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Acute Subdural Hematoma in Pigs: Role of Volume on Multiparametric Neuromonitoring and Histology

Ralph Timaru-Kast, Anke Meissner, Axel Heimann, Bernd Hoelper, Oliver Kempski, and Beat Alessandri

Abstract

Traumatic brain injury (TBI) is often complicated by acute subdural hemorrhage (ASDH) with a high mortality rate. The pathophysiological mechanisms behind such an injury type and the contribution of blood to the extent of an injury remain poorly understood. Therefore, the goals of this study were to establish a porcine ASDH model in order to investigate pathomechanisms of ASDH and to compare effects induced by blood or sheer volume. Thus, we infused 2, 5, and 9 mL of blood (up to 15% of intracranial volume), and we compared a 5-mL blood and paraffin oil volume to separate out effects of extravasated blood on brain tissue. An extended neuromonitoring was applied that lasted up to 12 h after injury and included intracranial pressure (ICP), cerebral perfusion pressure (CPP), tissue oxygen concentration ($ptiO_2$), biochemical markers (glutamate, lactate), somatosensory evoked potentials (SEP), brain water content, and histological assessment (Lesion Index [LI]). Volume-dependent changes were detected mainly during the first hours after injury. ICP increased to significant levels ($p < 0.05$) of 36.89 ± 1.59, 15.52 ± 0.48, and 11.25 ± 0.35 mm Hg after 9, 5, and 2 mL of subdural blood, respectively (sham, 4.85 ± 0.06 mm Hg). The $ptiO_2$ dropped drastically after 9 mL of subdural blood without recovery in both hemispheres to below 20% of baseline, but was affected little after 2 and 5 mL in the acute monitoring period (maximal drop to 71% of baseline). Later, 5 mL of blood led to a significant increase of $ptiO_2$ compared to 2 mL ipsilaterally ($p < 0.05$). Glutamate and lactate showed a comparable pattern with a long-lasting increase after 9 mL of blood and short-lasting changes after 2 and 5 mL. The two smaller volumes caused an increased brain swelling (2 mL, 80.60 ± 0.34%; 5 mL, 81.20 ± 0.66%; $p < 0.05$ vs. sham), a significant LI (sham, 6.4 ± 1.4; 2 mL, 30.0 ± 0.95; 5 mL, 32.1 ± 1.2; $p < 0.05$ vs. sham), and a reduced SEP amplitude (5 mL, $p < 0.05$ vs. baseline) at the end of the experiment. A 9-mL led to herniation during the experiment causing dramatical brain swelling and acute histological damage. Comparison of blood volume with paraffin oil showed no significance, indicating that volume alone determines the acute pathophysiological processes leading to a rapidly developing histological damage. Additional effects due to blood contact with brain tissue (e.g., inflammation) may be detected only at later time points (>12 h).

Key words: acute subdural hematoma; intracranial pressure (ICP); microdialysis; neuromonitoring; neurotransmitters; pig; ptiO2; SEP; somatosensory evoked potentials; tissue oxygen tension; traumatic brain injury

Introduction

UP TO 2% OF THE POPULATION is affected by traumatic brain injury (TBI), which constitutes the major cause of death and severe disability among young people (Bullock et al., 2006). TBI is often complicated by an acute subdural hematoma (ASDH) with a mortality rate of more than 50% despite early surgical intervention (Bullock et al., 2006; Depreitere et al., 2006; Murray et al., 1999). The subdural blood mass causes a dramatic elevation of intracranial pressure (ICP), a decrease of cerebral perfusion pressure (CPP), and ischemic local or global cerebral blood flow (CBF). The lack of substrates and reduced oxygen supply to the tissue (Bouma et al., 1992; Bryan et al., 1995) lead to metabolic derangement, which results in cytotoxic and vasogenic edema that promotes a vicious circle of further ICP increase (Bullock et al., 1991, 2006; Bullock, 1997; Reilly and Bullock, 1997), which is nurtured by a chain reaction of pathophysiological events (Alessandri et al., 1999; Bullock, 1997; Bullock et al., 1991; Kempski et al., 1990; Kochanek et al., 1995; Palmer et al., 1993).

The complexity of tissue reactions and therapy, compara-

Institute for Neurosurgical Pathophysiology, Johannes Gutenberg University, Mainz, Germany.

ble to those seen in patients, can ideally be studied in animal models with similar gyrencephalic brain structure where multiparametric neuromonitoring can be applied and a controlled injury can be induced. Alessandri et al. (2003) and Manley et al. (2006) have developed models of controlled cortical impact (CCI) injury in juvenile pigs that enables the application of clinically relevant multiparametric neuromonitoring, as it is frequently added to routine monitoring of trauma patients in neurointensive care units (Alessandri et al., 1999; Bullock et al., 2006; Hillered et al., 1990; Meixensberger et al., 2001; Shaw, 2002; Stocchetti et al., 2001; Van Santbrink et al., 1996; Zauner et al., 1997). Our CCI model allows the investigation of processes which lead to acute and delayed cell death. Thus, establishing an analogue model for ASDH that can be combined with a CCI would be desirable. However, the pathophysiological mechanisms behind such an injury type remain poorly understood, and it is still unclear to which extent blood contributes to the injury produced by sheer volume. Excitotoxic processes, increased pressure, vasoactive effects, and toxicity of the blood itself have been postulated to play an important role in creating tissue injury underneath. There is evidence showing toxic effects of blood clots on brain tissue after induction of ASDH by comparing blood with silicon oil (Yilmazlar et al., 1997). On the other hand, subdural blood causes only little damage if applied at an open craniotomy (Duhaime et al., 1994). This indicates that blood may only induce detrimental effect in combination with increased ICP. The goals of this study were to establish a porcine model of ASDH with different volumes of autologous venous blood and to investigate the role of blood in the acute changes following a traumatic ASDH. Inspired by the publication of Durham and Duhaime (2007) that indicates a maturation-dependent lesion growth in case of ASDH, we used young adult pigs (about 4 months old). The study was performed by employing extended neuromonitoring for all experiments, by recording ICP, CPP, brain tissue oxygen tension ($ptiO_2$), brain temperature, interstitial fluid constituents by microdialysis (lactate, glutamate), and electrophysiological analysis with cortical somatosensory evoked potentials (SEP). Samples for brain water content and later histological evaluation were also taken. With this ASDH model, we wanted to investigate the implications in TBI caused by blood and answer the following questions: (1) How do different subdural blood volumes influence neuromonitoring parameters? (2) Is it possible to distinguish the effects induced by blood from sole mass effects (paraffin oil) by means of extended neuromonitoring? A third goal we wanted to achieve was to separate out effects of TBI due to focal contusion from lesions induced by ASDH. Therefore, we combined in a second study this ASDH model with the CCI model of Alessandri et al. (2003). This study will be published in a second paper.

Methods

Animals and anesthesia

All experiments were carried out in accordance with the Animal Welfare Guidelines and were approved by the local ethics committee. Thirty-four adult male pigs (German breed), weighing in average 28.61 kg (±0.28), were randomly divided into five different groups: three groups with different volumes of subdural blood (ASDH 2 mL, $n = 8$; ASDH 5 mL, $n = 8$; ASDH 9 mL, $n = 3$), a group with 5 mL of inert paraffin oil injected into subdural space (PAR, $n = 4$), and a sham-operated group (sham, $n = 11$) was compared to all groups.

The animals were initially anesthetized by intramuscular injection of ketamine (15 mg/kg), azaperone (3 mg/kg), and atropine (1 mg). The totally intravenous anesthesia (TIVA) was induced through peripheral vein injection of 15 mg of piritramide (Dipidolor, Janssen-Cilag) and 40–50 mL of alpha-chloralose (5 g [Physalis, France] + 4 g Na2B4O7 [Merck] in 100 mL of saline solution 0.9 %, adjusted pH 7.4). Anesthesia was maintained by a slow continuous infusion of piritramide (1 mg/mL): 2–5 mL/h via ear-vein and alpha-chloralose (2 g/40 mL) 10–40 mL/h through iliac vein (after catheterization). All animals received an oral endotracheal intubation and artificial ventilation adapted to maintain physiological arterial pH, pCO_2 and pO_2 (FiO_2 31.3 ± 0.1%) by using a respiratory pump (Servo 900B, Siemens-Elema, AB, Sweden) throughout the experiment. Rectal temperature was monitored and held constant at 38°C by thermometer-controlled heating blankets (Homeothermic Blanket; Harvard Apparatus, South Natick, MA).

Surgical preparation

The left femoral artery and vein were cannulated using French (F) 8 catheters for blood pressure monitoring, drug infusion, and withdrawal of blood for blood gas analysis or subdural infusion. After surgical suprapubic catheterization of the bladder, the animal was turned. The head was fixed in a stereotactic frame and the skull was exposed. A large craniotomy (diameter 30 mm) for application of ASDH was drilled carefully over the left parietal cortex. At a distance of 5 mm posterior to the craniotomy ipsilaterally and at the same position on the contralateral hemisphere, small burr holes were prepared for neuromonitoring catheters. Brain tissue oxygen ($ptiO_2$, Licox) probes were inserted 15 mm into the brain tissue (white matter) as well as temperature probes and microdialysis catheters (CMA/70). An ICP sensor was lowered into the right lateral ventricle. After probe insertion, burr holes were closed using bone wax, whereas a cover made out of Alginat Quick (Demedis, Düsseldorf, Germany) was used to close the craniotomy. This cover was kept in place by a metal cylinder fixed to the stereotactic frame in order to prevent the release of pressure during and after injury. For SEP monitoring, two extradural electrodes were screwed into the scull with the recording electrode right above the left prefrontal cortex frontal of the craniotomy and the reference electrode about 2–3 cm further medio-frontal (Fig. 1).

Multiparametric monitoring

1. Physiological parameters. The monitoring included ventilation-parameters (CO_2, FIO_2), arterial blood gas analyses (paO_2, $paCO_2$, pH, lactate, glucose, electrolytes), rectal temperature, heart rate, and mean arterial blood pressures (MABP).

2. Neuromonitoring. ICP was measured by intraventricular catheter (saline-filled polyethylene [PE] tube, i.d. 0.58 mm) in the right lateral ventricle. Cerebral perfusion pres-

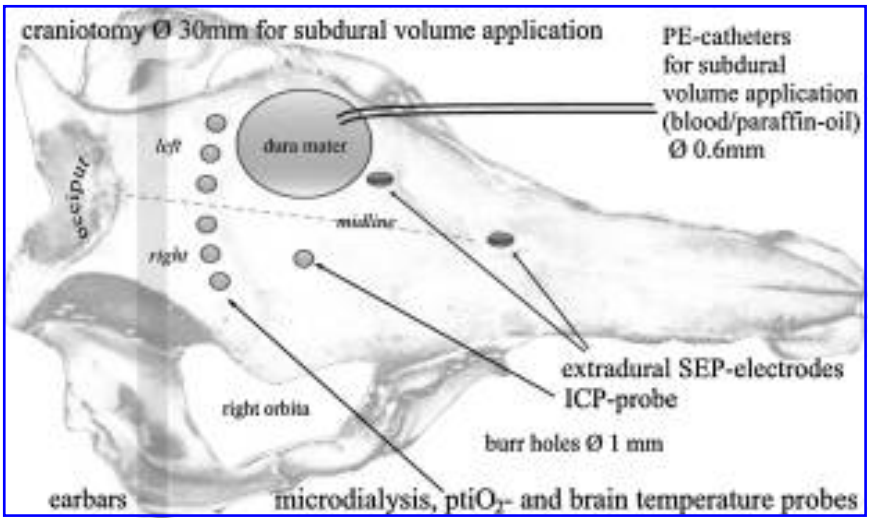

FIG. 1. Schematic drawing of position of neuromonitoring probes. The monitoring setup included microdialysis, brain tissue oxygen tension ($ptiO_2$), brain temperature, intracranial pressure (ICP; measured intraventricularly), and medianus nerve somatosensory evoked potentials (SEP; performed on the ipsilateral cortex, beneath craniotomy).

sure (CPP) was calculated as follows: MABP–ICP. The brain $ptiO_2$ and brain temperature were measured with Licox® probes (Licox CC1 SB, Licox LT; Integra Neurosciences Ltd., Hampshire, UK), and samples for the assessment of biochemical markers such as glutamate and lactate were collected through microdialysis catheters at a continuous flow of artificial cerebrospinal fluid (1 μL/min). The dialysate was collected in microdialysis vials (CMA 70 probe; Axel Semrau GmbH, Germany). Dialysate samples were taken every 15 min during baseline-period and the first 3 h post-ASDH induction, and thereafter every hour until the end of the experiment. All samples were frozen immediately at –20°C until analysis with an enzymatic analyzer (CMA 600). Continuous measurement of brain $ptiO_2$, ICP (and CPP) monitoring, and intracerebral temperature were recorded every minute. For the SEP of the left cortex, the right foreleg median nerve was stimulated with 1 Hz and 5 mA (each value was created by the average of 20 stimulations) every 15 min (Neuropack 2; Nihon Kohden, Japan). We analyzed latency and amplitude of the first cortical response to stimulation (N20).

Histology and water content

At 12 h after TBI, the animals were euthanized under deep alpha-chloralose anesthesia, and brains were removed immediately. One brain slice was taken underneath the injury for determination of cortical water content analysis using the wet-dry method (drying at 110°C for 24 h) (Alessandri et al., 2003). Percent water content and swelling were calculated. Adjacent to this brain section, two additional slices on each side were cut and then immersed in paraformaldehyde (mixture of 4% and 37% paraformaldehyde to achieve floating) for several days. Afterwards, they were embedded in paraffin. Coronal sections (10 μm thick) were cut from blocks. Two whole slices matching the frontal (anterior) position A12.5 mm and the occipital (posterior) position P4.5 mm according to the brain atlas of Felix et al. (1999) were stained with hematoxylin and eosin (H&E) and then examined histologically. In order to quantify the expansion and extent of cerebral damage a Lesion Index (LI) was created based on the Contusion Index for human and experimental non-missile head injury, introduced by Adams et al. (1985), where contusions are graded by depth and extent (index ranges from 0 to 12; applied in a porcine model of CCI injury by Alessandri et al. [2003]). This method has been modified and extended to describe further parameters of cortical lesions such as bleedings, edema, and neuronal death (necrosis), which are not due to contusion (Fig. 2).

Firstly, the derived LI included the extent (values, 1–3) and depth (values, 1–4) of damage. These values were multiplied by each other (extent × depth; maximum = 12). Secondly, values for intracranial bleedings (intracerebral, 0–3; subarachnoidal, 0–2), intensity of edema (0–3), and areas of contusion (0–1) were added [LI = (extent × depth) + intracranial bleeding + edema + contusion] (Fig. 2). However, actually, in this study no contusion was performed; we used this index for further studies with combinations of cortical contusion and ASDH. A LI was calculated for two anatomical brain locations (A12.5, P4.5), which were added for each hemisphere and pig [total LI = LI(A12.5)+LI(P4.5)]. Thus, the LI per hemisphere and animal ranges from 0 to 42.

Acute subdural hematoma

The ASDH was applied by perfusor syringe via PE tubing that was carefully placed within the craniotomy 2 mm into the subdural space fixed and sealed with acrylic glue (Histoacryl®, B. Braun, Melsungen, Germany). After insertion of tubings the craniotomy was closed immediately. Infusion flow was 0.5 mL/min. Non-heparinized autologous venous blood (2, 5, or 9 mL) or paraffin oil (5 mL) was used. Depending on the volume used, the infusion time lasted up to 18 min (Fig. 3). In order to prevent clotting during infusion of 5 and 9 mL of blood, a new syringe filled with freshly withdrawn blood was mounted on the pump every 5 min. As safeguard, we also inserted a second catheter into the subdural space. Sham pigs received all interventions, but no TBI.

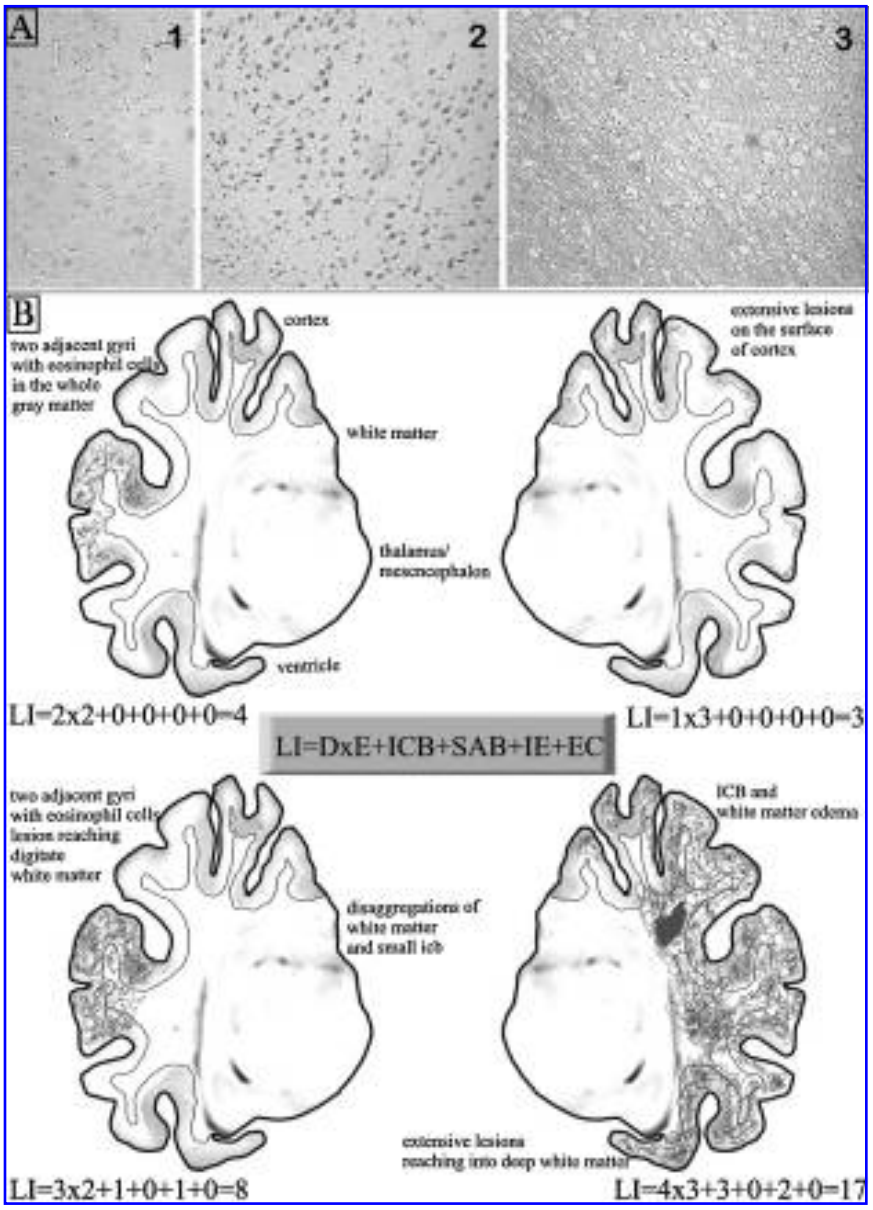

FIG. 2. (**A)** Examples of hematoxylin and eosin–stained cortical sections. (1) Cortex with vital neurons: basophil intracellular matrix, clear nucleoli and oval structure of cells. (2) Cortex with dead neurons: predominantly eosinophil cells, nucleoli disappeared, clear difference between matrix and cells. (3) Brain swelling: edematous white matter. Original magnification, ×100. (**B)** Schematic drawing to explain histological evaluation by the Lesion Index (LI), which was modified from Adams et al. (1985). Drawings show examples of coronal sections with different LIs. A LI is calculated as follows:

$$LI = [D \times E] + [ICB + SAB + IE + EC]$$

[D × E] − Multiplication of injury depth (D: 1 = partial thickness of the cortex; 2 = full thickness of the cortex; 3 = extending into digitate white matter; 4 = extending into deep white matter) and extent of damage (E: 1 = localized, i.e., one gyrus or two adjacent gyri; 2 = moderately extensive, i.e., involvement of the greater part of one surface of a lobe; 3 = extensive, i.e., more than one surface of a lobe).

[ICB + SAB + IE + EC] – Sum of intracranial hemorrhage and edema formation including intracerebral bleeding (ICB: 0 = none; 1 = minimal; 2 = medium, i.e., 1–2 gyri affected; 3 = large, i.e., more gyri, diffuse, whole section affected), subarachnoidal bleeding (SAB: 0 = none; 1 = minimal, i.e., blood in sulcus; 2 = extended SAB) intensity of edema (IE: 0 = none; 1 = minimal white matter disaggregations; 2 = medium edematous white matter; 3 = large diffuse edema formations) and existence of contusion (EC: 0 = absent; 1 = existent).

FIG. 3. Time course of the experiment. After 1-h baseline period, the craniotomy was opened at time point 0. For the acute subdural hematoma (ASDH) groups (ASDH 2, 5, 9 mL) and the paraffin oil group (PAR 5 mL), the ASDH injection started at time point +10 min. Note that in all groups the craniotomy was open for 6 min and that the infusion time varied because of a constant flow of 0.5 mL/h (i.e., 2 mL/4 min, 5 mL/10 min, and 9 mL/18 min). Neuromonitoring ended 10 h after injury or sham operation, and brains were removed 2 h later.

Study and monitoring protocol

After a probe equilibration period (up to 2 h) that was judged stable by physiological parameters and steady $ptiO_2$ values, a 60-min baseline period followed. This was followed by the opening of the craniotomy and the insertion of the ASDH catheters (time point 0). At time point 10 min, the ASDH injection was started, and a 10-h observation period followed (Fig. 3). Pigs were randomly assigned to different injury groups in order to establish two main lines of investigation:

1. To establish a porcine ASDH model, three groups received different volumes of autologous venous blood injected into subdural space, namely 2 mL (ASDH 2 mL, $n = 8$), 5 mL (ASDH 5 mL, $n = 8$), and 9 mL (ASDH 9 mL, $n = 3$).
2. To investigate volume effects, the ASDH 5-mL group was compared to an additional group with 5 mL of paraffin oil that was applied by the same means as blood (PAR 5 mL, $n = 4$). The experimental time course for each group is depicted in Figure 3. In all groups, parameters were recorded after induction of ASDH for 10 h.

However, this study represents the establishment of a new model with no reliable pre-existing data, the adequate dose of blood volume had to be found (9, 5, or 2 mL, or sham operation). Animals were randomly assigned to these groups. With 9 mL, all pigs herniated and we discontinued this group. The second step was the comparison of 5 ml blood and paraffin oil. Whereas no significant difference could be seen after four animals in each group, this series was also stopped.

Statistical analysis

Statistical analysis was performed for four different time periods within the entire monitoring time frame of 11 h, namely for baseline period (-60 min to time point 0 min post-TBI), and early (10–60 min post-TBI), intermediate (180–300 min post-TBI), and late (480–600 min post-TBI) post -traumatic period (Alessandri et al., 2003). Area-under-curve (AUC) was calculated for each animal and time period separately. For all neuromonitoring parameters, comparisons of groups and time points were made using one-way analysis of variance (ANOVA) with Student-Newman-Keuls post-hoc tests. Due to large baseline differences between animals in microdialysis, $ptiO_2$ and SEP parameters values were normalized to baseline and expressed as relative or percentage change to baseline. The mean of the last three measurements before injury was used for calculations. We used Sigma Stat 3.0 for all statistics.

Results

Physiological parameters

The physiological data is shown in Table 1. Except for the ASDH 9-mL group, all values were within physiologically ranges.

Comparison of subdural blood volumes

All pigs from the 9-mL group herniated shortly after infusion, but they were monitored for 10 h after blood infusion. However, this experimental group was discontinued, and while results are given descriptively and graphically, they are not included in the statistical analysis.

Intracranial pressure and cerebral perfusion pressure

As depicted in Figure 4, subdural blood infusion increased ICP volume-dependently. The elevation seen in the ASDH 9-mL group peaked out at 46.0 ± 10.3 mm Hg, remained at 35.89 ± 1.59 mm Hg during the first post-traumatic hour, and did not recover until the end of the observation period. The infusion of 2 and 5 mL of autologous blood also increased ICP (2-mL peak, 22.25 ± 1.75 mm Hg at 15 min; 5-mL peak, 29.38 ± 2.15 mm Hg at 21 min) significantly for a prolonged time (up to 5 h) when compared to sham. During the acute phase (0–60 min post-trauma), 5 mL increased ICP significantly (15.52 ± 0.48 mm Hg) compared to the elevation caused by 2 mL (11.25 ± 0.35 mmHg, $p = 0.024$).

Similarly to ICP, 9 mL of blood affected CPP massively, which did not recover over time and remained at ischemic levels. CPP changes after 2- and 5-mL infusion were not significant compared to sham and between the two ASDH groups.

ICP and CPP of the sham-operated group remained constant throughout the experiments at 4.85 ± 0.06 and 81.06 ± 0.53 mm Hg, respectively. CPP dropped at the end of the experiment due to MABP changes to 77.6 ± 4 mm Hg in the sham group (Fig. 4).

Tissue oxygen tension

Due to large differences in baseline values between animals and in the ipsi- and contralateral oxygen tissue con-

Table 1. Physiological Data from All Groups Included in the Study

Parameter	Group				
	Sham	*ASDH 2 mL*	*ASDH 5 mL*	*ASDH 9 mL*[a]	*Paraffin*
MABP (mm Hg)					
Baseline	91.4 ± 5	97.1 ± 5	94.7 ± 4	104.5 ± 0.5	96.7 ± 4
60 min post-injury	92.2 ± 7	96.5 ± 6	98.6 ± 3	86.0 ± 10	86.6 ± 6
600 min post-injury	73.3 ± 4	80.1 ± 5	79.6 ± 5	54.7 ± 27	80.0 ± 5
Rectal temperature (°C)					
Baseline	37.2 ± 0.3	37.3 ± 0.2	37.4 ± 0.5	38.4 ± 0.6	38.1 ± 0.4
60 min post-injury	37.7 ± 0.2	37.7 ± 0.2	37.5 ± 0.5	38.2 ± 0.2	38.0 ± 0.3
600 min post-injury	37.4 ± 0.2	37.7 ± 0.2	37.9 ± 0.3	38.6 ± 0.1	38.4 ± 0.5
pH					
Baseline	7.46 ± 0.01	7.46 ± 0.02	7.47 ± 0.02	7.46 ± 0.001	7.46 ± 0.007
60 min post-injury	7.45 ± 0.01	7.46 ± 0.02	7.46 ± 0.01	7.43 ± 0.02	7.45 ± 0.005
600 min post-injury	7.43 ± 0.02	7.45 ± 0.01	7.45 ± 0.01	7.39 ± 0.08	7.44 ± 0.01
pO_2 (mm Hg)					
Baseline	139 ± 6	141 ± 4	142 ± 12	141 ± 4	145 ± 6
60 min post-injury	142 ± 6	146 ± 5	133 ± 7	134 ± 9	128 ± 8
600 min post-injury	120 ± 16	135 ± 12	127 ± 11	122 ± 5	120 ± 11
PCO_2 (mm Hg)					
Baseline	46.0 ± 1.2	47.20 ± 0.9	43.8 ± 0.8	46.0 ± 1.3	43.3 ± 0.5
60 min post-injury	45.3 ± 1.8	46.8 ± 0.7	42.9 ± 1.2	48.6 ± 1.1	45.5 ± 2.6
600 min post-injury	41.1 ± 3.6	45.7 ± 0.7	43.5 ± 1.4	38.1 ± 3.7	47.4 ± 1.2
Glucose (mg/dl)					
Baseline	79.7 ± 10	89.0 ± 7	90.6 ± 5	98.0 ± 8	66.0 ± 4
60 min post-injury	73.3 ± 13	92.3 ± 6	82.9 ± 4	94.5 ± 6	83.5 ± 10
600 min post-injury	70.5 ± 7	88.5 ± 10	77.6 ± 4	88.3 ± 24	77.0 ± 4
Lactate (mM)					
Baseline	0.83 ± 0.13	0.81 ± 0.13	0.66 ± 0.07	0.70 ± 0.06	0.80 ± 0.14
60 min post-injury	0.75 ± 0.17	0.74 ± 0.11	0.61 ± 0.06	0.87 ± 0.18	0.80 ± 0.15
600 min post-injury	0.61 ± 0.09	0.70 ± 0.05	0.75 ± 0.08	2.17 ± 0.88	0.78 ± 0.05

Values are given as mean ± SEM. Note that there are no significant differences between groups at any analyzed time point.
[a]Note that the ASDH 9 mL groups was not included in the statistical analysis.
ASDH, acute subdural hematoma; MABP, mean arterial blood pressure.

centration, and in order to compare $ptiO_2$ values, we analyzed the relative changes of oxygen levels within the tissue. Absolute baseline values were counted as 1 (one). Ipsi- and contralateral $ptiO_2$ values are depicted in Figure 5 as relative changes to baseline values. Ipsilateral absolute baseline values of tissue oxygen were 26.00 ± 1.06 mm Hg for the sham group, and 25.86 ± 1.68, 21.53 ± 1.42, and 22.07 ± 1.20 mm Hg for the ASDH 2-, 5-, and 9-mL groups, respectively. In the contralateral hemisphere, baseline values were 29.17 ± 1.67 mm Hg (sham), 19.57 ± 0.99 mm Hg (2 mL), 24.61 ± 1.16 mm Hg (5 mL), and 20.10 ± 2.71 mm Hg (9 mL). Similarly to the time course seen with CPP, $ptiO_2$ dropped drastically after the infusion of 9 mL of subdural blood and did not recover in both hemispheres, but was affected only marginally after 2 mL and even 5 mL of blood in the acute monitoring period. Later, 5 mL of blood led to an increase of $ptiO_2$, which was significant compared to the ASDH 2-mL group in the ipsilateral hemisphere ($p < 0.05$; Fig. 5).

Glutamate and lactate in dialysate

Alike the $ptiO_2$ values, we analyzed dialysates as relative changes from baseline (=1), since we detected in six of 34 pigs glutamate levels of >50 μM. These high baseline glutamate levels, however, did not correlate with the outcome parameters. Absolute baseline values were very high and as follows:

Sham: ipsilateral 34.60 ± 6.68 μmol/L, contralateral 21.96 ± 5.11 μmol/L
ASDH 2 mL: ipsilateral 23.61 ± 4.65 μmol/L, contralateral 24.18 ± 3.72 μmol/L
ASDH 5 mL: ipsilateral 16.52 ± 4.69 μmol/L, contralateral 17.15 ± 3.43 μmol/L
ASDH 9 mL: ipsilateral 15.53 ± 3.78 μmol/L, contralateral 11.60 ± 1.55 μmol/L

The infusion of 9 mL dramatically elevated glutamate in both hemispheres (ipsilateral after 30 min peak at 57 times) and remained pathologically high throughout the experiment. ASDH 2 and 5 mL caused a significant elevation of glutamate if compared to sham animals during the first 60 min after injury ($p < 0.05$), but normalized later. Contralateral ASDH 2 and 5 mL had no significant effect on glutamate levels at any time period (Fig. 5).

Baseline lactate values read as follows:

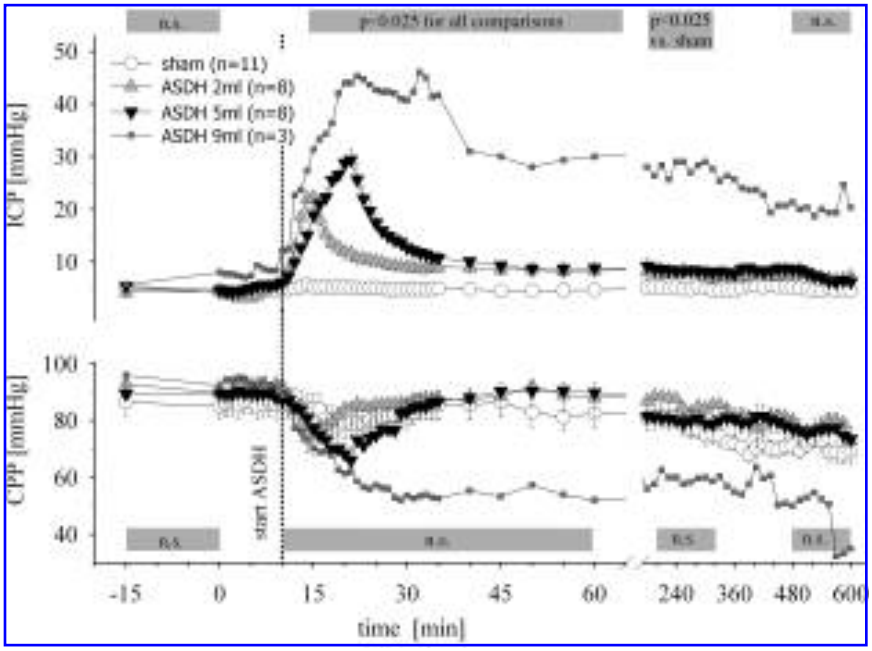

FIG. 4. Time course of intracranial pressure (ICP; **top**) and cerebral perfusion pressure (CPP; **bottom**) before and after subdural infusion of autologous blood (acute subdural hematoma [ASDH] 2, 5, or 9 mL). The vertical line indicates the starting point of ASDH induction. Gray bars show the statistically analyzed time periods (baseline, 10–60 min, 180–300 min, and 480–600 min). Note that the ASDH 9-mL group was not included in the statistical analysis. Values are given as mean ± SEM (n.s. = not significant).

Sham: ipsilateral 1.94 ± 0.22 mmol/L, contralateral 1.76 ± 0.19 mmol/L
ASDH 2 mL: ipsilateral 2.02 ± 0.14 mmol/L, contralateral 2.67 ± 0.26 mmol/L
ASDH 5 mL: ipsilateral 2.07 ± 0.13 mmol/L, contralateral 2.60 ± 0.24 mmol/L
ASDH 9 mL: ipsilateral 1.31 ± 0.09 mmol/L, contralateral 2.29 ± 0.28 mmol/L

There was no significant change in lactate levels in the contralateral hemisphere for all groups. Ipsilaterally, ASDH 5 mL caused elevation to two times of baseline in the acute period ($p < 0.05$ vs. sham). Only in 9-mL ASDH did levels remain high up to the end (Fig. 5).

Somatosensory evoked potentials

The bar charts show change of amplitude L1-L2 in percent relative to baseline values (Fig. 6). Sham-operated animals keep 100% of baseline up to 10 h, whereas 5 mL of blood reduces SEP amplitude significantly to baseline from the first hour after injury on ($p < 0.05$). ASDH 2 mL is reduced only at the beginning, recovers after 2 h, and remains at almost baseline level. Latency of the signal could not be assessed because no amplitude could be detected in several animals, especially in the early phase of post-traumatic monitoring.

Brain water content

As depicted in Figure 7, subdural infusion of 2 and 5 mL blood increased ipsilateral water content significantly ($p < 0.03$; with sham, 79.22 ± 0.19%; ASDH 2 mL, 80.60 ± 0.34%; and ASDH 5 mL, 81.20 ± 0.66%). The contralateral water content was not elevated significantly when compared to sham-operated animals (sham, 79.61 ± 0.26%; ASDH 2 mL, 80.14 ± 0.19%; and ASDH 5 mL, 80.10 ± 0.36%).

Lesion index

As can be seen in Figure 8, all sizes of subdural hematoma caused highly significant lesions in both hemispheres ($p < 0.001$ vs. sham; ipsilateral: sham 6.36 ± 1.41; ASDH 2, 5, 9 mL: 30.00 ± 0.95, 32.13 ± 1.20, 36.00 ± 2.30; contralateral: sham 8.18 ± 0.83; ASDH 2, 5, 9 ML: 23.88 ± 1.41, 26.00 ± 1.36, 34.67 ± 2.67). Both the ASDH 2- and 5-mL groups had significantly larger LI in the injured hemisphere when compared to the contralateral hemisphere (Fig. 8).

Blood versus volume effects

The effect of autologous venous blood and paraffin oil on physiological and on neuromonitoring parameters is shown in Tables 1 and 2. The subdural infusion of a 5-mL volume of blood or paraffin oil produced almost identical changes.

Discussion

Despite two decades of clinical and experimental investigations of TBI, the pathophysiological mechanisms remain poorly understood. It remains unclear to which extent blood constituents, in addition to sheer volume, are worsening the outcome of patients and how to monitor closely progression of brain damage. Successful pathophysiological and pharmacological animal studies have not translated into neuroprotective clinical therapies and have not improved outcome in patients with TBI (Manley et al., 2006). Although only a

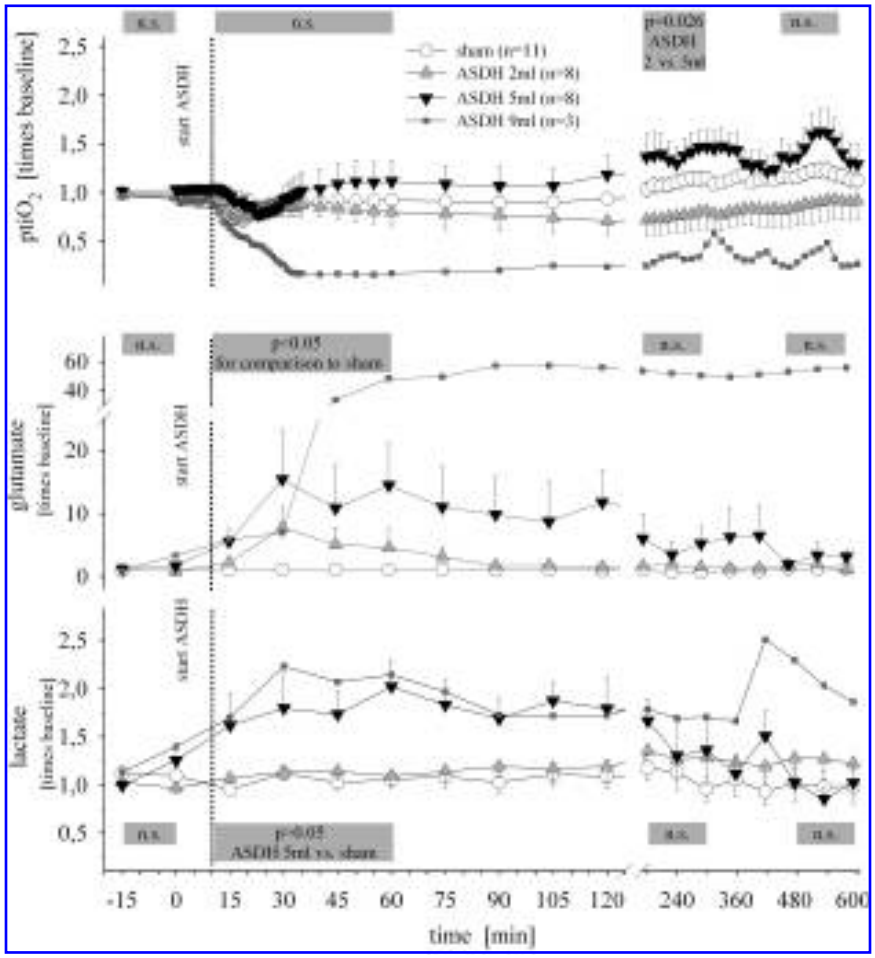

FIG. 5. Relative tissue oxygen concentration ($ptiO_2$; **top**), glutamate (**middle**), and lactate (**bottom**) values (mean ± SEM) in the ipsilateral hemisphere. The vertical line indicates the starting point of subdural infusion of autologous blood (acute subdural hematoma [ASDH]; 2, 5, or 9 mL). Gray bars show the statistically analyzed time periods, namely a baseline period, and an early (10–50 min), an intermediate (180–300 min), and a late (480–600 min) post-injury period. Note that the ASDH 9-mL group was not included in the statistical analysis (n.s. = not significant).

few attempts have been made to study them simultaneously under controlled conditions in small animal models (Sawauchi et al., 2003), TBI models of large animals are rare albeit important for understanding complex relationships between neuromonitoring parameters (Alessandri et al., 2003; Durham and Duhaime, 2007; Manley et al., 2006; Zauner et al., 1995; Zauner et al., 2002). Difficulties in translating results of experiments in rodents into clinical practice could be caused by biomechanical, structural, and cellular differences between the lissencephalic rodent brain and the gyrencephalic human brain as postulated by Manley et al. (2006). Besides having many important structural similarities to the human brain, the porcine brain is of a size (in our study 70 g) that allows employment of multimodal neuromonitoring as used in trauma patients in intensive care units, i.e., simultaneous monitoring of ICP, interstitial fluid constituents by microdialysis, brain $ptiO_2$, and temperature, as well as electrophysiological monitoring such as EEG and SEP (Alessandri et al., 1999; Bullock et al., 2006; Hillered et al., 1990; Meixensberger and Roosen, 1998; Shaw, 2002; Stocchetti et al., 2001).

Establishing a porcine model of ASDH for multiparametric monitoring

We aimed to establish a reproducible swine model of ASDH by injecting different volumes of autologous venous blood into the subdural space. Zwetnow et al. (1993) applied a blood volume that corresponded to 15% (12 mL blood) of the intracranial volume which was lethal in mechanically ventilated pigs. Chiari et al. (2000) used, similarly to Orlin et al. (1992), subdural rubber balloons for the application of 15-mL volumes, which were lethal. On the basis of these published studies using pigs or piglets which showed that a mass greater than 15 mL is lethal in most cases, we decided to apply 9 mL as a maximal volume, and 5 or 2 mL for comparison. A volume of 9 mL is approximately the threshold volume of supratentorial volume tolerance around 10% of intracranial volume (Zwetnow et al., 1993). Consequences were distinct ICP increase without recovery in parallel to irreversible ischemic CPP levels and $ptiO_2$ decrease to hypoxic values. Glutamate release with high toxic levels throughout the experiment and elevation of ipsilateral parenchymal lac-

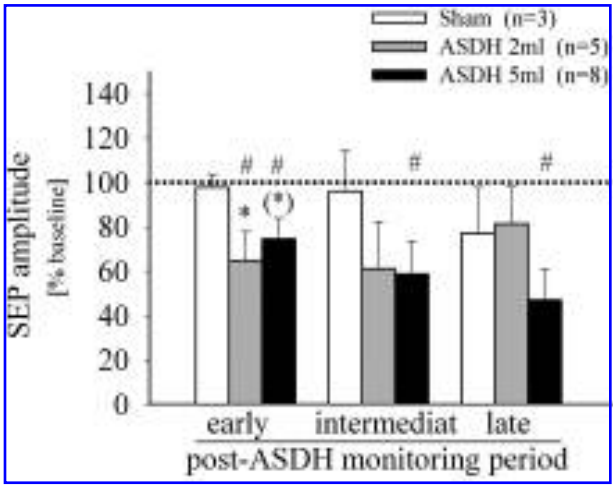

FIG. 6. Percent relative changes from baseline (100%) of somatosensory evoked potentials (SEP) amplitudes during the early (10–60 min post-trauma), intermediate (180–300 min), and late (480–600 min) period after sham operation and acute subdural hematoma (ASDH 2, 5 mL). Differences to sham are indicated by $^{*}p < 0.05$) and $(^{*})p = 0.071$. Significant differences to the appropriate baseline are indicated by $^{\#}p < 0.05$.

tate followed. All three animals with 9-mL ASDH herniated during the experiment, causing dramatical brain swelling and acute histological damage. We consequently had to discontinue this group and continued with the application of 2- or 5-mL subdural ASDH volumes. They led to volume-dependent increases in ICP in the acute phase. However, there was no significant decreases in CPP and $ptiO_2$, which were only affected marginally. Brain tissue oxygen monitoring is known as a reliable method to evaluate cerebral oxygenation after severe TBI (Kiening et al., 1998; Sarrafzadeh et al., 2000) and supplements traditional ICP-CPP-monitoring. Critical hypoxic levels are reached below 10 mm Hg, and are often accompanied by a CPP of <60 mm Hg, indicating ischemic conditions. Nonetheless, high oxygen levels alone do not necessarily indicate viable tissue. The $ptiO_2$ levels in the 5-mL group increased after 4 h. This could be caused by hypometabolism, hyperperfusion, or both. If tissue at risk is not metabolizing, $ptiO_2$ will be elevated to "healthy" levels. Clausen et al. (2001) demonstrated in a cat study that disturbed mitochondrial function in the monitored cortical area leads to distinct increase of $ptiO_2$. The elevation of $ptiO_2$ at later time points after ASDH may therefore indicate a loss of vasomotor reactivity and/or that mitochondrial dysfunction creates elevated tissue oxygen concentration due to decreased oxygen consumption. Alterations in energy metabolism could also be demonstrated in severely head-injured patients where an initial hyperglycolysis turns into hypoglycolysis within hours to days after TBI (Bergsneider et al., 2000). In addition, Unterberg et al. (1993) reported that the effect of increasing CPP as therapy on $ptiO_2$ depends on the type of intervention in patients, i.e., despite a global increase of CPP is achieved with each therapy, $ptiO_2$ did not respond uniformly (Kiening et al., 1997). Although CPP reflects sufficient global oxygen delivery, $ptiO_2$ is measured locally, and the distance to the infusion site (approximately 10 mm) has to be taken into consideration (Fig. 1). This could possibly lead to false interpretation of global brain tissue oxygenation if an insult is too moderate.

Ischemia, TBI, and hypoxic oxygen levels are well-known factors that can induce release of excitotoxic glutamate and lactate. It has been reported that CPP below 70 mm Hg correlates with increased glutamate levels in patients (Vespa, 2003) and that high glutamate levels correspond to injury type and outcome (Bullock et al., 1990, 1998; Zhang et al., 2001). Additionally, early high extracellular lactate has been reported to relate closely to poor outcome (Goodman et al., 1999). ASDH with 9 mL induced ischemic CPP and $ptiO_2$, which closely corresponds with long-lasting high glutamate and lactate levels. With the weaker insult by 2 and 5 mL, effects on glutamate and lactate were only short-lasting, if present at all. As seen in other animal models and patient studies (Hlatky et al., 2004), our data show tendencies of volume- dependent elevations of excitotoxic and ischemic markers. It is not possible to distinguish between injury severity below an ASDH of 5 mL, which reflects about 7% of intracranial volume. Furthermore, there is no clear correlation between high ICP or ischemic oxygen levels and glutamate or lactate levels respectively and our data showed no distinct changes of glucose and pyruvate levels after ASDH induction (data not shown). In line with clinical studies, these markers are of limited use in therapy strategies if TBI is not severe enough. (Hillered et al., 2006; Meixensberger et al., 2001).

Severe brain injury is known to cause cytotoxic and vasogenic edema formation. Swelling of brain tissue is also a critical factor after traumatic ASDH that is related to poor outcome (Gennarelli, 1993; Server et al., 2001). The assessment of brain water content reveals volume-dependent edema formation early after ASDH. In porcine models of epidural balloon expansion (Bauer et al., 1999) and fluid-percussion in-

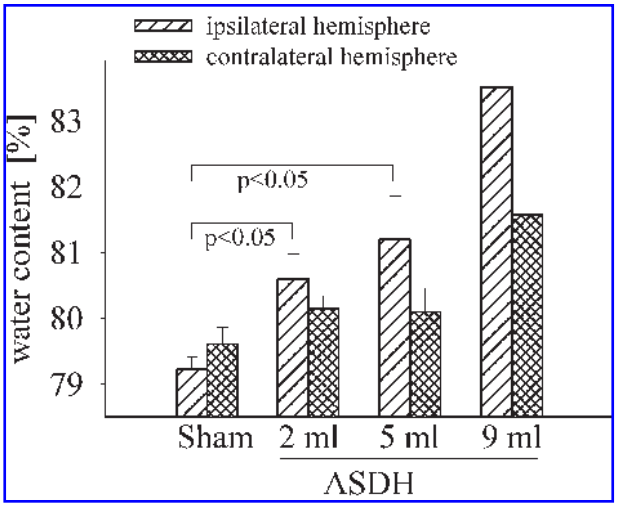

FIG. 7. Water content of ipsilateral and contralateral cortical brain tissue after sham operation or infusion of 2, 5, or 9 mL of autologous venous blood (acute subdural hematoma [ASDH] 2, 5, and 9 ml). Water content was assessed using the "wet-dry weight" method. Values are given as mean ± SEM. Note that samples of only one ASDH 9-mL pig could be collected and thus has not been tested.

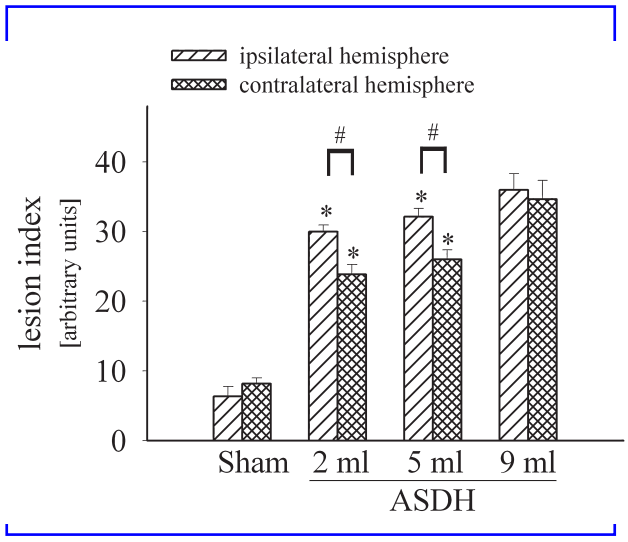

FIG. 8. Lesion Index (LI) of sham, and acute subdural hematoma (ASDH) 2, 5, and 9 mL (mean ± SEM). ASDH induced a significant damage in the ipsi- and contralateral hemisphere when compared to sham tissue. Asterisks indicate significant differences to sham animals for the ipsilateral and contralateral LI (all $p < 0.01$). #Significant difference between the ipsi- and contralateral LI ($p < 0.05$). Values are given as mean ± SEM. Note that the ASDH 9-mL group has not been included in the statistical analysis.

jury (Fritz et al., 2005), a secondary ICP elevation due to brain swelling occurred in animals 24 h after the insult. Thus, monitoring of brain edema might be a valuable tool for prognostic purpose. We found only a weak correlation between brain water content and histological damage (i.e., LI) on the ipsilateral side ($r^2 = 0.359$). Evaluation of histological damage by our LI showed significant difference to sham in all groups with a pronounced difference in the ipsilateral hemisphere. But there was no significant difference between ASDH groups. Assessments at later time points might reveal larger differences between groups, since clear demarcation of injured and uninjured tissue in pigs after several post-injury days has been reported (Bauer et al., 1999; Duhaime et al., 2000; Durham and Duhaime, 2007; Fritz et al., 2005).

After head injury, SEP is a dependable and invariable procedure that is much less susceptible to anesthesia than electroencephalography (EEG). SEP may provide worthwhile information about the site and nature of changes in cerebral activity not obtainable with the EEG. The prognostic value of SEP is superior to that of the EEG when used to assess severe head injury in patients. All components of the cortical SEP are most likely generated by activity conducted centrally through the dorsal column system, traversing the specific lemniscal or spinothalamical tracts. The P15 is the first deflection seen in the SEP monitor and arises from the caudal medulla. This is followed by N20, which is the first cortical peak and arises presumably from the postcentral gyrus. Later peaks likely arise from association cortex or reverberating circuits between cortex and subcortical structures (Moulton, 1997; Shaw, 2002). Functional deficits shown by amplitude decrease of SEP are seen after 2 mL in the acute phase with a full recovery to baseline levels after 2 h. ASDH 5 mL causes significant decrease of SEP amplitudes throughout the experiment with complete loss of cortical response in several animals. This shows the importance of specific functional measurement of SEP as an applicable and reliable acute neuromonitoring parameter.

ICP that was elevated in the acute phase, and SEP with a distinct decrease throughout the experiment in the 5-mL group, were the two most distinguishable parameters when comparing ASDH 2 and 5 mL. There was no volume-dependent effect seen for the injury-induced decrease of $ptiO_2$ and CPP. Neither water content nor histology showed significant differences between the two volumes. On the other hand, 9 mL produced clear and long-lasting changes in all central nervous parameters. Unfortunately, a statistical comparison to ASDH 2 and 5 mL is problematic because of the small number of animals in the 9-mL group. The post-injury time frame is too short for clear demarcations of necrotic area and penumbra. Therefore, the LI can indicate histological damage but cannot differentiate between the effect of 5 and 2 mL of blood volume after 12 h. Even in rat models of ASDH, lesions grow over a period of 24 h (Alessandri et al., 2006; Duhaime et al., 1994; Yilmazlar et al., 1997). Similarly, onset of diffuse brain swelling due to cerebral hyperemia in head-injured patients usually occurs within 24 h of injury, during which time autoregulation is lost (Meixensberger and

Table 2. Comparison of Pathophysiological Changes in the Ipsilateral Hemisphere after Subdural Infusion of 5 mL of Autologous Blood or Paraffin Oil

	Parameter							
Group	*ICP peak*	*CPP peak*	*$ptiO_2$ peak*	*SEPs first hour*	*Glutamate[a] first hour*	*Lactate[a] first hour*	*Water content ipsilateral*	*Lesion index ipsilateral*
Sham	5.2 ± 0.6	84.4 ± 5.2	25.4 ± 2.8	105.4 ± 11	8.4 ± 3.7	1.6 ± 0.4	79.2 ± 0.2	6.4 ± 1.4
ASDH 5 mL	29.4 ± 2.2*	66.0 ± 5.1	16.7 ± 3.3	60.0 ± 11*	67.9 ± 27*	3.9 ± 0.7*	81.2 ± 0.7*	32.1 ± 1.2*
Paraffin 5 mL	25.8 ± 2.1*	67.3 ± 3.9	16.9 ± 2.7	48.7 ± 10*	65.5 ± 23*	3.6 ± 0.3**	81.0 ± 0.1*	30.0 ± 0.8*

CPP, cerebral perfusion pressure (mm Hg); ICP, intracranial pressure (mm Hg); $ptiO_2$, partial tissue pressure of oxygen (mm Hg); SEP, sensory evoked potential (% change from baseline); ipsi, ipsilateral; contra, contralateral.

[a]For glutamate and lactate, the mean release during the first hour after injury was calculated.

Values are given as mean ± SEM and compared statistically with a one-way AVOVA with a Student-Newman-Keuls post-hoc test for group differences.

$p < 0.05$ versus sham.

Roosen, 1998). Thus, histopathological lesion demarcation in big animal models requires a survival period of at least 24 h, and up to 72 h after which clear necrotic core has developed. Visualization of a volume-dependent effect on histological outcome will only be possible with longer observation periods when using a porcine ASDH model. Our results indicate that there is a volume tolerance between 2 and 5 mL, in the case of CPP and $ptiO_2$, whereas function is more severely affected after 5 mL or larger volumes. This highlights the importance of SEP as a routine monitoring parameter at least in large animal models of TBI. But also ICP, CPP, $ptiO_2$, and even glutamate are parameters which relate to the severity of injury when compared to the 9 mL of subdural blood. Volumes higher than the threshold for volume tolerance of 10% intracranial volume led to herniation in our model. Therefore, only smaller volumes are of interest for investigations of reversible and curable injury. Since we found a relatively high variability of the data within each group, we would recommend a different procedure for injury induction in further studies. Within our volume range of subdural blood between 5 and 9 mL, trauma severity should be guided by monitoring parameters such as ICP, CPP, or $ptiO_2$. In this way, variability can be reduced, which will not only improve interpretation of multiparametric parameters but also allow the introduction of therapeutical measures in this model.

Blood versus volume effects

In order to investigate acute effects of blood constituents in comparison to effects of sheer volume, we infused 5 mL of autologous blood or paraffin oil into the subdural space. The assessed neuromonitoring parameters were not distinguishable between the two groups during the acute and 10-h posttraumatic period. This was also the case for brain swelling and histological outcome. Yilmazlar et al. (1997) showed that blood caused extensive ischemic damage in underlying cortical tissue after 24 h when compared to silicon oil as volume. On the other hand, Duhaime et al. (1994) postulated that blood alone without pressure does not induce significant damage underneath the subdural hematoma. We can add that as a result of our studies there is no evidence that shows that blood itself causes distinctly different pathophysiological patterns and lesions in contrast to volume alone in the early stage after injury. Within the first 12 h, it seems that ICP is the main cause for the neuropathophysiological changes and consequently for the acute neuronal damage. However, in agreement with the study from Yilmazlar et al. (1997), we could demonstrate recently that acute pathophysiological changes of ICP, CPP, $ptiO_2$, and CBF were not distinguishable between blood and volume, but that at 24 h, a significant larger lesion was found in the blood infused ASDH group in rats (Alessandri et al., 2006). This implies strongly again that a longer survival period is necessary in our pig model in order to sort out the effect of blood on lesion growth.

Conclusion

We presented a new multiparametric porcine model of ASDH with a threshold blood volume for long lasting neuromonitoring effects between 5 and 9 mL (7–13% of intracranial volume). The present study showed that ASDH as a standardized brain injury induces well-known pathophysiological processes that are volume-dependent and comparable to those found in clinical routine. The similar effects of 5 mL of blood and paraffin oil indicate that the acute changes of parameters such as ICP determine processes leading to a "subacute" histological damage found after 12 h. Additional effects due to blood contact with brain tissue (e.g., inflammation) may be detected only at later time points (>12 h). Further studies with long-term survival experiments must be performed to elucidate the correlation between monitoring parameters and histological or functional outcome, and to investigate the pathophysiological consequences of blood constituents.

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Author Disclosure Statement

No conflicting financial interests exist.

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Address reprint requests to:
Beat Alessandri, M.D.
Institute for Neurosurgical Pathophysiology
Johannes Gutenberg University
Langenbeckstrasse 1
Mainz, Rheinland-Pfalz, Germany

E-mail: alessandri@nc-patho.klinik.uni-mainz.de