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ORIGINAL ARTICLE

Experimental Subarachnoid Hemorrhage: Double Cisterna Magna Injection Rat Model—Assessment of Delayed Pathological Effects of Cerebral Vasospasm

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Abstract Aneurysmal subarachnoid hemorrhage comprises of an early phase after the bleeding and a late phase of delayed consequences of the bleeding. The development of delayed injury mechanisms, like the reduction of cerebral blood flow (CBF) due to cerebral vasospasm (CVS), seems mainly to depend on the amount and the duration of the subarachnoid blood clot. The reduction of CBF may lead to cerebral ischemia and delayed neurological deterioration. The rat double cisterna magna injection model reproduces the time course of the delayed consequences of CVS and imitates the clinical setting more precise than other rodent subarachnoid hemorrhage models. Therefore, the rat double cisterna magna injection model seems to be predisposed to be used to mimick the delayed consequences of subarachnoid hemorrhage. We reviewed the existing literature on this animal model and propose a standard protocol including technical considerations, as well as advantages and limitations of this model.

Keywords Animal model · Cerebral vasospasm · Delayed ischemic neurologic deficit · Double-hemorrhage model · Intracranial aneurysm · Subarachnoid hemorrhage

Background

Historical Background of the Model

Aneurysmal subarachnoid hemorrhage (SAH) is a severe disease. Concerning their chronological order, the consequences of pathophysiological changes following SAH are often divided into early and delayed effects of the bleeding. Several experimental designs in different animal models exist to investigate these effects of early brain injury and delayed effects of cerebral vasospasm (CVS) due to SAH on neurological deterioration [1–3].

In 1979, the induction of SAH has been firstly described in a rat model [4]. It has been further refined into vessel disruption and blood injection models [5–7]. Concerning blood injection models, methods that include single- or double-injection strategies have been reported [5, 7]. However, the capability of rodents to rapidly clear blood in the subarachnoid space presents a difficulty in single-injection models [8]. Concerning single-injection models, the development of CVS has been described with a peak less than an hour after injection and with a second peak after day one or two [5, 9, 10].

Therefore, a double hemorrhage technique was introduced to better mimic delayed effects of SAH.

Materials and Methods

After its introduction, several technical modifications have been reported concerning the rat double hemorrhage model. To give a valuable overview, we performed a systematic review of the literature.

MEDLINE was searched for published studies based on the rat double hemorrhage model. The keywords "experimental subarachnoid hemorrhage," "double injection model," and "rats" were used. Identified studies from the MEDLINE search were further evaluated for inclusion in the systematic review. Two reviewers independently extracted data (V.B. and P.S.). Full-text versions were obtained from all studies that were considered to be potentially relevant by both reviewers. The methods of the used models had to be

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Table 1 Results from the systematic review concerning the rat double hemorrhage model

First author, year [Ref.]	Short description	Animal breed, gender	Animal weight (g)	Time of 2nd SAH (h)	Method of anesthesia	Baseline parameters during SAH	Outcome measures	Technical considerations
Rickels [11]	DHM with injection of CSF-venous blood mixture into cisterna magna	SPF, male	250–300	24	Xylazine and ketamine i.p.	Body temperature, MABP	Local CBF, ECG, extra-cellular potassium and calcium conc.	Placing into stereotaxic frame, exposure of AOM, insertion of a 25 G needle into cisterna magna and withdrawal of 0.1 ml CSF, 0.4 ml venous blood from retrobulbar venous plexus and mixing with CSF, induction of SAH via injection of 0.1 ml CSF-venous blood-mixture into cisterna magna, head down positioning for few min, and 2nd SAH in the same fashion after 24 h
Widenka [13]	DHM with injection of autologous arterial blood into cisterna magna	Wistar, male	250–300	24	Chloral hydrate i.p.	Body temperature, heart rate, MABP, paO_2, $paCO_2$, and pH	Angiography for verification of CVS (7 days after 2nd SAH) and microscopic analysis of section of ICA, MCA, and BA	Prone position within stereotaxic frame, exposure of AOM via suboccipital incision, injection of 0.1 ml arterial blood-CSF-mixture as bolus into cisterna magna using 25 G needle following aspiration of 0.1 ml CSF, wound closure after 5 min and head down positioning for another 5 min, and 2nd SAH in the same fashion after 24 h
Ryba [12]	DHM with injection of autologous arterial blood into cisterna magna and previous ligation of both CCA	Wistar, male and female	280–350	48	Ketamine and xylazine i.p.	n.a.	Neurological assessment, EM of BA, and immunoassay of NOS and ß-APP (7 days after 2nd SAH)	Ligation of both ACC 14 days before SAH-induction, prone position with fixed head in stereotaxic frame, injection of 0.1 ml non-heparinized autologous arterial blood into cisterna magna using 28 G needle over 1 min , and 2nd SAH in the same fashion after 48 h
Suzuki [9]	DHM with injection of autologous arterial blood intos cisterna magna	Sprague–Dawley, male	350–450	48	Chloral hydrate i.p.	Rectal temperature and MABP	Angiography for verification of CVS (d0–d14) and PCR for detection of expression of heme-oxygenase-1 in BA and brain tissue	Prone position within stereotaxic frame with flexion-angle of 30°, midline incision from cal-varium to the lower cervical spine, injection of 0.3 ml autologous arterial blood with 27 G needle into cisterna magna over 10 min, and 2nd SAH in the same fashion after 48 h
Miyagi [15]	DHM with injection of autologous arterial blood into cisterna magna	Sprague–Dawley, male	350–400	48	Ketamine and xylazine i.p.	Body temperature	Neurological assessment, morphological analysis of BA using light micro-scope and TEM, and PCR for detection expression of rho-A and rho-kinases on d3, d5, and d7	Midline incision from vertex to the lower cervical spine, injection of 0.3 ml autologous arterial blood with 27 G needle into cisterna magna over 3 min using surgical micro-scope following withdrawing of 0.3 ml CSF, head-down positioning for 30 min after injection, and 2nd SAH in the same fashion after 48 h
Lefranc [14]	DHM with injection of autologous arterial blood into cisterna magna	n.a.	n.a.	48	Ketamine and xylazine i.p.	n.a.	Angiography of BA for verification of CVSand immunohistochemistry of BA for detection of expression of S-100B in BA	Midline incision from vertex to the middle cervical spine, exposure of AOM using surgical microscope, injection of 0.3 ml autologous arterial blood with 27G needle into cisterna magna, head-down positioning for 30 min after injection, and 2nd SAH in the same fashion after 48 h

Table 1 (continued)

First author, year [Ref.]	Short description	Animal breed, gender	Animal weight (g)	Time of 2nd SAH (h)	Method of anesthesia	Baseline parameters during SAH	Outcome measures	Technical considerations
Satoh [16]	DHM with injection of autologous arterial blood into cisterna magna	Sprague–Dawley, male	260–340	48	Ketamine and xylazine i.p.	Rectal temperature	Lumen cross-section area of BA, immunohisto-chemistry for detection of MAPKs in BA, and western blotting for detection of expression of MAPKs in BA	Midline incision in the neck in prone position, exposure of AOM using surgical microscope, injection of 0.35 ml autologous arterial blood with 25 G needle into cisterna magna over 3 min after withdrawal of 0.1 ml CSF, head-down positioning for 30 min after injection, and 2nd SAH in the same fashion after 48 h
Aladag [18]	DHM with injection of autologous heparinized venous blood into cisterna magna	Wistar, male	225–250	48	Ketamine and xylazine i.p.	Body temperature	Lumen cross-section area of BA and wall-thickness and biochemical analysis	Antibiotic prophylaxis before surgery, injection of 0.3 ml autologous heparinized venous blood into cisterna magna with 27 G needle over 10 min, and 2nd SAH in the same fashion after 48 h
Vatter [22]	Modified DHM with injection of autologous arterial blood into cisterna magna	Sprague–Dawley, male	250–410	24	Midazolam and ketamine i.p.	Body temperature	Neurological assessment, DSA of BA, and MRI with evaluation of rrCBF and rrCBV	Prone position with fixed head in stereotaxic frame, injection of 0.2 ml autologous arterial blood into cisterna magna using PE-10-catheter, withdrawing of 0.1 ml CSF before blood injection, and 2nd SAH in the same fashion after 24 h
Takata et al. [21]	DHM with injection of autologous arterial blood into cisterna magna	Wistar, male	300–350	48	1.6–1.8 % isofluran via endotracheal tube	Pericranial temperature, MABP, paO_2, $paCO_2$, pH, Hkt, and blood glucose	Rotarod, vertical screen, and balance beam tests; Morris water maze; diameter of BA and MCA; and rCBF	Prone position with fixed head in stereotaxic frame, injection of 0.5 ml autologous arterial blood into cisterna magna using PE-10 catheter over 10 min, before blood-injection withdrawing of 0.2 ml CSF, head down position after injection, and 2nd SAH-induction after 48 h with 0.3 ml autologous arterial blood
Lee [19]	DHM with injection of autologous arterial blood into cisterna magna	Sprague–Dawley, male	280–350	24	2.25–2.75 % isoflurane via endotracheal tube	Body temperature, MABP, paO_2, and $paCO_2$	Diameter and lumen cross-section areas of BA and rCBF	Injection of 0.2 ml autologous arterial blood into cisterna magna with PE-10 catheter. 2nd SAH-induction after 24 h with 0.1 ml autologous arterial blood
Lu [20]	DHM with injection of autologous arterial blood into cisterna magna	Sprague–Dawley, male	300–350	48	Pentobarbital i.p.	Body temperature, MABP, paO_2, and $paCO_2$	Lumen cross-section areas of BA, immunohistochemistry, western blot, and PCR for detection of MCP-1	Injection of 0.3 ml autologous arterial non-heparinized blood into cisterna magna with 27 G needle over 2 min, head down position after injection, and 2nd SAH in the same fashion after 48 h
Wang [29]	DHM with injection of autologous arterial blood into cisterna magna	Sprague–Dawley, male	300–350	48	Pentobarbital i.p.	n.a.	Lumen cross-section areas of BA and immunohistochemistry and western blot for detection of Nrf2	Injection of 0.3 ml autologous arterial non-heparinized blood into cisterna magna with 27 G needle over 2 min, head down position after injection, and 2nd SAH in the same fashion after 48 h

Table 1 (continued)

First author, year [Ref.]	Short description	Animal breed, gender	Animal weight (g)	Time of 2nd SAH (h)	Method of anesthesia	Baseline parameters during SAH	Outcome measures	Technical considerations
Güresir [24]	Modified DHM with injection of auto-logous arterial blood into cisterna magna and previous ligation of left CCA	Sprague–Dawley, male	280–360	24	Midazolam and ketamine, i.p.	Body temperature, systolic BP, paO_2, $paCO_2$, and art. pH	Neurological assessment, evaluation of rrCBF and rrCBV with MRI, histology of cross-sections for evaluation of BA-diameter, and histological assessment of brain tissue damage within hippocampus and adjoining cortex	Ligation of the left CCA using ventral approach, prone position with fixed head in stereotaxic frame, injection of 0.25 ml autologous arterial blood into cisterna magna using PE-10 catheter, before blood-injection withdrawing of 0.1 ml CSF, head down position after injection, and 2nd SAH in the same fashion after 24 h
Cai [23]	DHM with injection of autologous arterial blood into cisterna magna	Sprague–Dawley, male	350–400	24	Xylazine and ketamine i.p.	Body temperature	Neurological assessment, ICP, CBF, synchrotron radiation angiography, and histological examination of ACA and MCA	Injection of 0.2 ml autologous arterial blood into cisterna magna with PE-10 catheter after aspiration of 0.1 ml CSF, head down position after injection, and 2nd SAH in the same fashion after 24 h
Hänggi [26]	DHM with injection of autologous arterial blood into cisterna magna	Wistar, male	250–350	24	Xylazine and ketamine i.p.	n.a.	Neurological assessment, DSA, and immunohistochemistry	Injection of 0.2 ml autologous arterial blood into cisterna magna with 27 G needle, before blood-injection withdrawing of 0.2 ml CSF, head down position after injection, and 2nd SAH in the same fashion after 24 h
Raslan [28]	Modified DHM with injection of autologous arterial blood into cisterna magna with stereotaxic device	Sprague–Dawley, male	280–350	24	2.5–3 % isoflurane via endotrachel tube	Body temperature, MABP, paO_2, $paCO_2$	Neurological assessment, rCBF, and histological examination of BA	Injection of 0.2 ml non-heparinized autologous arterial blood into cisterna magna over 3 min, blood injection performed with stereotactically controlled PE-10 catheter, before blood-injection withdrawing of 0.1 ml CSF; and 2nd SAH in the same fashion after 24 h, but using injection with 0.1 ml non-heparinized autologous arterial blood
Güresir [25]	Modified DHM with injection of autologous arterial blood into cisterna magna	Sprague–Dawley, male	245–385	24	Midazolam and ketamine, i.p.	Body temperature, systolic BP, Hkt, paO_2, $paCO_2$, and art. pH	Neurological assessment, concentration of EPO in arterial blood and CSF, evaluation of CBF and CBV with MRI, and diameter of BA	Prone position with fixed head in stereotaxic frame, injection of 0.25 ml autologous arterial blood into cisterna magna using PE-10 catheter, before blood-injection withdrawing of 0.1 ml CSF, head down position after injection, and 2nd SAH in the same fashion after 24 h

Ref. reference, *SAH* subarachnoid hemorrhage, *h* hours, *DHM* double hemorrhage model, *CSF* cerebrospinal fluid, *CCA* common carotid artery, *i.p.* intraperitoneal, *MABP* mean arterial blood pressure, *CBF* cerebral blood flow, *AOM* atlantooccipitale membrane, *n.a.* not applicable, *EM* electron microscopy, *TEM* transmission electron microscopy, *DSA* digital substraction angiography, *MRI* magnetic resonance imaging, *CBV* cerebral blood volume

described in detail. Any disagreement between the reviewers concerning article inclusion or exclusion was resolved by consensus of a third author (E.G.).

Results

We included 20 studies based on the rat double hemorrhage model [9, 11–29]. Details on included studies and technical modifications are given in detail in Table 1. In the following description of the double hemorrhage rat model, several technical considerations from included studies of the review of the literature are described complementary to our own experiences.

Animals

Adult male Sprague–Dawley or Wistar rats, usually weighing 225 to 450 g, are commonly used for the double hemorrhage model [7, 17, 22, 24].

Anesthesia, Analgesia, Monitoring, and Perioperative Care

Intraperitoneal application of midazolam (1 mg/kg body weight) and ketamine (100 mg/kg body weight) is used for anesthesia because it appears to be safe and practicable [17, 22, 25, 30–32]. Sedated with this medication, animals are able to breathe spontaneously and sufficient. Furthermore, circulation parameters are stable, and a derangement of cerebral blood flow (CBF) is not observed using this medication [33]. Nevertheless, intubation and mechanical ventilation have been suggested in order to ensure stable physiological conditions [34]. On the other hand, optimal physiological conditions in intubated and mechanical ventilated rodents might retain several acute effects of SAH induction in these animals. However, there are several modifications reported concerning anesthesia: intraperitoneal application of ketamine and xylazine [11, 12, 14–16, 18, 23, 26], chloral hydrate [9, 13], pentobarbital [20, 29], or isoflurane via an endotracheal tube [19, 21, 28] (Table 1).

During the procedure, the body temperature of the animals should be maintained at approximately 37 °C, most commonly by a heading pad. Several other parameters are used to monitor animals during the procedure: middle arterial blood pressure [9, 11, 13, 19–21, 28], systolic blood pressure [24, 25], heart rate [13], paO_2 and $paCO_2$ [13, 19–21, 24, 25, 28], hematocrit [21, 25], or blood glucose [21]. An overview of physiological parameters is provided in Table 2.

After induction of SAH, application of 5 ml crystalloid solution and 0.0125 mg Fentanyl subcutaneously is recommended. In order to provide sufficient analgesia, animals should receive 5 ml crystalloid solution and 0.0125 mg Fentanyl subcutaneously twice a day during the following observation or treatment course.

Table 2 Physiological parameters

Parameter	Reported values	References
Body weight (g)	225–450	Rickels et al. [11], Widenka et al. [13], Suzuki et al. [9], Ryba et al. [12], Miyagi et al. [15], Satoh et al. [16], Aladag et al. [18], Vatter et al. [22], Takata et al. [21], Lee et al. [19], Lu et al. [20], Güresir et al. [24], Cai et al. [23], Hänggi et al. [26], Raslan et al. [28], and Güresir et al. [25]
Body temperature (in °C)	37	Rickels et al. [11], Widenka et al. [13], Suzuki et al. [9], Miyagi et al. [15], Satoh et al. [16], Aladag et al. [18], Vatter et al. [22], Lee et al. [19], Lu et al. [20], Güresir et al. [24], Cai et al. [23], and Güresir et al. [25]
Mean arterial blood pressure **Systolic blood pressure	82–120 mmHg 110–115 mmHg	Rickels et al. [11], Widenka et al. [13], Prunell et al. [35], Cai et al. [23], and Güresir et al. [25]**
ICP	5–7 mmHg	Prunell et al. [35] and Cai et al. [23]
pCO_2	40–42 mmHg	Widenka et al. [13] and Güresir et al. [25]
pO_2	79–98 mmHg	Widenka et al. [13] and Güresir et al. [25]
pH	7.38–7.4	Widenka et al. [13] and Güresir et al. [25]
Blood glucose	96 mg/dl	Takata et al. [21]
Hematocrit	41–48	Prunell et al. [35] and Güresir et al. [25]
Cross-section diameter		
BA	145–291 μm	Aladag et al. [18], Lee et al. [19], Güresir et al. [24], and Güresir et al. [25]
MCA	225 μm	Cai et al. [23]
ACA	219 μm	Cai et al. [23]
CSF (total volume)	396 μl	Prunell et al. [35]

ICP intracranial pressure, *BA* basilar artery, *MCA* middle cerebral artery, *ACA* anterior cerebral artery, *CSF* cerebrospinal fluid

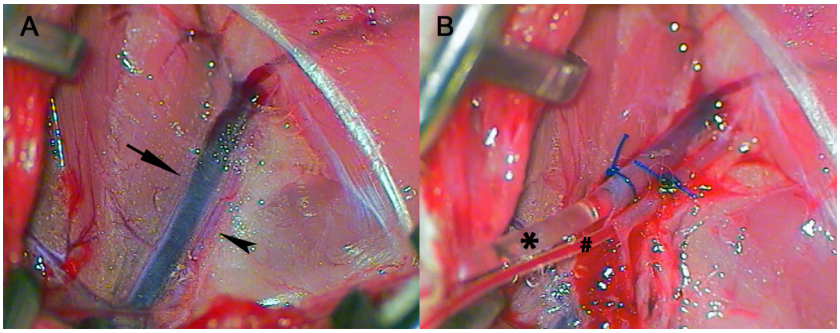

Fig. 1 **a** Preparation of the femoral vein (*arrow*) and femoral artery (*arrow head*). A portex tube is inserted into the femoral artery (*hash mark*) for measurement of blood gas values, for control of blood pressure, and for blood sample withdrawal. **b** The femoral vein is cannulated with another tube (*asterisk*) for administration of additional medication or infusions

SAH Induction

After onset of anesthesia, the left femoral artery is punctuated and a tube (Portex® polythene tube, luminal diameter, 0.96 mm) is inserted for measurement of blood gas values, circulation parameters, and as source of the later injected autologous blood. For additional medication or infusions, another tube is inserted in the left femoral vein. Both tubes are plugged after irrigation with saline infusion, stored under the skin, and left inside the vessels until the animals are sacrificed (Fig. 1).

After insertion of both tubes into the vessels and stable conditions, animals are positioned in a stereotactic frame [9, 11–13, 21, 22, 24, 25].

A medial incision is performed after infiltration of the skin and the muscles with local anesthesia (e.g., mepivacaine 1 %). Skin incision should cover the suboccipital region and the arch of C1 (Fig. 2a). Acromiotrapezius muscle is cut strictly along the midline to avoid bleeding. Afterwards, the suboccipital region, the atlanto-occipital membrane and the C1 arch are exposed in a stepwise fashion. After careful surgically disclosure of the atlanto-occipitale membrane, a tube (Portex® polythene tube; luminal diameter, 0.28 mm) is inserted in the cisterna magna (Fig. 2b/c) [22, 24, 25]. Using this tube, 0.1 ml of cerebrospinal fluid is withdrawn. Thereafter, 0.2 ml autologous arterial blood from the femoral artery approach is injected into the cisterna magna through the suboccipital tube to induce the SAH. Takata et al. reported the use of 0.5 ml autologous arterial blood during the initial SAH induction, and 0.3 ml for the second SAH induction [21]. Furthermore, the injection of heparinized venous blood or of a CSF-/blood-mixture has been reported [11, 13, 18]. Additionally, various time ranges for the blood injection have been described: 1 [12], 2 [20, 29], 3 [15, 16, 28], and 10 min [9, 18, 21]. To ensure an optimal subarachnoid distribution of the administered autologous blood, animals are then brought in a head down position and kept in this position for approximately 15 min. Nevertheless, other time periods for optimal subarachnoid distribution of the administered blood have been reported: 5 [13] and 30 min [14–16].

The identical surgical procedure was then repeated 24 h after initial SAH for induction of the second SAH [11, 13, 19, 22–26, 28]. Several other reports suggested a second SAH 48 h after initial SAH [9, 12, 14–16, 18, 20, 21, 29]. For the second SAH induction, several other groups reported the use of a reduced amount of blood compared to the initial SAH induction [19, 21, 28].

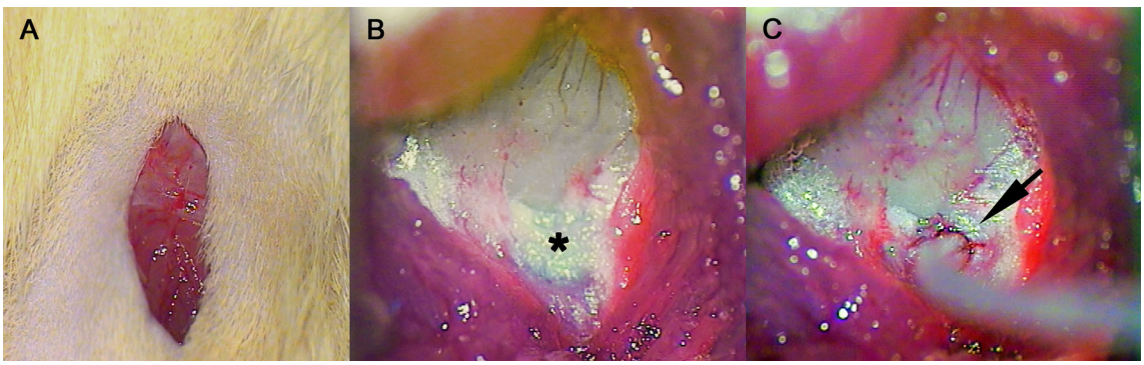

Fig. 2 Preparation of cisterna magna: suboccipital skin incision (**a**), the nuchal muscle layers were divided in the midline and the atlanto-occipital membrane (*asterisk*) was exposed (**b**). After insertion of a catheter into the cisterna magna (*arrow*), 0.1 ml CSF is withdrawn followed by injection of 0.2 ml autologous arterial blood to induce SAH (**c**)

Table 3 Expected pathophysiological parameters

Parameter	Expected pathophysiological changes	References
Acute changes		
Mean arterial blood pressure	⇔ ⇓ ⇑	Widenka et al. [13] and Güresir et al. [25] Rickels et al. [11] Prunell et al. [35]
Intracranial pressure	⇑⇑	Prunell et al. [35] and Cai et al. [23]
% of cerebral blood flow reduction	⇓⇓ (20–75 % of baseline)	Prunell et al. [35], Vatter et al. [22], Takata et al. [21], Lee et al. [19], Güresir et al. [24], Cai et al. [23], Raslan et al. [28], and Güresir et al. [25]
Mortality	0 % 10–50 %	Suzuki et al. [9] and Ryba et al. [12] Aladag et al. [18], Vatter et al. [22], Lu et al. [20], Wang et al. [29], Güresir et al. [24], Hänggi et al. [26], and Cai et al. [23]
Neurological deficits		
Acute	⇑	Ryba et al. [12] and Hänggi et al. [26]
Delayed	⇑	Vatter et al. [22], Güresir et al. [24], and Güresir et al. [25]
Time course of DCVS (angiographic)		
Peak onset (d)	d1 d3 d5 d7	Lefranc et al. [14] Miyagi et al. [15], Vatter et al. [22], Lu et al. [20], Güresir et al. [24], and Güresir et al. [25] Ryba et al. [12], Suzuki et al. [9], Satoh et al. [16], Lee et al. [19], and Wang et al. [29] Rickels et al. [11] and Widenak et al. [13]
Max. DCVS (d)	d5	Miyagi et al. [15], Vatter et al. [22], Güresir et al. [24], and Güresir et al. [25]
Cross section diameter		
BA	⇓⇓	Suzuki et al. [9], Miyagi et al. [15], Satoh et al. [16], Aladag et al. [18], Vatter et al. [22], Takata et al. [21], Lee et al. [19], Lu et al. [20], Wang et al. [29], Güresir et al. [24], and Güresir et al. [25]
MCA	⇓	Widenka et al. [13], Takata et al. [21], and Cai et al. [23]
ACA	⇓	Cai et al. [23]
Histological changes	wall thickness ⇑ corrugation of IEL ⇑	Satoh et al. [16], Aladag et al. [18], Lu et al. [20], Wang et al. [29], and Güresir et al. [25] Ryba et al. [12], Miyagi et al. [15], Satoh et al. [16], Lee et al. [19], Lu et al. [20], and Wang et al. [29]
Quantitative data on neuronal cell count		
Apoptotic index neuronal cells		
Time point after SAH	d35[d], d3[e] and d5[e,f]	[d]Takata et al. [21], and [e,f]Güresir et al. [24, 25]
Hippocampus CA1	⇓[d] d3: ⇔[e], d5: ⇓[e,f]	[d]Takata et al. [21] [e,f]Güresir et al. [24, 25]
Hippocampus CA2	d3: ⇔[e], d5: ⇓[e,f]	[e,f]Güresir et al. [24, 25]
Hippocampus CA3	d3: ⇔[e], d5: ⇓[e,f]	[e,f]Güresir et al. [24, 25]
Hippocampus CA4	d3: ⇔[e], d5: ⇓[e,f]	[e,f]Güresir et al. [24, 25]
Cortex	⇓[d] d3: ⇔[e], d5: ⇓[e,f]	[d]Takata et al. [21] [e,f]Güresir et al. [24, 25]
Semi-quantitative data on micro-thrombembolism		[a]Prunell et al. [35], [b]Lee et al. [31], and [c]Hänggi et al. [26]
Hippocampus CA1	14 %[a]	[a]Prunell et al. [35]
Hippocampus CA2	n.a.	n.a.
Hippocampus CA3	n.a.	n.a.
Dentate gyrus	n.a.	n.a.
Cortex	9 %[b], 20–40 %[c], 23–25 %[g]	[b]Lee et al. [31], [c]Hänggi et al. [26], [g]Wang et al. [29]
Brain water content (%)	79.5	Lee et al. [19]

Parameters expected to be ⇓ = decreased, ⇔ = without change (not significant), and ⇑ = increased after induction of SAH

DCVS delayed cerebral vasopasm, *BA* basilar artery, *MCA* middle cerebral artery, *ACA* anterior cerebral artery

The superscript letters in the "expected pathophysiological changes" refer to the authors who analyzed those changes

Table 4 Advantages and limitations

Advantages	Limitations
Experimental setting manageable and can be established in most centers	Seems less suitable to study acute brain injury
Good imitation of CVS and delayed ischemic lesions	Variations in amount of blood in subarachnoid space
CVS, reduction of CBF are more pronounced compared to single-injection models	High mortality rate
Cost effective	

CVS cerebral vasospasm, *CBF* cerebral blood flow

Treatment and/or Observation Course After SAH Induction

During the following treatment or observation course, several changes in physiological parameters are expected and have been investigated. Table 3 provides an overview concerning the expected pathophysiological parameters in the rat double hemorrhage model.

Possible outcome measures include neurological assessment with investigation of motor deficits [12, 22, 36]. Furthermore, several radiological investigations were established using digital subtraction angiography (DSA) or magnetic resonance imaging in order to detect and/or monitor CVS and consecutive impairment of cerebral perfusion [5, 17, 22, 37]. In order to verify morphological damage after SAH, histological analyses were performed for the rat double hemorrhage model [17, 24]. Considering the more distinctive sensitivity of the hippocampus and the adjoining cortex areas, vital neurons were counted to depict CVS-related cerebral ischemia.

Furthermore, functional investigations of cerebral arteries were performed to analyze possible drug effects on relaxation or contractibility of cerebrovascular structures [25, 27, 38, 39].

Technical Considerations

An initial small skin incision assures a decrease of surgical trauma and therefore reduction of stress to the animals. The use of a flexible polythene tube for injection of autologous blood into the cisterna magna might minimize the risk of brainstem injuries. The leakage of injected arterial blood can be avoided by adding fibrillar haemostypticum patches (Tabotamp®) and cotton. To avoid collateral damage, injection of autologous blood itself should be carried out slowly with injection volumes no greater than 0.25 ml.

Advantages and Limitations of the Model

Investigation of pathological consequences of aneurysmal subarachnoid hemorrhage is a multifactorial challenge involving consideration of early and delayed effects on neurological deterioration.

When dividing pathological changes after SAH into early and delayed effects, several authors stated that the rat double hemorrhage model seems more eligible to imitate delayed effects of SAH [6, 31, 35, 40] (Table 4). Regarding the emulation of early effects of SAH, perforation models seem to be more suitable [31]. However, concerning the subarachnoid blood distribution, a great variation in amount of subarachnoid blood in injection and perforating models has been reported [35, 40]. While reflecting the clinical setting, great variation of subarachnoid blood volume might lead to the need of larger experimental groups to retain the standard protocol. Furthermore, it has been reported that only a fraction of the previously injected autologous blood was found intracranially when compared to other models [35, 40]. As mentioned above, this

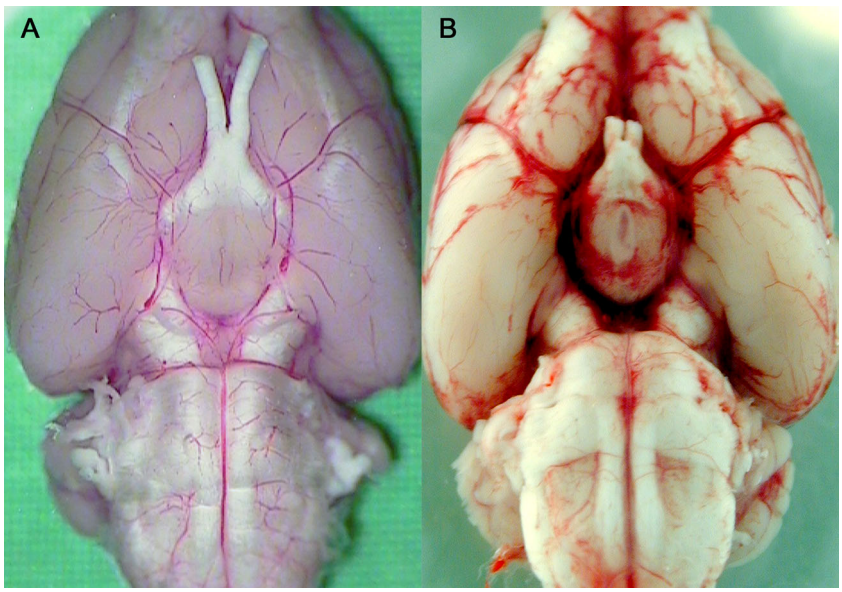

Fig. 3 Basal view of the brain in a sham-operated animal (**a**). Distribution of the blood in the subarachnoid space demontrated by basal view of the brain after induction of SAH (**b**)

might be controlled by adding fibrillar haemostypticum patches to avoid leakage and therefore to better steer the amount of blood injected into the cisterna magna (Fig. 3). The reported high mortality rate in the rat double hemorrhage model has been criticized, but also indicates the induction of severe experimental SAH and is comparable to the clinical situation in humans.

Nevertheless, besides some limitations the rat double hemorrhage model is a feasible, effective, and customizable rodent model for experimental SAH (Table 4).

First, the experimental setting is cost-effective [41], manageable, and can be established in most centers. As mentioned above, this model provides a good imitation of the clinical setting and time course of delayed effects of CVS in humans suffering from SAH. Eventually, the degree of severity and characteristics of CVS as well as the reduction of CBF are more pronounced in the rat double hemorrhage model when compared to single injection models [6]. As mentioned above, development of CVS was reported shortly after injection and after day one or two in single injection models [5, 9, 10]. In contrast, maximal CVS onset has been reported on day 5 or day 7 after initial SAH in the double hemorrhage model [6, 22]. Despite the rats capability for rapid clearance of blood after subarachnoid hemorrhage, the second SAH induction during the conduction of the double hemorrhage models seem to secure an adequate amount of periarterial blood for longer time period compared to single injection models [6].

Furthermore, the overall mortality rate in double hemorrhage models has been described as high as 50 % [17, 22, 24, 25]. This might even be increased by further invasive diagnostic procedures, e.g., DSA. As described before, death mostly occurred immediately after SAH induction or within 6 h [31]. Nevertheless, the high mortality rate in the double hemorrhage model seems to indicate the successful induction of a severe experimental SAH.

In conclusion, the double hemorrhage model is a well established rodent model to simulate the delayed effects of SAH, and to investigate the use of drugs on morphological ischemic, functional, and delayed consequences of SAH.

Conflict of Interest Erdem Güresir, Patrick Schuss, Valeri Borger, and Hartmut Vatter declare that they have no conflict of interest.

Compliance with Ethics Requirements All institutional and national guidelines for the care and use of laboratory animals were followed.

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