten zwischen dem 4. und 14. Tag ausbildet.[22]

Jedoch zeigt sich bereits im Anschluss an die Akutphase eine zerebrale Minderperfusion, obwohl die größeren Arterien zu diesem Zeitpunkt noch keine pathologischen Vasokonstriktionen aufweisen.[6,34] In den vergangenen Jahren konnte sowohl experimentell als auch klinisch ein Vasospasmus der kleineren Arteriolen und eine Abnahme der pialen Kapillardichte nachgewiesen werden.[40,41] Diese Befunde werden unter dem Begriff des Mikrovasospasmus zusammengefasst (Abbildung 2).

Dieser Mikrovasospasmus wird momentan intensiv erforscht und so konnte gezeigt werden, dass es schon in den ersten Stunden der Akutphase zu Konstriktionen der subarachnoidalen und pialen Arteriolen kommt, die durchschnittlich zu einem um etwa 30% kleineren Gefäßlumen führten und besonders in den kleineren Arteriolen stärker ausgeprägt war.[42] Da nach dem Hagen-Poiseuille-Gesetz kleine Lumenverengungen eine starke Reduktion der Durchblutung auslösen, tragen diese frühen Störungen der Mikrozirkulation zu einer deutlichen zerebralen Minderperfusion bei.[42] So bewirkt die Abnahme des Gefäßlumens um 30% bereits einen mehr als vierfach erhöhten Gefäßwiderstand sowie eine Abnahme der Gewebeperfusion auf weniger als 25% des Normalwertes und liegt somit nur knapp über der Ischämiegrenze von 20%.[28,42]

Außerdem konnte nachgewiesen werden, dass sich in spastischen Gefäßen mehr Mikrothromben bilden und dass diese Arteriolen eine verminderte Reaktivität für CO_2 aufweisen. CO_2 ist ein selektiver zerebraler Vasodilatator und entscheidend verantwortlich für die Autoregulation der zerebralen Durchblutung.[42,43] Ein Störung dieser Abläufe begünstigt ebenfalls eine zerebrale Minderperfusion (Abbildung 2).

Hirnödem

Bei einem Hirnödem kommt es durch Zunahme des zerebralen Wassergehalts zu einer Schwellung des Hirnparenchyms und somit zu einer Steigerung des intrakraniellen Drucks.[44,45] Bei etwa 20% der Patienten lässt sich innerhalb der ersten 5 Tage ein globales

Hirnödem mittels CT darstellen, was ebenfalls ein Prädiktor für ein schlechteres klinisches Outcome ist.[46]

Bei der Entstehung eines Hirnödems muss zwischen einer zytotoxischen und einer vasogenen Form unterschieden werden. Das vasogene Ödem entsteht infolge einer Permeabilitätsstörung der Blut-Hirn-Schranke (Abbildung 2, „*BBB leakage*“), wodurch es zum Austritt eines proteinreichen Exsudats ins Parenchym kommt.[28,45] Beim zytotoxischen Ödem hingegen schwellen vorwiegend Astrozyten, da sie entsprechend ihrer physiologischen Funktion versuchen, die Homöostase im Extrazellulärraum aufrecht zu erhalten.[47] Sie nehmen von Neuronen freigesetzte Metabolite, wie z.B. Glutamat, H^+ und Kalium entlang ihres transmembranären Natriumgradienten auf, wodurch sich ihre intrazelluläre Osmolarität erhöht und Wasser osmotisch nachfließt, sodass die Zellen anschwellen.[45,47] Nach SAB tritt dieser Zustand vor allem in der Akutphase ein, da es während der temporären globalen Ischämie zu vermehrt anaeroben Prozessen sowie zu unkontrollierten Depolarisationen von Neuronen kommt (Abbildung 2, „*Cortical Spreading Depression*“).[28,45] Auf Grund der nach wenigen Minuten einsetzenden Reperfusion können sich diese Prozesse wieder rückbilden, sodass im weiteren Verlauf die vasogene Form ursächlich für die Ödementstehung ist, welche sich experimentell an einem gesteigerten Austritt von Albumin aus den Gefäßen ins Parenchym nachweisen ließ.[45,48] Weitere Publikationen zeigten auch eine aktive Degradation der Basallamina durch Kollagenasen und Matrix-Metalloproteinasen, wodurch die Entstehung des vasogenen Ödems verursacht bzw. verstärkt wird.[48,49] Außerdem löst das extravasale Blut eine Entzündungsreaktion aus, die ebenfalls die Permeabilität der Blut-Hirn-Schranke erhöht.[45,46]

Inflammation

Inflammatorische Prozesse nach SAB wurden bislang wenig untersucht. Die Entzündungsreaktionen lösen bereits in den ersten 10 Minuten eine vermehrte Leukozyten- und Makophagen-Rekrutierung aus (Abbildung 2), welche für den Abbau des Hämatoms benötigt werden.[50,51] Die Aktivität der Zellen lässt sich an Hand eines Anstiegs von pro-inflammatorischen Zytokinen (z.B. Tumornekrosefaktor-α oder Interleukin-6) im Liquor nachweisen.[52] Darüber hinaus kann es bei schweren Verläufen zu einer systemischen inflammatorischen Reaktion kommen, welche sich negativ auf die Mortalität und die Morbidität auswirkt.[52]

1.4 Tiermodelle

Die frühe experimentelle SAB Forschung wurde vorrangig an größeren Versuchstieren durchgeführt, wie z.B. Schafe, Hunde, Katzen oder Kaninchen.[53] Ein weit verbreitetes Modell war das zweizeitige Injektionsmodell beim Hund, bei dem autologes Blut peripher gewonnen und danach subarachnoidal appliziert wird.[53] In den letzten Jahren wurden nun vermehrt SAB Modelle bei Mäusen etabliert, da auf Grund der Möglichkeit genetisch veränderte Tiere zu verwenden, die Rolle verschiedener Proteine und Signalkaskaden besser untersucht werden kann.[53] Jedoch sind hierbei die mikrochirurgischen Ansprüche an den Experimentator besonders groß.[53]

Das Injektionsmodell, wie oben bereits kurz skizziert, wurde bei der Maus erstmals 1985 angewandt und ist am schnellsten zu erlernen und durchzuführen.[54] Dabei unterscheidet sich je nach Modell der Injektionsort. Einfach zu punktieren ist die Cisterna magna, welche aber nicht dem typischen Blutungsort einer spontanen SAB entspricht.[53] Daher wurde eine Abwandlung der Methode entwickelt, bei der autologes Blut in die perichiasmatische Region appliziert wird.[55] Allerdings muss dafür eine Nadel durch das Hirnparenchym gestochen werden, was die spätere Beurteilung des Hirnschadens erschwert.

Der große Nachteil der Injektionsmodelle besteht in dem fehlenden Gefäßdefekt, welcher bei einer Ruptur eines Aneurysmas entsteht. Um diesem Umstand Rechnung zu tragen, wurde das endovaskuläre Perforationsmodell entwickelt.[53,56,57] Dabei wird ein starres Monofilament über die A. carotis externa retrograd eingebracht und über die A. carotis interna zur Schädelbasis vorgeschoben. Auf Höhe der Bifurkation der A. cerebri media und A. cerebri anterior wird die Gefäßwand des Circulus arteriosus Willisii perforiert und eine SAB ausgelöst (siehe Publikation I).[58,59] Dieses Modell bildet die pathophysiologischen Umstände sowie die klinische Situation der Patienten sehr gut ab, da es auch zu spontanen Rezidivblutungen kommen kann.[58]

Ein vielfach beschriebener Nachteil des endovaskulären Perforationsmodells ist eine geringe Reproduzierbarkeit auf Grund des durch die blinde Punktion nicht kontrollierbaren Blutungsvolumens.[53] Mit einer präzisen und standardisierten chirurgischen Technik und ausreichendem Training lassen sich diese in der Literatur beschriebenen Schwierigkeiten allerdings fast vollständig vermeiden (siehe Publikation I).[58]

1.5 Therapeutische Maßnahmen

Das wichtigste Ziel der SAB Therapie besteht in der Verhinderung einer Rezidivblutung. Dazu wird die mittels CT oder Angiographie identifizierte Blutungsquelle je nach Lokalisation und Konfiguration entweder nach operativer Eröffnung der Schädelkalotte mittels eines Titanclips verschlossen oder endovaskulär mittels Coiling versorgt, wozu eine dünne Platinspirale in das rupturierte Aneurysma eingebracht und dieses dadurch obliteriert wird.[13,26] Darüber hinaus erhalten SAB Patienten eine umfassende intensivmedizinische Behandlung.[13]

Die weiteren therapeutischen Maßnahmen dienen hauptsächlich der Vermeidung oder der Therapie einer verzögerten zerebralen Ischämie infolge von Vasospasmen der großen Arterien.[13] Zum einen wird die Triple-H-Therapie eingesetzt, bei der man mittels Hypervolämie, Hypertonie und Hämodilution einer zerebralen Minderperfusion entgegenwirkt.[60] Andererseits gibt es pharmakologische Ansätze zur Spasmolyse. Der Calcium-Antagonist Nimodipin wird verwendet, um die Kontraktion der glatten Muskulatur innerhalb der Gefäßwände zu inhibieren und so den Vasospasmus zu lösen.[13] Jedoch können systemische Nebenwirkungen auftreten, welche zu einem Blutdruckabfall und damit zu einer Verschlechterung der zerebralen Perfusion führen.[61]

Ein weiterer therapeutischer Ansatzpunkt ist die Vermeidung oder Verminderung einer intrakraniellen Hypertension.[28] Pharmakologisch wird mittels des osmotischen Diuretikums Mannitol versucht dem Hirnparenchym Wasser zu entziehen und somit den intrakraniellen Druck zu senken.[13] Eine chirurgische Möglichkeit der effektiven Drucksenkung ist die dekompressive Kraniektomie, bei der die Schädelkalotte großflächig eröffnet wird. Dadurch wird der normalerweise abgeschlossene intrakranielle Raum eröffnet, wodurch es zu einer Druckanpassung mit der Umgebung und somit zu einer intrakraniellen Drucksenkung kommt.

1.6 Dekompressive Kraniektomie

Bereits vor über 100 Jahren wurden von Theodor Kocher zum ersten Mal die positiven Effekte einer Kraniektomie bei erhöhtem intrakraniellem Druck beschrieben.[62] Die Methode wurde in der Folge bei verschiedensten Pathologien mit erhöhtem ICP eingesetzt.[63,64] Da es aber weder standardisierte Protokolle noch verlässliche Studienergebnisse gab, hat es

große Unterschiede bei den Operationstechniken gegeben u.a. in der Lokalisation und Größe der entnommenen Kalottenanteile, mit oder ohne Eröffnung der Dura, uni- oder bilaterale Dekompression.[63,64] Diese hohe Variabilität spiegelte sich bei den beobachteten Komplikationen wider, sodass es keinen wissenschaftlichen Konsens zum Nutzen einer dekompressiven Kraniektomie gab und die Methode daher über die Jahre an Bedeutung verlor.[63,64]

Eine dekompressive Kraniektomie war nur noch indiziert, wenn konservative Therapiemaßnahmen versagten und sich eine unkontrollierbare intrakranielle Hypertonie einstellte.[63] Die Indikationsstellung zu solch späten Zeitpunkten hatte allerdings zur Folge, dass es auf Grund der bereits entstandenen Sekundärschäden zu keiner Symptomverbesserung kam, obwohl der ICP gesenkt wurde.[65]

Da aber regelmäßig auch Fallstudien mit positiven Ergebnissen publiziert wurden, ist die dekompressive Kraniektomie nie völlig aufgegeben worden.[63] Infolge von Verbesserungen im Bereich der Neurochirurgie, der Neuroradiologie und Fortschritten in der Neurointensivmedizin ließen sich Komplikationen im Rahmen einer dekompressiven Kraniektomie besser vermeiden oder behandeln.[63]

In den letzten beiden Jahrzehnten hat man begonnen, die Effekte einer dekompressiven Kraniektomie systematisch in größeren Studien zu untersuchen.[63] So zeigte sich, dass eine frühzeitige dekompressive Kraniektomie nach Schädel-Hirn-Trauma bei Kindern eine langfristige Verbesserung des funktionellen Outcomes bewirkt.[66] Aktuelle multizentrische Studien wie die DECRA Studie sowie die RESCUEicp Studie untersuchten diese Ergebnisse in größeren Populationen (155 respektive 408 Patienten).[67-69] In den Studien führte die dekompressive Kraniektomie zu einer Verminderung der intrakraniellen Hypertension und zu einer geringeren Mortalität.[67-69] In der RESCUEicp Studie wurde die 6-Monats-Mortalität von 49% auf 27% gesenkt.[69] Allerdings wiesen die kraniektomierten Patienten ein schlechteres durchschnittliches funktionelles Outcome auf.[67,69] Somit konnte keine der beiden Studien eine klare Empfehlung für den Einsatz der dekompressiven Kraniektomie nach Schädel-Hirn-Trauma aussprechen.[68,69]

Bei großen ischämischen Infarkten der A. cerebri media kann sich durch ein ausgedehntes Hirnödem ebenfalls eine relevante intrakranielle Hypertension entwickeln.[70] Die dekompressive Kraniektomie erwies sich in diesen Fällen als gute Therapieoption und wurde daraufhin in die Therapierichtlinien der USA aufgenommen.[70]

Bei Patienten mit SAB wird die dekompressive Kraniektomie meist als Second-Line-Therapie eingesetzt, sobald sich die intrakranielle Hypertension nicht mehr konservativ senken lässt und sich der Zustand des Patienten verschlechtert.[63] Daher gibt es nur wenige Daten zum Einsatz einer frühzeitigen dekompressiven Kraniektomie als First-Line-Therapie. Die bisherigen Fallauswertungen und Metaanalysen sind nicht eindeutig und lassen noch keine abschließende Bewertung zu, aber sie zeigen durchaus positive Effekt in Bezug auf das Überleben und das funktionelle Outcome.[71-74]

1.7 Ziele der vorliegenden Arbeit

Im vorliegenden Promotionsvorhaben zum Thema SAB wurde die Rolle der post-hämorrhagischen intrakraniellen Hypertension bei der Entstehung des frühen Hirnschadens sowie sein Einfluss auf das spätere funktionelle Outcome untersucht. Dazu wurde zunächst das bestehende SAB Mausmodell an das benötigte intra-operative multimodale Monitoring angepasst, wofür eine standardisierte und gut adjustierbare Anästhesie mit einer mechanischen Ventilation benötigt wurde. Zusätzlich mussten für die Bewertung des funktionellen Outcomes entsprechende neurologische Testverfahren als auch histopathologische Auswertungen erarbeitet und etabliert werden. Aus diesen Vorarbeiten ergab sich ein standardisiertes Protokoll, welches später in Form der Publikation I der vorliegenden Dissertation veröffentlicht wurde.[58]

Nach Etablierung dieser Methoden wurde die Rolle des intrakraniellen Drucks bei SAB im Mausmodell untersucht. Dabei sollte differenziert werden, wie sich der frühe ICP-Anstieg, einhergehend mit einer globalen Ischämie, als auch die spätere kontinuierliche intrakranielle Hypertension auf die Entwicklung und das Ausmaß der EBI auswirken. Hierfür wurde die dekompressive Kraniektomie zu unterschiedlichen Zeitpunkten eingesetzt, um eine Entlastung der intrakraniellen Hypertension zu erreichen. Dafür wurde die Größe und Position der Kraniektomie optimiert, sodass eine möglichst große bilaterale Kraniektomie möglich war, welche keinen Einfluss auf Kontrollgruppen hatte.

Insgesamt gab es vier unterschiedliche Versuchsgruppen. Eine Sham-Gruppe durchlief dieselben operativen Schritte, jedoch ohne Induktion einer SAB. In den übrigen drei Gruppen wurde bei den Mäusen eine SAB induziert. Davon wurde eine Gruppe nicht kraniektomiert, eine Gruppe wurde 15 Minuten nach der SAB kraniektomiert und eine

Gruppe wurde bereits vor der SAB kraniektomiert. Durch die beiden letztgenannten Gruppen sollte eine Differenzierung zwischen der initialen globalen Ischämie und der späteren zerebralen Minderperfusion entsprechend des zeitlichen Verlaufs des intrakraniellen Drucks ermöglicht werden. Die Ergebnisse dieser Versuche wurden in Form der Publikation II der vorliegenden Dissertation veröffentlicht.[75]

2 Zusammenfassung

Die Subarachnoidalblutung (SAB) ist eine Form des Schlaganfalls und weist eine hohe Mortalität und Morbidität auf. Fast ein Viertel aller Patienten versterben prähospital und die Mortalität im ersten Monat beträgt beinahe 50%. Haupttodesursachen sind die initiale globale Ischämie sowie der frühe Hirnschaden (EBI). Zu den zentralen pathologischen Prozessen zählen der erhöhte intrakranielle Druck (ICP) und die reduzierte zerebrale Durchblutung (CBF), welche zu sekundären Schädigungen und neuronalem Zelltod führen. Eine Methode zur Reduzierung des intrakraniellen Drucks ist die dekompressive Kraniektomie (DC). Sowohl bei Schädel-Hirn-Traumata als auch bei großen ischämischen Infarkten hat die DC einen positiven Einfluss auf den Krankheitsverlauf, jedoch sind die Ergebnisse bei SAB nicht eindeutig. In der vorliegenden Arbeit wurde untersucht, ob eine DC die post-hämorrhagische intrakranielle Hypertension senken und dadurch positiv auf den Krankheitsverlauf nach experimenteller SAB wirken kann.

Für die Studien wurde bei männlichen C57BL/6 Mäusen eine SAB mittels endovaskulärer Perforation des Circulus arteriosus Willisii ausgelöst. Zunächst wurde ein standardisiertes Protokoll mit multimodalem intraoperativem Monitoring etabliert und gezeigt, dass für das endovaskuläre Perforationsmodell eine gute Reproduzierbarkeit erreichbar ist. Außerdem sind typische neurologische Defizite und histopathologische Veränderungen bei den Versuchstieren nachweisbar (siehe Publikation I).

Bei der Studie zur Untersuchung der Rolle der intrakraniellen Hypertension gab es insgesamt vier Versuchsgruppen (siehe Publikation II): Sham operiert, SAB, DC vor SAB und DC 15 min nach SAB. Um eine möglichst große Reduktion des ICPs zu erreichen, wurden über beiden Hemisphären Teile der Schädelkalotte entfernt. Intra-operativ wurden ICP, CBF, Herzfrequenz, Sauerstoffsättigung und endexspiratorischer pCO_2 bis 45 min nach SAB aufgezeichnet. Die Tiere wurden täglich neurologisch untersucht. Nach sieben Tagen wurden die Gehirne entnommen und an Hand von koronaren Paraffinschnitten wurden die Hydrozephalusentwicklung, die Corpus callosum Dicke und das Überleben hippocampaler Neurone ausgewertet.

Obwohl die intrakranielle Hypertension durch die DC gesenkt wurde, führte dies nicht zu einer Verbesserung der zerebralen Minderperfusion nach SAB. Darüber hinaus kam es sogar zu vermehrten Rezidivblutungen, zu einer höheren Mortalität und zu stärkeren neurologischen Defiziten. Typische histopathologische Veränderungen nach SAB sind

Hydrozephalusentwicklung und neuronale Schädigung. Diese Pathologien konnten in unserem Tiermodell ebenfalls untersucht werden (siehe Publikation I und II). Zusätzlich stellten wir eine lateralisierte Schädigung der weißen Substanz fest. Jedoch hatte die DC keinen Einfluss auf das Ausmaß dieser Pathologien (siehe Publikation II).

Insgesamt führte die DC zwar zu einer deutlichen Reduktion des post-hämorrhagischen ICP, hatte aber keinen positiven Effekt nach experimenteller SAB - im Gegensatz zum ischämischen Schlaganfall und Schädel-Hirn-Trauma. Im Gegenteil, dekomprimierte Tiere hatten mehr Rezidivblutungen und ein schlechteres funktionelles Outcome. Diese Ergebnisse deuten darauf hin, dass der erhöhte ICP kurz nach SAB wichtig ist für die Blutstillung und deshalb in der akuten Phase nicht reduziert werden sollte. Daher sollte bei der Erwägung einer DC bei SAB Patienten sowohl der Zeitpunkt als auch das Ausmaß der Blutung besonders berücksichtigt werden.

3 Summary

Subarachnoid hemorrhage (SAH) is a stroke subtype associated with high mortality and morbidity. Nearly one quarter of all patients die before hospitalization and the one-month mortality rate is almost 50%. Initial global ischemia and the following early brain injury (EBI) are the main causes of death. The major pathophysiological features are elevated intracranial pressure (ICP) and subsequently reduced cerebral blood flow (CBF) - leading to secondary injuries and neuronal cell death. A way to reduce ICP is decompressive craniectomy (DC). Beneficial effects were reported in traumatic brain injury and malignant ischemic stroke, but regarding SAH the results are controversial. In this study, we wanted to evaluate whether DC is able to reduce post-hemorrhagic intracranial hypertension and thereby improving outcome following experimental SAH.

SAH was induced in male C57BL/6 mice via endovascular Circle of Willis perforation. Initially, a standardized protocol with multimodal intraoperative monitoring was established and a good reproducibility for this endovascular perforation model could be demonstrated. Also typical neurological deficits and histopathological changes could be found (see publication I).

The study for the evaluation of the role of intracranial hypertension included the following four groups (see publication II): Sham surgery, SAH, DC before SAH, and DC 15 min after SAH. In order to achieve sufficient ICP reduction, skull bone was removed over both hemispheres. During surgery ICP, CBF, heart rate, oxygen saturation, and end-tidal pCO_2 were monitored until 45 min after SAH induction. Following surgery, neurological function was evaluated daily for 7 days. Finally, brains were harvested and hydrocephalus formation, corpus callosum thickness, and survival of hippocampal neurons were evaluated on paraffin-embedded coronal brain sections.

Although DC relieved intracranial hypertension, there was no improvement in cerebral hypoperfusion after SAH. Moreover, it even led to a higher incidence of rebleeding, to a higher mortality rate and to more severe neurological impairments. At the histopathological level SAH results in hydrocephalus formation and neuronal damage. These pathologies were also present in our animal model (see publication I and II). In addition, we could identify lateralized white matter damage in SAH pathology. All these histopathological features were unaffected by DC (see publication II).

Overall, DC markedly reduced post-hemorrhagic ICP but had no beneficial effect after experimental SAH - in contrast to ischemic stroke and traumatic brain injury. On the contrary, decompressed animals had more rebleedings and worse functional outcome. These results suggest that elevated ICP shortly after SAH is important for cessation of the hemorrhage and should not be reduced acutely. Therefore DC in SAH patients needs to be considered carefully with special emphasis on timing and degree of bleeding.

4 Literaturverzeichnis

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5 Publication I

Protocol for the Induction of Subarachnoid Hemorrhage in Mice by Perforation of the Circle of Willis with an Endovascular Filament

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Abstract

Genetically engineered mice are a valuable tool to investigate the molecular and cellular mechanisms leading to brain damage following subarachnoid hemorrhage (SAH). Therefore, several murine SAH models were developed during the last 15 years. Among those models, the perforation of the Circle of Willis by an endovascular filament or "filament model" turned out to become the most popular one, since it is believed to reproduce some of the most prominent pathophysiological features observed after human SAH. Despite the importance of the endovascular filament model for SAH research, relatively few studies were published using this technique during the past years and a number of laboratories reported problems establishing the technique. This triggered discussions about the standardization, reproducibility, and the reliability of the model. In order to improve this situation, the current paper aims to provide a comprehensive hands-on protocol of the murine endovascular filament model. The protocol proved to result in induction of SAH in mice with high intrapersonal and interpersonal reproducibility and is based on our experience with this technique for more than 10 years. By sharing our experience with this valuable model, we aim to initiate a constantly ongoing discussion process on the improvement of standards and techniques in the field of experimental SAH research.

Keywords: Subarachnoid hemorrhage; mouse; model; endovascular; filament; Circle of Willis perforation

Background

Subarachnoid hemorrhage (SAH) is a subtype of stroke with a devastating outcome and a high socio-economic impact which equals almost that of ischemic stroke [1]. Therefore, it is surprising that SAH is by far less frequently investigated than other neuro-vascular diseases. Consequently, several important aspects of the molecular and cellular pathophysiology of SAH are not well defined, a fact which significantly impedes the development of novel therapeutic strategies [2]. One of the many reasons for this situation could be that murine models of SAH are technically demanding and have so far been difficult to standardize [3].

Since the late 1970s when Barry and colleagues published one of the first reports on the experimental induction of SAH in "small animals" [4], several techniques for SAH induction in rodents became popular. Several laboratories studied SAH in mice by directly injecting blood or blood components into the cisterna magna [5], by opening an intracisternal vein [6], by injecting blood into the basal cisterns [7], or by perforating the Circle of Willis at the skull base using an endovascular filament inserted through the external carotid artery [8-16]. Although none of these models fully recapitulate the sequels of human SAH at least for the acute phase after SAH, the endovascular Circle of Willis perforation model, or "filament model," became one of the most popular murine SAH models. The main reason for this popularity is that it mimics the burst of a cerebral aneurysm and most of its sequels reasonably well and is therefore believed to have a superior clinical relevance as compared to all other available models [17]. Good examples for the translational potential of the filament model are reports demonstrating delayed cerebral vasospasm [8, 12-14], neurological dysfunction [9, 11-13, 15], brain edema formation [9, 11, 18-20], and a clinically relevant mortality of approximately 30 % [9, 11, 15, 19, 21].

Despite these very positive and useful aspects of the filament model, important features of the model are still not fully standardized between laboratories. This makes results difficult to compare and sometimes hard to reproduce. In contrast to other well-standardized murine models of acute brain injury, e.g., ischemic stroke, successful induction and the severity of the insult are often not monitored intraoperatively leading to heterogeneous results with large standard deviations and questionable statistical power. This makes randomized study designs difficult to perform and limits the value of this otherwise clinically highly relevant animal model [3]. Therefore, the aim of this paper is to suggest a

protocol which may facilitate a better standardization of the murine endovascular filament SAH model between individual researchers and between different laboratories.

Protocol

Experimental Approach

Before starting any animal experiment, a proper sample size calculation should be performed and animals should be randomly assigned to experimental groups by an investigator blinded to the treatment and/or the genotype of the animals. These measures may be considered as being time consuming or distrustful on the first sight; however, it should be taken into consideration that a biased experiment is a much greater waste of time and resources. Personal bias is a normal, unintentional behavior of every motivated and dedicated scientist who wants to achieve novel results. Therefore, stringent randomization and blinding protocols should be an implicitness for every researcher keen to publish meaningful and sustainable results in high-quality journals [22].

Another important point which needs to be considered long before starting experiments on transgenic animals is the choice of proper controls. This is particularly important for studies using cerebro-vascular disease models since the cerebro-vascular anatomy is very different between mouse strains commonly used to produce transgenic animals, i.e., C57BL/6 and SV129 mice [23]. Hence, the same procedure may result in completely different results when performed on different strains of mice, and completely different results may be obtained when transgenic animals, which are in most cases a mixture of C57BL/6 and SV129 mice, are compared to a wrong wild-type mouse line. In order to avoid this potential confounder, we would recommend using appropriately backcrossed transgenic mouse lines (>10 generations) for experiments or littermates as controls for transgenic mice.

Pre-operative Care

It is well known from studies in humans and animals that pre-operative conditions such as housing or stress may have a significant impact on brain function and on outcome after surgery [24]. Accordingly, it is highly recommended not to disrupt well-established social interactions between animals, e.g., by separating groups of mice which grew up together or

by adding dominant males to well-established groups of animals, and to keep mice under the same housing conditions for at least 1 week prior to surgery.

Another potentially important confounder is access to food and water prior to surgery since even short-term fasting before surgery may significantly alter study results [25]. We recommend allowing mice full access to food and water prior to surgery. This results in sufficient hydration and relatively homogenous blood glucose levels, which are also known to have a large impact on the development of brain injury [26, 27].

Pre-medication and Anesthesia

Animals should be brought to the surgery room only briefly before surgery, and anesthesia should be induced with no delay and with as little stress to the animal as possible. We would recommend inducing anesthesia in a small chamber, using 4 % isoflurane in 30 % oxygen until the animal loses consciousness. Animals are then weighted, preemptive post-operative analgesia is induced with carprofen (4 mg/kg s.c.), and anesthesia is maintained by intraperitoneal injection of fentanyl (0.05 mg/kg), midazolam (5 mg/kg), and medetomidine (0.5 mg/kg) as previously described [28]. Immediately thereafter, mice are intubated and mechanically ventilated (MiniVent 845, Hugo Sachs Elektronik/Harvard Apparatus) because SAH induces global cerebral ischemia for 2-3 min which results in respiratory dysfunction or even failure [8]. Intubation can be performed either oro-tracheally or by tracheotomy. For survival surgery, we perform oro-tracheal intubation as previously described [18-20, 28] and recently shown in a video publication [21]. As soon as mice are incubated and connected to the ventilator, the animal is placed on a heating pad pre-heated to 37 °C and a rectal temperature probe is inserted for monitoring and maintenance of body temperature. This is particularly important for mice because they quickly lose temperature during anesthesia [28].

The suggested anesthetics have relatively little impact on systemic blood pressure and cerebral blood flow. Specifically, the maintenance of a physiological and homogenous systemic blood pressure is important for the standardized induction of SAH since in addition to the size of the filament used for perforation of the Circle of Willis, the systemic blood pressure plays an important role for the severity of SAH. Another advantage of this anesthesia protocol is that it can be antagonized immediately after termination of surgery

(see below). This allows the animals a rapid gain of consciousness, motor activity, and control of body temperature.

Intraoperative Monitoring

The endovascular filament approach induces hemorrhage without visual control. Therefore, it is important to monitor the induction of hemorrhage in real time. Proper monitoring of SAH induction avoids post hoc exclusion of animals which had no hemorrhage and - according to our experience even more importantly - prevents pushing the filament too far and thereby causing additional mechanical brain damage at the perforation site.

Monitoring the decrease of cerebral blood flow (CBF) which occurs after SAH is one possible option to monitor Circle of Willis perforation (CWp); however, we observed at different occasions that CBF may decrease without SAH [19]. This was most likely due to vasoconstriction of intracerebral vessels induced by the mechanical stimulation of the endothelium with the endovascular filament. Therefore, we suggest to monitor SAH directly by the effect of the evolving hematoma on intracranial pressure (ICP). After switching from CBF to ICP monitoring, the rate of false positively monitored SAHs dropped to zero. For this purpose, the medial part of the left temporal muscle is detached from the skull bone, a small hole is drilled into the temporal bone, and an ICP probe (ICP Express, Codman) is introduced between the bone and brain into the epidural space. The sensor is fixed with dental cement (Carboxylatzement, Speiko, Germany; Fig. 1) and ICP is recorded using a data acquisition system (PowerLab, ADInstruments). As soon as the ICP starts to rise sharply (Fig. 3a), a bleeding into the subarachnoid space takes place [21]. Upon withdrawal of the filament, the ICP rises to values close to the systemic blood pressure. Animals not showing this sharp increase in ICP or showing an increase below 50 mmHg even after a second (and final) perforation attempt should be excluded from the study. Within 5 min after the initial bleeding, values drop to around 30 mmHg. Within another 20-min observation period, ICP values stabilize around 25 mmHg (Fig. 3a). One day after the bleeding, the ICP is still elevated to 10 mmHg whereas 3 days after the hemorrhage, it normalizes at 5 mmHg [19].

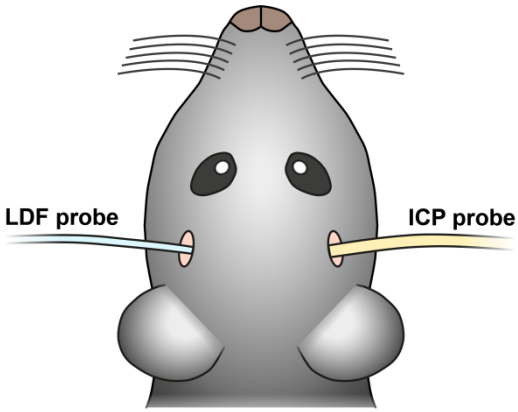

Fig. 1 Probe positions for physiological monitoring. The laser Doppler flowmeter (*LDF*) probe is glued on the left temporal bone above the MCA territory. The intracranial pressure (*ICP*) probe is placed in the epidural space through a small borehole at the right temporal bone (adapted from Schuller et al. [21])

Another important parameter which determines the amount of bleeding after SAH is the systemic blood pressure [21]. The higher the blood pressure during the bleeding, the more blood is extravasating. Therefore, noninvasive blood pressure monitoring with a cuff placed around the tail of the mouse (Coda monitor, Kent Scientific) during the procedure helps to standardize the bleeding volume. The noninvasive measurement is important since this allows long-term survival of the mice after surgery without the risk of hind limb ischemia due to femoral artery catheterization. Animals with a mean arterial pressure under 60 mmHg should be excluded from the study.

Next to ICP and systemic blood pressure, also the arterial pCO_2 needs to be measured and controlled. CO_2 is a strong and specific dilator of cerebral vessels and therefore arterial pCO_2 may also critically determine bleeding intensity after SAH. Arterial pCO_2 can be reliably measured in the inspired and expired air by a microcapnometer (Capnograph 340, Hugo Sachs Elektronik/Harvard Apparatus) connected to the ventilation tube [28]. Values should be adjusted to 25-30 mmHg. This results in arterial pCO_2 values in the physiological range (35-45 mmHg).

In order to receive additional information about regional cerebral blood flow (rCBF), a laser Doppler probe is glued on the temporal bone with cyanoacrylate glue (Fig. 1) and laser Doppler flux is measured through the intact bone. Laser Doppler recordings drop once the filament reaches the bifurcation of the MCA and reaches values close to zero when SAH occurred (Fig. 3b). As mentioned above, a drop of rCBF does not necessarily indicate vessel

perforation. The heart function can be monitored by pulsoximetry on the hind paw (Mouse STAT, Kent Scientific). This noninvasive measurement provides peripheral oxygen saturation and heart rate. The high ICP after SAH induces a Cushing response, i.e., an increase in blood pressure (data not shown) and a decrease in heart rate (Fig. 3c).

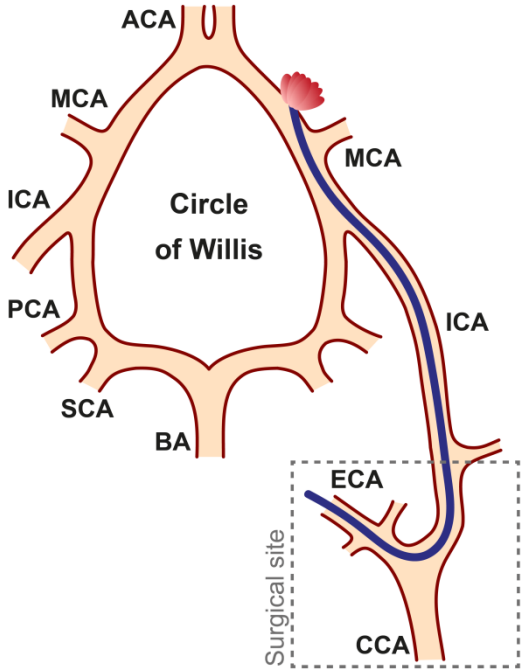

Fig. 2 Scheme of SAH induction by perforation of the Circle of Willis. Via the surgical site at the neck, the left carotid arteries are visualized. A stiff filament (5-0 prolene, 12 mm) is introduced in the ECA, placed into the ICA and further advanced toward the Circle of Willis. By gently pushing forward, the vessel wall close to the MCA can be perforated to induce a SAH. *ACA = anterior cerebral artery, BA = basilar artery, CCA = common carotid artery, ECA = external carotid artery, ICA = internal carotid artery, MCA = middle cerebral artery, PCA = posterior cerebral artery, SCA = superior cerebellar artery* (adapted from Schuller et al. [21])

SAH Induction

First, the animal is placed in a supine position and the neck is exposed. The skin is opened in the midline. Afterward, a blunt dissection through connective tissue between the salivary glands is performed. The external, internal, and common carotid artery and their branches are exposed and partly mobilized. The external carotid artery is ligated with a silk filament and another silk filament for fixation of the perforation filament is prepared. The common and internal carotid arteries are temporarily closed with micro clips. A stiff and blunted filament (Prolene 5-0) is inserted into the external carotid artery and fixed with the pre-

arranged silk filament [8, 19, 21]. After removal of the micro clips, the filament is advanced into the ICA and then further toward the brain stem (Fig. 2). A sudden increase of the ICP together with a drop of the rCBF indicates vessel perforation at the Circle of Willis (Figs. 2 and 3a, b).

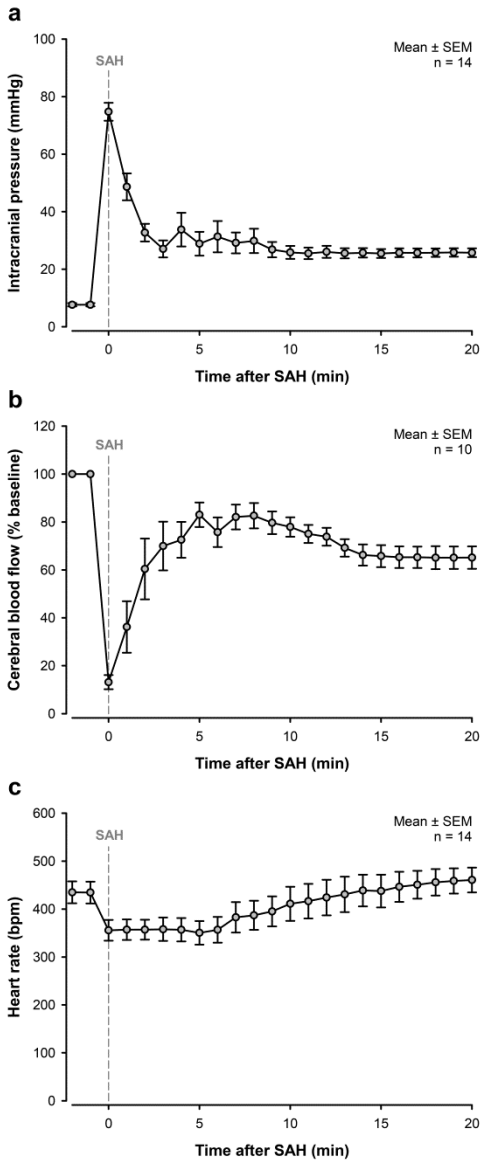

Fig. 3 Intraoperative monitoring. **a**-**c** Continuous recording of intracranial pressure (*ICP*), cerebral blood flow, and heart rate during SAH induction (indicated by *dashed line*). SAH induction results in an immediate strong increase of ICP (**a**) which leads to a global cerebral ischemia at the same time (**b**). After a few minutes, ICP is decreasing again but stays elevated (**a**). Also, the cerebral blood flow is stabilizing but remains reduced (**b**). A drop in heart rate can be a consequence of the Cushing response to elevated intracranial pressure (**c**)

Once the ICP rises, the filament is withdrawn immediately from the internal carotid artery. If the ICP does not rise, the filament needs to be withdrawn completely and a second attempt to introduce the filament in to the internal carotid artery and the perforate the vessel may be performed. If this does not result in SAH, the animal needs to be excluded from the study. After SAH, the external carotid artery is ligated and the skin wound sutured. The physiologic parameters and especially the ICP are monitored for another 20 min after bleeding induction to screen for potential re-bleedings which are detected by additional sharp increased of ICP. With this technique, a preferential distribution of blood along subarachnoid vessels (Fig. 4) with little to no variation between different animals can be achieved [21].

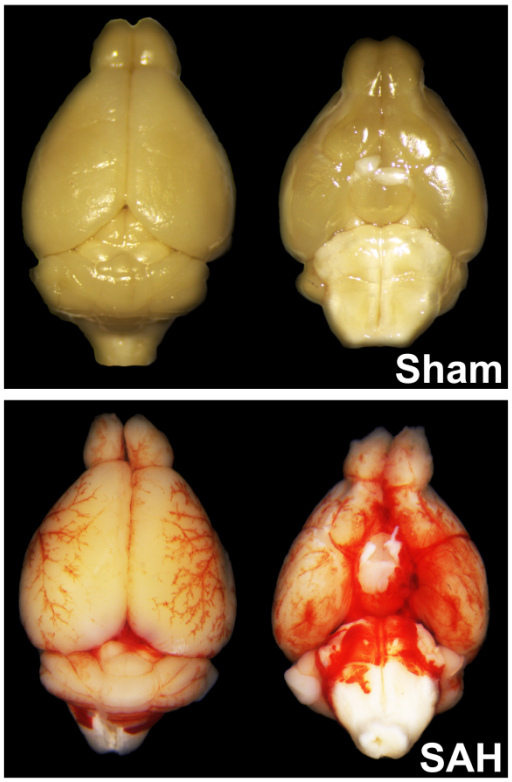

Fig. 4 Blood distribution along arteries after SAH. Brains were removed directly after surgery to evaluate blood distribution along brain-supplying arteries. Mice were transcardially perfused with saline so that no blood remained inside the vessels. In sham-operated animals, there is no visible blood while after SAH, blood is surrounding the vessels and is distributed along the arteries (adapted from Schuller et al. [21])

Post-operative Care

At the end of the surgery, anesthesia is terminated with a subcutaneous injection of atipamezol (2.5 mg/kg), flumazenil (0.5 mg/kg), and naloxon (1.2 mg/kg). In addition, 0.2 ml saline is injected subcutaneously to substitute for a possible volume loss during surgery. Mice are extubated as soon as they show motor activity. Afterward, animals are kept alone in a pre-heated incubator at 30 °C for 24 h to prevent hypothermia and are then returned together with their cage mates to their home cage. Dry and soaked food pellets together with easily accessible water are provided.

Mice are observed daily for a period of 7 days. They receive daily subcutaneous injections of carprofen (4 mg/kg) and 0.2 ml saline. In our hands, this procedure results in a mortality rate of approximately 30 % mainly during the first 1-5 days after surgery (Fig. 5a). Thereafter, mice survive long term.

Neurological deficits are assessed daily for 7 days or longer using a neuroscore adjusted to the neurological deficits observed after SAH (Table 1). After SAH, mice usually score between 10 and 20 points. To receive reliable results, mice should be familiarized with the test procedure for 2 days prior to surgery. Mice not achieving the best possible performance before SAH (0 points) should be excluded from further assessment. After SAH, mice are most impaired on the first post-operative day (Fig. 5b). On the following days, mice start to recover gradually which results in lower scores.

As a sensitive indicator for general well-being, the body weight is assessed daily. The biggest loss of bodyweight can be observed on the first 3 days after surgery (Fig. 5c). Afterward, mice start to gain weight again and can almost reach their initial weight.

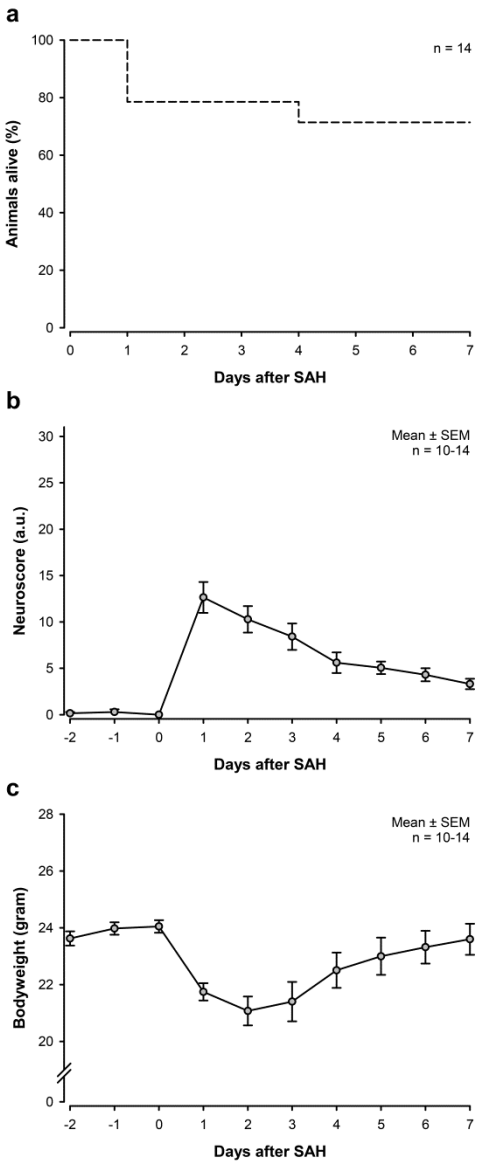

Fig. 5 Post-operative outcome. **a** Mortality after SAH by perforation of the Circle of Willis. In this model, a mortality rate of about 30 % in the first week can be observed. **b** Daily neurological examinations. The applied neuroscore (best, 0; worst, 31; see Table 1) shows that mice after SAH induction have the biggest deficits on the first post-operative day and that they can recover gradually over time. **c** Bodyweight changes after SAH induction. Loss of bodyweight can be observed on the first 3 days after surgery. Afterward, mice start to gain weight again

Table 1 Neuroscore for SAH

Task	Criteria	Score
Consciousness	Spontaneous exploration	0
	Movements after tactile stimulus	1
	No movements (comatose)	2
Whisker movements	Present	0
	Absent	1
Hearing (turning to hand clapping)	Directed	0
	Undirected	1
	Absent	2
Motor function (per limb)	Normal	0
	Stiff	1
	Paralyzed	2
Mod. Bederson score	No obvious deficits	0
	Flexed forelimbs	1
	Lowered resistance to lateral pushing	2
	Circling if pulled by tail	3
	Spontaneous circling	4
	No spontaneous activity	5
Placing test[a]	Present	0
	Absent	1
Beam walk (3cm, 2cm, 1cm)	Normal movements	0
	Improper paw placing	1
	Circling on beam	1.5
	No movements	2
	Falling off after few steps	3
	Falling off immediately	4
Total		0-31

[a] Front paws reaching ground when lifted by tail

Histology

A feasible way to analyze brain damage after SAH is on formalin-fixed and paraffin-embedded brain tissue. Animals are killed by transcardial perfusion with 20 ml of saline followed by 20 ml of 4 % paraformaldehyde (PFA) in phosphate-buffered saline (PBS) at a pressure of 120 mmHg. Brains are harvested and then stored in 4 % PFA in PBS (4 °C, 24 h)

for post-fixation. Afterward, brains are embedded in paraffin and 4-µm coronal sections are prepared using a microtome. On cresyl violet stained coronal sections, different regions of interest can be selected in the hippocampus to quantify viable pyramidal neurons (Fig. 6) [19].

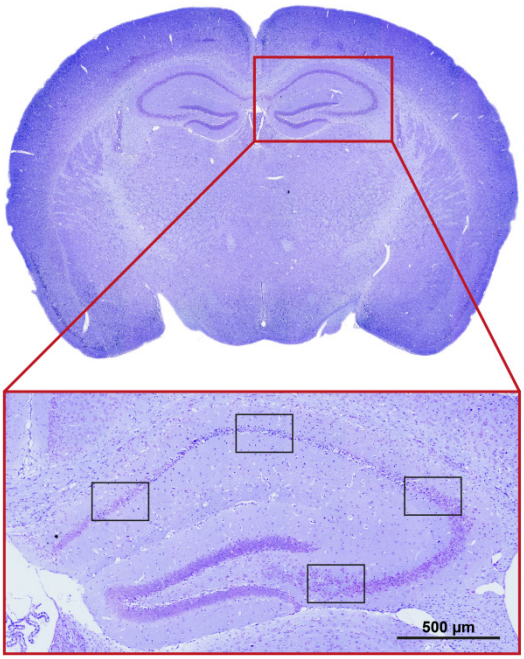

Fig. 6 Histopathological evaluation. Neuronal damage can be evaluated, e.g., on cresyl violet stained coronal sections by counting pyramidal neurons in the CA1 region of the hippocampus

Summary and Conclusion

Here we present a well-established and standardized protocol for the induction of subarachnoid hemorrhage in mice by perforation of the Circle of Willis with an endovascular filament, the so-called filament model. Based on multiple publications with this protocol over the past 10 years and on repetitive validation experiments performed in our laboratory on a regular basis, the protocol proved to have a high intrapersonal and interpersonal reproducibility. Key parameters which need to be taken into consideration for reproducible induction of SAH with this method are sample calculation, randomized and blinded study design, intubation and ventilation, injection anesthesia, maintenance of physiological systemic blood pressure and arterial pCO_2, monitoring of intracranial

pressure, and a proper surgical technique. When adhering to this protocol, researchers with preexisting experience in mouse handling and surgery should be able to reproducibly induce SAH in mice after a training period of about 6 months.

Conflict of Interest

Dominik Bühler, Kathrin Schüller, and Nikolaus Plesnila declare that they have no conflict of interest.

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6 Publication II

Effect of Decompressive Craniectomy on Outcome Following Subarachnoid Hemorrhage in Mice

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Abstract

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 four groups: Sham surgery, SAH, DC after SAH, and DC before SAH. DC was performed either 15 min before or after SAH induction. ICP, cerebral blood flow, heart rate, oxygen saturation, and end-tidal pCO_2 were monitored for 45 min. Following surgery, neurological function was evaluated daily for seven days. After sacrifice hippocampal neurons, corpus callosum thickness, and ventricular volume were evaluated on PFA-fixed coronal brain sections.

Results: Although DC reduced SAH-induced ICP, it yielded no beneficial effect with respect to post-hemorrhagic hypoperfusion; moreover, DC increased the incidence of re-bleeding, induced more severe neurological impairments, and caused higher mortality. Post-SAH mice that survived seven days had no histopathological 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 re-bleeding, and poorer outcome. Thus, elevated post-hemorrhagic ICP plays an important role in controlling bleeding following 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.

Keywords: Subarachnoid hemorrhage; decompressive craniectomy; intracranial pressure; mouse model; functional outcome

Introduction

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

EBI is the predominant cause of death following 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 outcome;[6-8] however, how and to what extent the initial global ischemia and/or subsequent elevated ICP contribute to EBI 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 injury (TBI)[10,11] 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 four experimental groups: Sham surgery, SAH, DC after SAH, and DC before SAH (Figure 1). All assessments were performed by an investigator blinded with respect to the treatment group. Because the first five mice in the DC before SAH group experienced high morbidity and failed to reach the 7-day endpoint, for ethical reasons we stopped this group after five mice. In addition, a DC plus sham surgery group with six animals was performed to evaluate potential effects due to craniectomy under normal conditions.

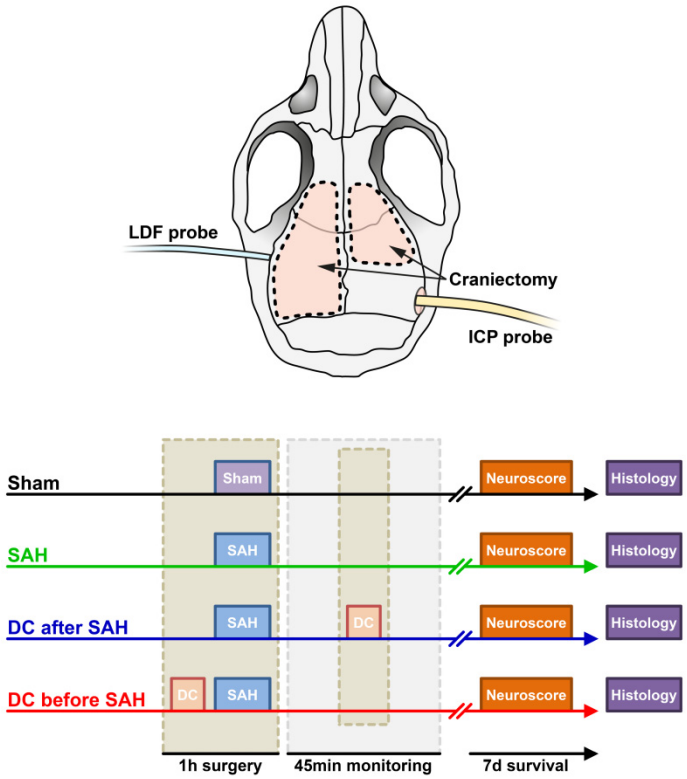

Figure 1. Study design. All four study groups had a 1 h surgery period followed by a 45 min monitoring phase. Sham animals underwent the same surgery but without SAH induction. After surgery, daily neurological examinations were performed. Seven days after surgery, the mice were sacrificed and the brains were harvested for histological evaluations. The craniectomy sites and probe positions for physiological monitoring are indicated in the top panel. *DC = decompressive craniectomy; ICP = intracranial pressure; LDF = laser Doppler flowmeter.*

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 pCO_2 was measured using microcapnometry (CI240, Columbus Instruments, Columbus, OH). A thermostatically regulated feedback-controlled heating pad (FHC, Bowdoin, ME) was used to maintain a rectal temperature of 37 °C. Preemptive postoperative analgesia was provided with carprofen (4 mg/kg s.c.).

For continuous monitoring of ICP, a cranial burr hole was drilled under continuous cooling with saline, and an ICP transducer (Codman, Johnson & Johnson, Berkshire, UK) was introduced in the epidural space over the right hemisphere (Figure 1). Regional cerebral blood flow (CBF) was measured continuously using a flexible laser Doppler flowmetry (LDF) probe (PeriFlux System 5000, Perimed, Järfälla, Sweden) glued onto the left temporal bone above the territory of the left MCA (Figure 1). The ICP and LDF probes were removed at the end of the monitoring period.

Decompressive Craniectomy

DC was performed either before or 15 min after SAH induction (Figure 1). In order to achieve a sufficient reduction in ICP, the skull bone was removed over both hemispheres (left: 8x4 mm; right: 4x4 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 towards 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. Re-bleeding 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 h after surgery. For a period of seven 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 two days prior to surgery until seven days after surgery using a global SAH neuroscore (see Table I in the online-only Data Supplement; adapted from Bühler 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) in order 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 sacrificed under anesthesia by transcardial perfusion with 4% paraformaldehyde (PFA). The brains were harvested and post-fixed in 4% PFA for 24 h. 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, two 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 (i.e., 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 two 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 mm x 0.2 mm) were selected in the CA1, CA2, and CA3 regions of the hippocampus, and viable pyramidal neurons were counted as previously described.[19-21] Neurons were counted in three sections (at 50-µm intervals), and the results are expressed as the mean of these three sections.

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 one-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.

Results

Physiological Monitoring

SAH induction caused an immediate increase in ICP (from a baseline of 5 mmHg to >50 mmHg; $P<0.001$ vs. Sham; Figure 2A). The three SAH groups did not differ significantly with respect to post-SAH ICP, even though one group of animals had a DC before SAH induction (58±3 mmHg for SAH, 55±3 mmHg for DC after SAH, 51±5 mmHg for DC before SAH; $P=0.49$). After 15 minutes, ICP had decreased similarly among the three SAH groups (20±2 mmHg for SAH, 18±1 mmHg for DC after SAH, 20±1 mmHg 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 mmHg at 15 min, 8±1 mmHg at 30 min, 10±1 mmHg at 45 min; *P*<0.001; Figure 2B). A similar time course was observed in the DC before SAH group (20±1 mmHg at 15 min, 11±2 mmHg at 30 min, 11±2 mmHg at 45 min; *P*<0.05). 45 minutes after SAH, ICP was significantly lower in both the DC after SAH and the DC before SAH groups compared to the SAH group (17±2 mmHg for SAH, 10±1 mmHg for DC after SAH, 11±2 mmHg for DC before SAH; *P*<0.01; Figure 2A,B).

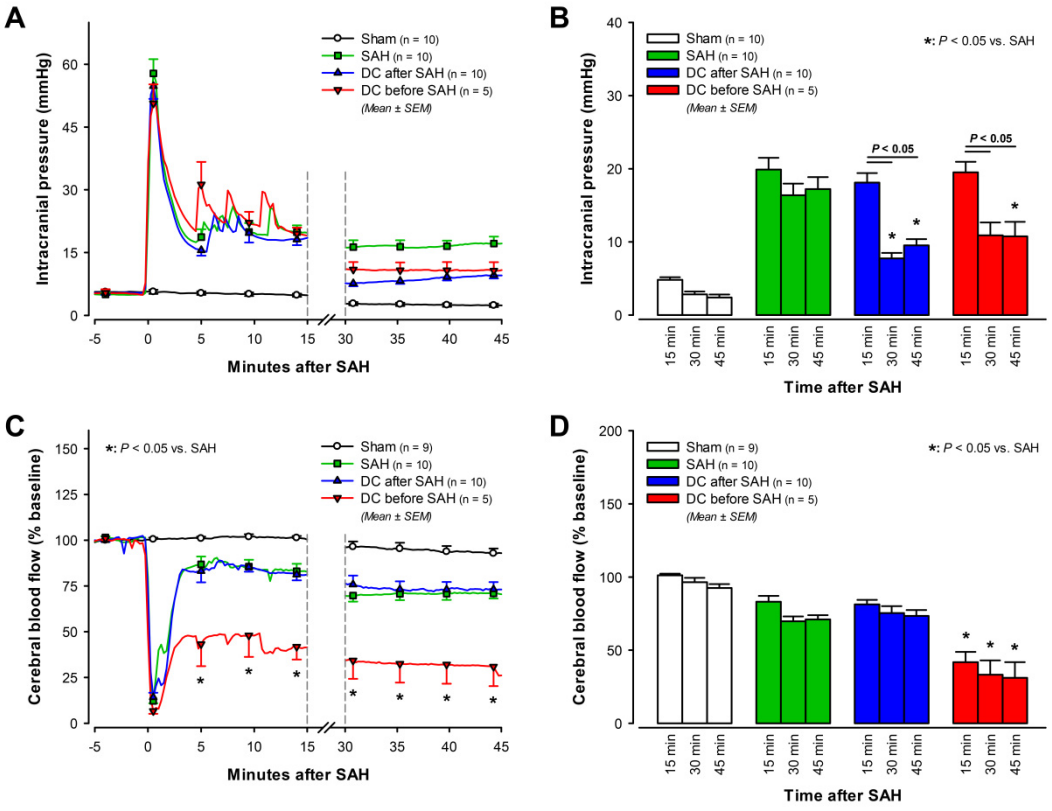

Figure 2. Intraoperative monitoring of intracranial pressure (ICP) and cerebral blood flow (CBF). Continuous ICP and CBF recordings (**A,C**) revealed that SAH induced a strong increase in ICP (to approximately 60 mmHg), which led to transient global cerebral ischemia. ICP then decreased and CBF recovered. Both 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**).

SAH induction also caused a dramatic concomitant decrease in ipsilateral CBF to <20% of baseline (*P*<0.001 vs. Sham; Figure 2C). All three SAH groups had a similar initial drop in CBF; however, 15 minutes after SAH, the DC before SAH group recovered significantly less than the other two SAH groups (83±4% for SAH, 81±3% for DC after SAH, 42±7% for DC before SAH; *P*<0.01). This difference remained throughout the 45-minute observation period (Figure 2C,D). Despite the significant reduction in ICP, CBF did not improve in the DC after SAH group (81±3% at 15 min, 75±5% at 30 min, 73±4% at 45 min; *P*=0.28; Figure 2C,D). DC performed in mice with sham surgery had no significant influence on these physiological parameters or on the following functional and histological outcome (see Figure I and Figure II in the online-only Data Supplement).

All animals in the study had similar physiological parameters throughout anesthesia (see Figure III in the online-only Data Supplement). The observed decrease in heart rate following SAH induction is part of the Cushing response to elevated intracranial pressure.

Re-bleeding

The mice in the DC before SAH group had a higher incidence of re-bleeding (80%) compared to the SAH group (50%) and the DC after SAH group (40%) (Figure 3A). In addition, these mice also showed a higher rate of re-bleedings per animal (0.5±0.2 for SAH, 0.4±0.2 for DC after SAH, 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 (i.e., higher 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 five days following surgery.

A loss in body weight could be seen in all four study groups; however, the mice in the two groups that underwent DC had the largest decrease (Figure 4B). In addition, these two groups recovered their body weight significantly more slowly than the Sham and SAH groups.

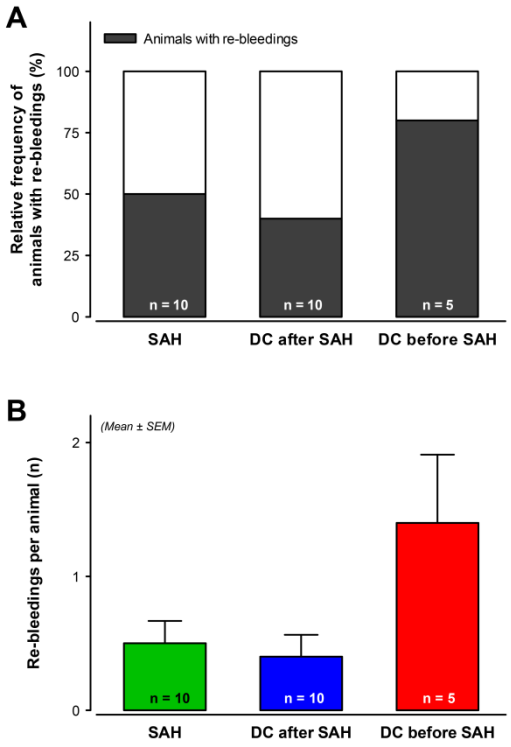

Figure 3. Incidence of re-bleeding. **A**, The mice in the DC before SAH group had a higher incidence of re-bleeding than the mice in the DC after SAH or SAH groups. **B**, Mice with DC before SAH had a higher rate of re-bleedings per animal.

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, due to ethical considerations and regulatory issues, only five animals were used in this group. In addition, because all five mice in this group died before the 7-day endpoint, no histomorphometry or histopathology data were available for this group.

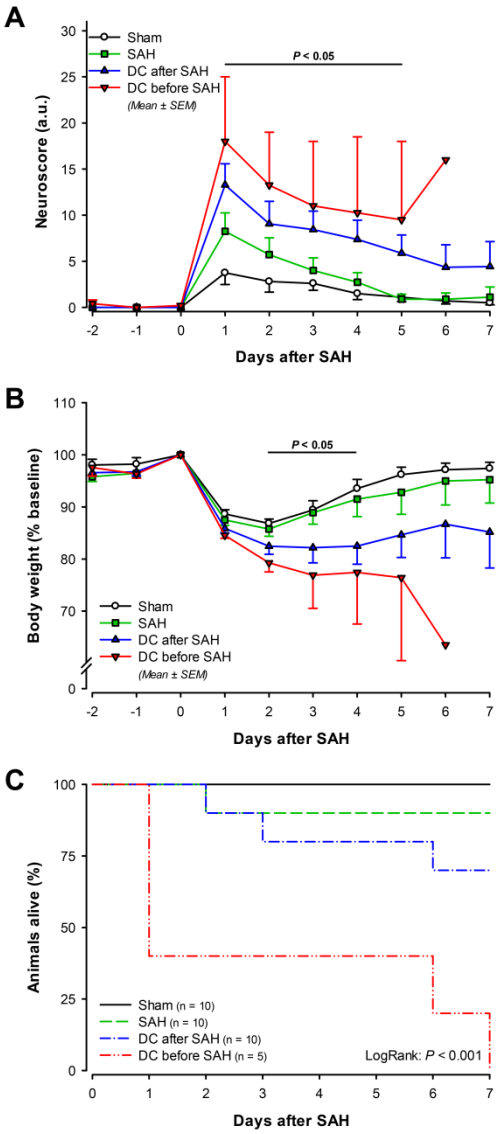

Figure 4. Functional outcome. **A**, All groups with SAH had a significantly worse neuroscore (best score: 0, worst score: 31; see Table I in the online-only Data Supplement) on the first day after surgery, and all groups recovered gradually. The mice with DC had significantly worse neuroscores in the first five days compared to the SAH group. **B**, All groups had a considerable decrease in body weight in the first three days following 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 seven days after hemorrhage.

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 to 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.

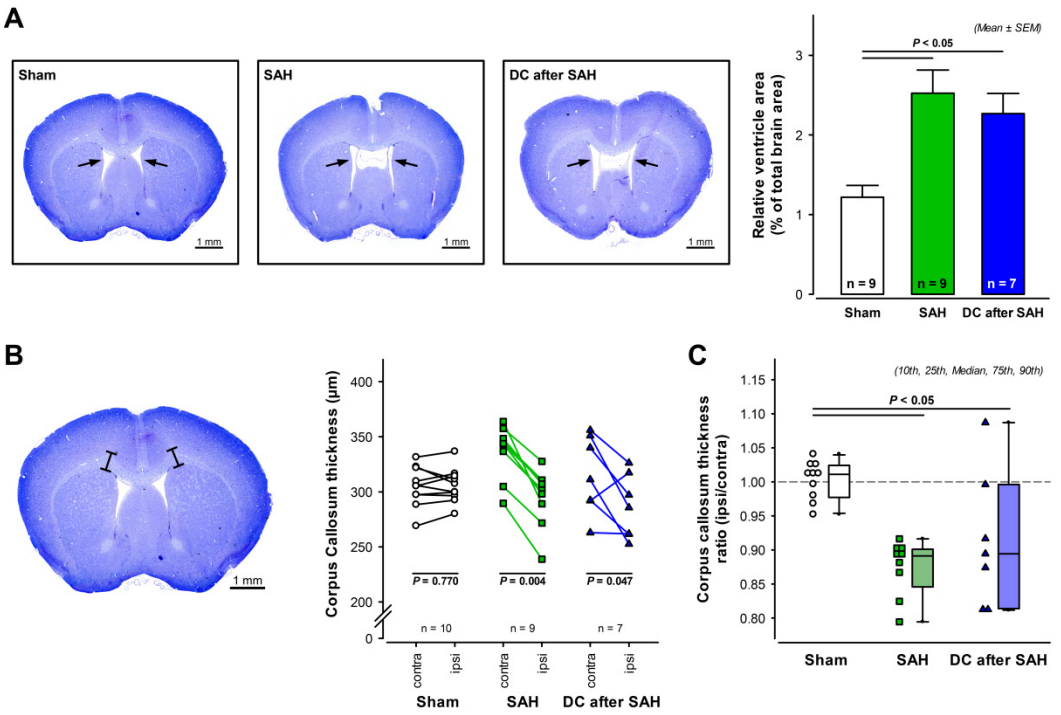

Figure 5. Histomorphometric evaluation seven days after surgery. **A**, Relative ventricle area (ventricle area divided by total brain area) was measured in cresyl violet-stained coronal sections. Both the SAH and the DC after SAH groups had significantly enlarged ventricles (arrows), a sign of hydrocephalus. DC had no effect on the extent of SAH-induced hydrocephalus. Evaluation of SAH-induced white matter damage - measured by corpus callosum thickness (**B**) and ipsilateral/contralateral ratio (**C**) - showed a lateralized effect ipsilateral to SAH induction.

Histopathology

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

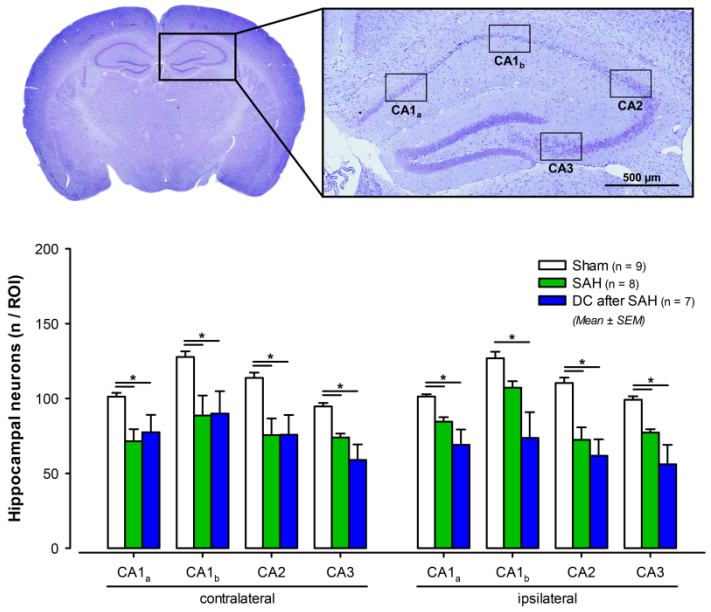

Figure 6. Histopathological evaluation of hippocampal neurons seven days after surgery. Neuronal damage was assessed by counting the surviving hippocampal pyramidal neurons in several regions of interest (ROI) in cresyl violet-stained coronal sections (**top**; adapted from Bühler et al.[21]). SAH led to a significant reduction in the number of viable neurons throughout the hippocampus (**bottom**). DC after SAH did not improve the survival of hippocampal neurons. **P*<0.05

Discussion

Performing decompressive craniectomy - either before or after SAH induction - reduced ICP significantly; however, the mice in the DC groups experienced a higher incidence of re-bleeding, 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 upon ICP, but is dependent

upon 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 re-bleeding that led to a high mortality rate and poor functional outcome. Using a different SAH model (e.g., injecting a predetermined volume of blood) would not have revealed these results, since in these models re-bleeding does not occur.

One goal of our study - specifically, to clarify the role of early ICP-induced global cerebral ischemia in brain damage following SAH - could not be achieved. The primary reason why we did not reach this goal was that both endpoints required for determining such a correlation were not reached. First, the mice maximally decompressed prior to 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 two other important points. First, in addition to its detrimental effect (in terms of rapidly inducing global ischemia), the initial peak in ICP following SAH has also a beneficial aspect, as it helps stop bleeding from the injured vessel and helps prevent re-bleeding. These findings support the concept of a "brain tamponade"[24] in which high ICP stops post-SAH bleeding, and these results are consistent with previous findings from our laboratory, which show that preventing re-bleeding is highly beneficial following 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 microvasospasm[22,23] 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 following SAH, we were able to investigate further effects of DC on functional outcome. Performing established neurological examinations[21] 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 (due to 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 towards 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 seven 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 TBI[10,11] 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.[30] However, only a few case reports describe the application of DC in SAH patients,[14-18] and the results of these reports are controversial due to different time points and indications for DC. For example, in patients with intractable intracranial hypertension and/or signs of brain swelling, DC can have a beneficial effect on functional outcome,[16-18] whereas DC has no benefit in cases of delayed cerebral ischemia.[18] 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 craniectomized patients[14] which is consistent with our results, despite the very early, clinically not relevant time points of DC in the current study. Therefore, randomized controlled clinical trials are urgently needed in order 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 prior to SAH resulted in a higher incidence of re-bleeding and a higher mortality rate. Therefore, because post-hemorrhagic ICP elevation seems to play an important role in the cessation of bleeding, it should not be reduced directly following SAH. Mechanistically, our data suggest that the hypoperfusion observed directly after SAH is not caused by elevated ICP, but is likely due to microcirculatory dysfunction. This hypothesis is based on the finding that DC did not affect

post-hemorrhagic 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.

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Disclosures

None.

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Supplemental Table

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

Task	Criteria	Score
Consciousness	Spontaneous exploration	0
	Movements after tactile stimulus	1
	No movements (comatose)	2
Whisker movements	Present	0
	Absent	1
Hearing (turning	Directed	0
to hand clapping)	Undirected	1
	Absent	2
Motor function	Normal	0
(per limb)	Stiff	1
	Paralyzed	2
Mod. Bederson	No obvious deficits	0
score	Flexed forelimbs	1
	Lowered resistance to lateral pushing	2
	Circling if pulled by tail	3
	Spontaneous circling	4
	No spontaneous activity	5
Placing test[a]	Present	0
	Absent	1
Beam walk	Normal movements	0
(3cm, 2cm, 1cm)	Improper paw placing	1
	Circling on beam	1.5
	No movements	2
	Falling off after few steps	3
	Falling off immediately	4
Total		0-31

[a] Front paws reaching ground when lifted by tail

Supplemental Figures

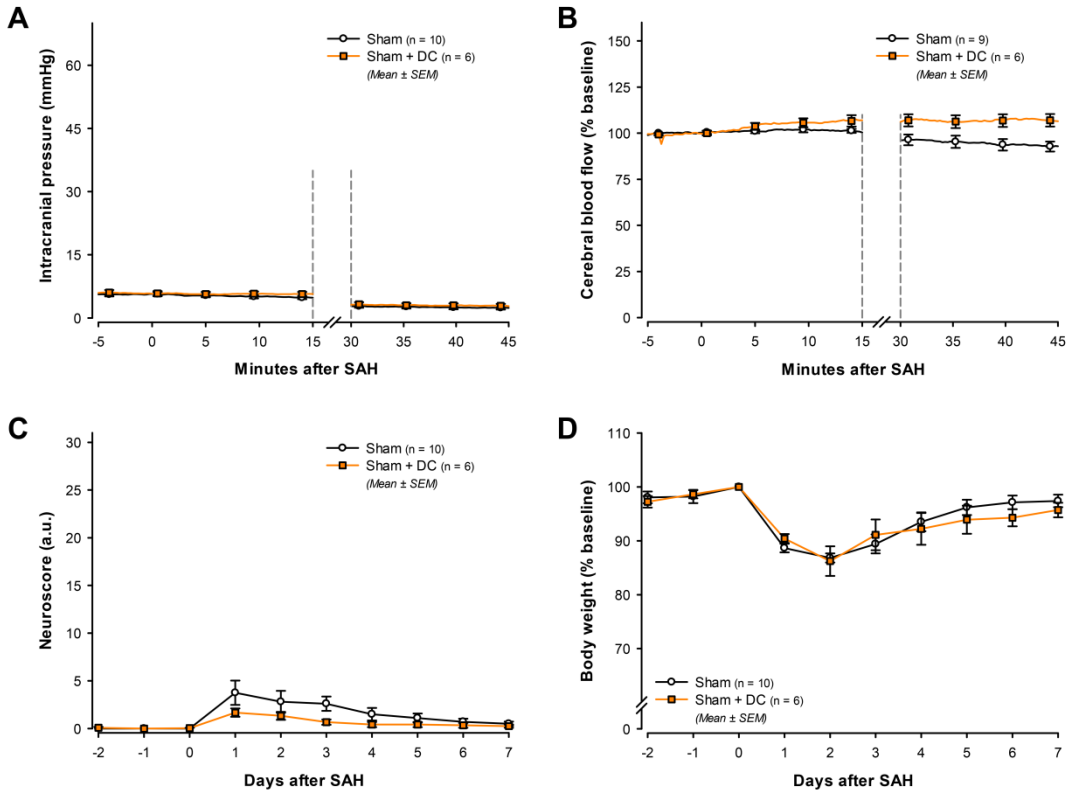

Figure I. Effects of DC in sham-operated animals on physiological and functional outcome. During surgery, there were no major alterations in ICP (**A**) or CBF (**B**). DC had also no influence on neurological outcome (**C**) or body weight recovery (**D**) after sham-surgery.

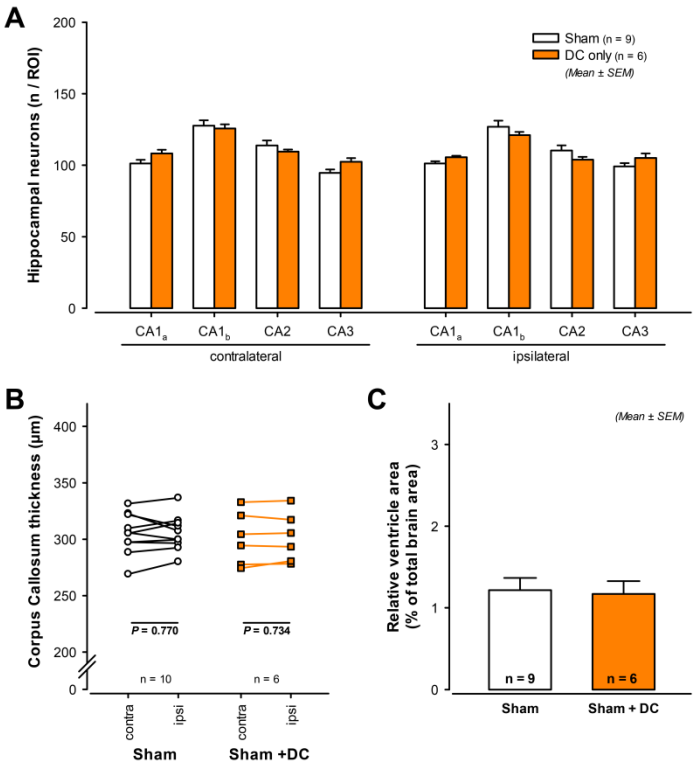

Figure II. Histological evaluation of DC in sham-operated animals seven days after surgery. DC had no influence on the number of hippocampal pyramidal neurons (**A**). There were also no alterations in corpus callosum thickness (**B**) or in relative ventricle area (**C**).

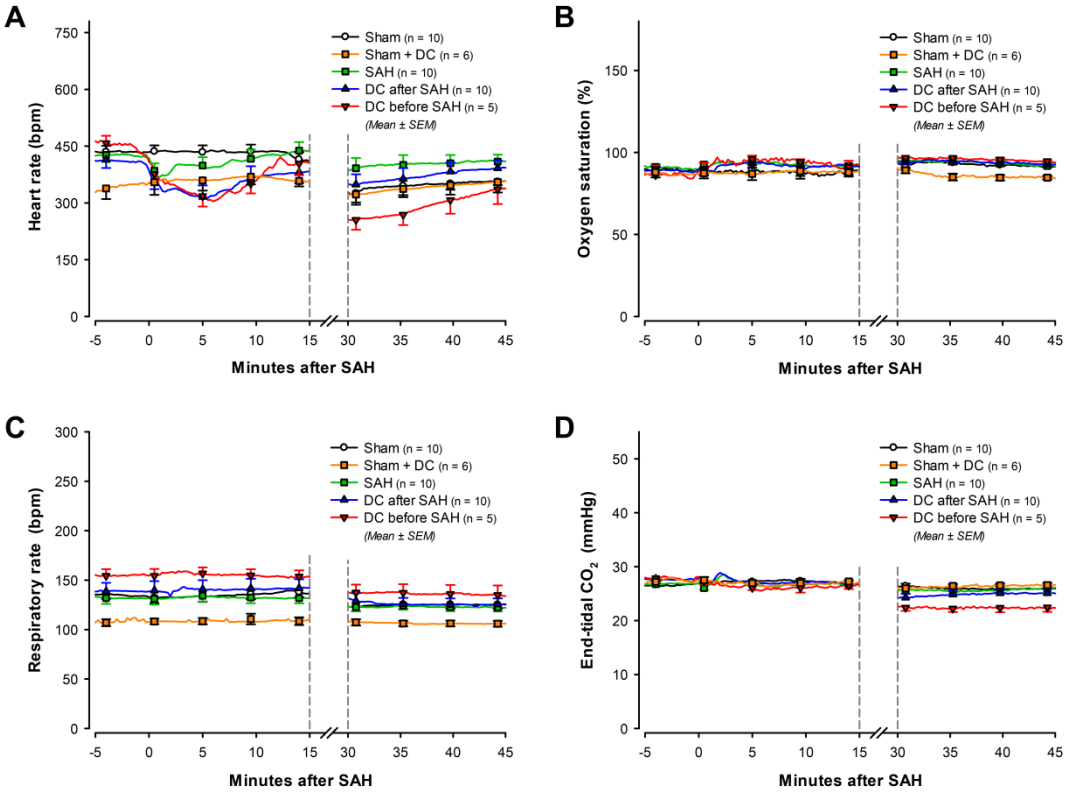

Figure III. Physiological monitoring during surgery. **A**, Cushing response to elevated ICP resulted in a drop in heart rate. The oxygen saturation (**B**), respiratory rate (**C**), as well as the end-tidal CO_2 (**D**) were in comparable ranges between the groups and constant during the whole surgery.

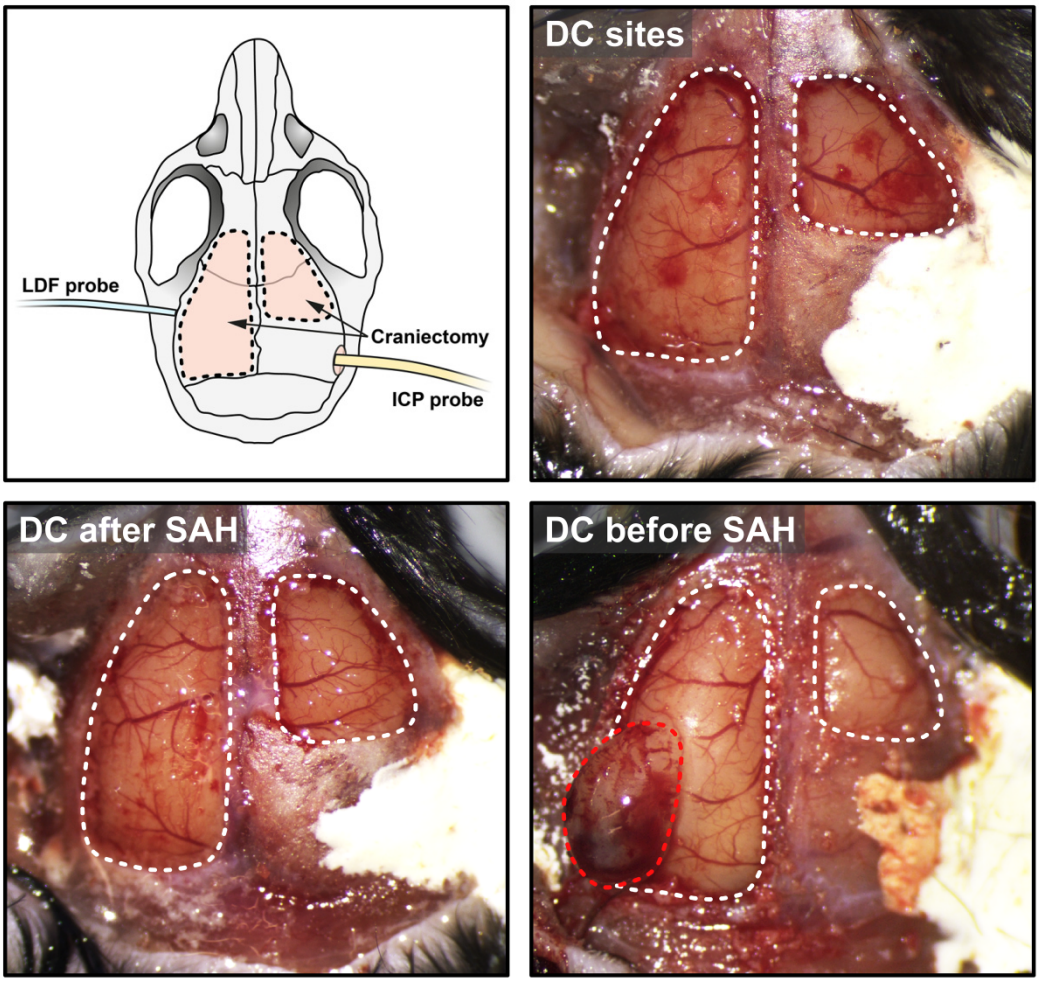

Figure IV. Craniectomy sites and outcome after SAH induction. **Top left,** Craniectomy sites and probe positions for physiological monitoring are indicated. **Top right,** Craniectomy (white dashed line) above both hemispheres without opening the dura mater. The skull above the superior sagittal sinus 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

1. Buhler D, Schuller K, Plesnila N. Protocol for the induction of subarachnoid hemorrhage in mice by perforation of the circle of willis with an endovascular filament. *Transl Stroke Res*. 2014;5:653-659

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