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Experimental Research

Neurochemical monitoring using intracerebral microdialysis during systemic haemorrhage*

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Summary

Background. Intracerebral microdialysis is a sensitive tool to analyse tissue biochemistry, but the value of this technique to monitor cerebral metabolism during systemic haemorrhage is unknown. The present study was designed to assess changes of intracerebral microdialysis parameters both during systemic haemorrhage and after initiation of therapy.

Methods. Following approval of the Animal Investigational Committee, 18 healthy pigs underwent a penetrating liver trauma. Following haemodynamic decompensation, all animals received a hypertonic-hyperoncotic solution and either norepinephrine or arginine vasopressin, and bleeding was subsequently controlled. Extracellular cerebral concentrations of glucose (Glu), lactate (La), glycerol (Gly), and the lactate/pyruvate ratio (La/Py ratio) were assessed by microdialysis. Cerebral venous protein S-100B was determined. Haemodynamic data, blood gases, S-100B, and microdialysis variables were determined at baseline, at haemodynamic decompensation, and repeated after drug administration.

Results. Microdialysis measurements showed an increase of La, Gly, and La/Py ratio at BL Th compared to BL (mean ± SEM; La 2.4 ± 0.2 vs. $1.4 \pm 0.2\,\text{mmol} \cdot \text{l}^{-1}$, $p < 0.01$; Gly 37 ± 7 vs. $27 \pm 6\,\mu\text{mol} \cdot \text{l}^{-1}$, n.s.; La/Py ratio 50 ± 8 vs. 30 ± 4, $p < 0.01$), followed by a further increase during the therapy phase (La $3.4 \pm 0.3\,\text{mmol} \cdot \text{l}^{-1}$; Gly $69 \pm 10\,\mu\text{mol} \cdot \text{l}^{-1}$; La/Py ratio 58 ± 8; $p < 0.001$, respectively). Cerebral venous protein S-100B increased at decompensation and after therapy, but decreased close to baseline values after 90 min of therapy.

Conclusions. In this model of systemic haemorrhage, changes of cerebral energy metabolism detected by intracerebral microdialysis indicated anaerobic glycolysis and degradation of cellular membranes throughout the study period.

Keywords: Cerebral ischaemia; microdialysis; systemic haemorrhage; lactate

Abbreviations

art	arterial
CO	cardiac output
CPP	cerebral perfusion pressure
cv	cerebral venous
CVP	central venous pressure
FiO_2	fraction of inspired oxygen
Glu	glucose
Gly	glycerol
ICP	intracranial pressure
La	lactate
La/Py ratio	lactate/pyruvate ratio
MAP	mean arterial blood pressure
$p(cv\text{-}a)CO_2$	cerebral venous – arterial partial pressure difference of carbon dioxide
S_jO_2	jugular venous oxygen saturation

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Introduction

Reliable monitoring of cerebral oxygenation and metabolism is an issue of paramount importance in critical care and anaesthesia, as systemic haemorrhage can result in an impaired balance of oxygen demand and supply that puts viable brain tissue at risk of ischaemia [4, 5]. This should be even more pronounced, due to constant growth of the elderly population, with cerebrovascular co-morbidity and a higher susceptibility to cerebral ischaemia of trauma victims will be more common in the future [31]. Systemic haemodynamic parameters, blood gases, and blood lactate levels can only contribute limited information regarding imbalance of microcirculation and cellular nutrition [16]. Furthermore, measurements of jugular venous oxygen saturation (S_jO_2) may reflect cerebral oxygenation, but do not give information about metabolism on a cellular level. Increased protein S-100B has been reported as a reliable biochemical marker of brain tissue damage after various regional and global cerebral ischaemic conditions [3, 7, 20]. However, protein S-100B release during systemic haemorrhage is still controversial [21].

Intracerebral microdialysis is a sensitive established technique to analyse brain tissue biochemistry in neurointensive care [28]. Extracellular glucose, lactate and the lactate/pyruvate ratio are reliable markers of tissue acidosis and cell ischaemia, while elevated glycerol levels may indicate cell membrane damage [27]. Accordingly, compared to monitoring cerebral oxygenation by cerebral venous blood gases or S_jO_2, intracerebral microdialysis may be more useful in assessment of cerebral tissue vulnerability, as markers of cerebral metabolism and direct cell damage are determined in addition. The effect of systemic haemorrhage and resuscitation on brain damage has already been evaluated by different methods of molecular biology, apoptosis markers and histo-morphological changes [30, 32]. While most of these methods were performed subsequent to the experiments, microdialysis provides real-time, continuous information on pathophysiological processes in the target organs at the bedside [15].

The purpose of this study was to assess changes of cerebral metabolism by intracerebral microdialysis parameters, and to determine the extent of cerebral cell damage in an animal model of systemic haemorrhage. We hypothesised that intracerebral microdialysis would be superior to traditional systemic and cerebral venous blood gases.

Materials and methods

This project was approved by the Animal Investigation Committee of the University Schleswig-Holstein, Campus Kiel, Germany, and animals were managed in accordance with the American Physiologic Society and institutional guidelines. The study was performed according to the Utstein-style guidelines [14] on 18 healthy pigs (German domestic pigs), ranging from 12 to 16 weeks of age, of either gender, and weighing 42 to 46 kg. Anaesthesia was used in all surgical interventions, all unnecessary suffering was avoided, and research was terminated if pain or fear resulted. The animals were fasted overnight, but had free access to water.

Surgical preparation

The pigs were premedicated with azaperone (neuroleptic agent; $8\,mg \cdot kg^{-1}$ intramuscular) and atropine ($0.05\,mg \cdot kg^{-1}$ intramuscular) 1 h before surgery, and anaesthesia was induced with a bolus dose of ketamine ($2\,mg \cdot kg^{-1}$ intramuscular), propofol ($1–2\,mg \cdot kg^{-1}$ intravenous), and sufentanil ($0.3\,\mu g \cdot kg^{-1}$ intravenous) [29]. After endotracheal intubation during spontaneous respiration, the pigs were ventilated with a volume-controlled ventilator (Siemens SV 900C, Germany) with 30% oxygen at 20 breaths/min, and with a tidal volume adjusted to maintain normocapnia ($paCO_2$ 35 to 40 mm Hg). Ventilation was monitored using an inspired/expired gas analyser that measured oxygen and end-tidal carbon dioxide ($petCO_2$) (M-PRESTN; Datex-Ohmeda Inc.; Helsinki, Finland). Anaesthesia was maintained with a continuous infusion of propofol (6 to $8\,mg \cdot kg^{-1} \cdot h^{-1}$) and sufentanil ($0.3\,\mu g \cdot kg^{-1} \cdot h^{-1}$); muscle relaxation was provided by a continuous infusion of pancuronium ($0.2\,mg \cdot kg^{-1} \cdot h^{-1}$). Ringer's solution ($10\,ml \cdot kg^{-1} \cdot h^{-1}$) was administered continuously throughout the preparation phase to replace fluid loss during instrumentation. A standard lead II electrocardiogram (ECG) was used to monitor cardiac rhythm; depth of anaesthesia was judged according to blood pressure, heart rate, and bispectral index (BIS*XP*, Aspect Medical Systems, Natick, MA) during the preparation phase. Additional propofol and sufentanil was administered if cardiovascular variables or electroencephalography indicated a reduced depth of anaesthesia.

Measurement

Intracranial pressure (ICP; Ventrix, Integra NeuroSciences, Plainsboro, NJ, USA) was measured within

the subdural space. For sampling of cerebral venous blood, a burr hole was drilled into the skull over the midline, and a 4F catheter was placed into the sagittal sinus. One 7F saline-filled catheter was advanced via the femoral artery for measurement of mean arterial blood pressure (MAP), and withdrawal of arterial blood samples. Additionally, an 8.5F catheter was inserted into the superior caval vein in order to measure core temperature, central venous pressure (CVP) and for drug administration; a 7.5F multilumen, flow-directed, fibreoptic pulmonary artery catheter (Edwards Swan Ganz Combo EDV Thermodilution Catheter, Baxter Laboratories, Irvine, USA) was placed in the pulmonary artery to measure cardiac output (CO). All catheters were flushed with isotonic saline containing $5\,U \cdot ml^{-1}$ heparin at a rate of $3\,ml \cdot h^{-1}$ to prevent obstruction during the preparation phase. Body temperature was maintained between 38.0 and 39.0 °C with a heating blanket. Each animal received an intravenous bolus of heparin ($100\,U \cdot kg^{-1}$) to prevent intracardiac clot formation.

Microdialysis

Another skull burr hole (left hemisphere, 10 mm paramedian and 10 mm rostral of the coronal suture) was prepared to insert a microdialysis catheter into the cerebral cortex 10 mm below the dura mater (CMA 71; membrane length 10 mm, CMA/Microdialysis, Stockholm, Sweden). The catheter was perfused with isotonic Ringer's solution (Perfusion Fluid CNS; CMA/Microdialysis, Stockholm, Sweden) at a flow rate of $1\,\mu l \cdot min^{-1}$ to obtain a concentrated dialysate with a recovery rate of approximately 30% [12, 13]. Accordingly, the microdialysis samples were collected at intervals of 30 min. The concentrations of brain tissue glucose (Glu), lactate (La), pyruvate (Py), and glycerol (Gly) were analysed (CMA 600, CMA/Microdialysis, Stockholm, Sweden), and the lactate/pyruvate ratio (La/Py ratio) was calculated.

After instrumentation for cerebral and haemodynamic variables, and a two hours equilibration phase, a midline laparotomy was performed; propofol infusion was adjusted to $4\,mg \cdot kg^{-1} \cdot h^{-1}$, and infusion of Ringer's solution was stopped prior to induction of haemorrhage. All parameters were in stable condition over five minutes, before the experiment was started. Baseline cerebral (ICP, CPP, calculated by MAP minus ICP or CVP, whatever was higher), and haemodynamic (MAP, HR, CO) parameters were assessed.

Blood samples

Arterial (a) and cerebral venous (cv) blood gas samples were obtained to analyse haemoglobin content (Hb), pH, partial pressure of oxygen (pO_2), partial pressure of carbon dioxide (pCO_2), base excess (BE), oxygen saturation (SO_2), and lactate (Lac) (ABL System 615; Radiometer Medical Inc.; Copenhagen, Denmark). Cerebral venous – arterial partial pressure difference of carbon dioxide was calculated as $p(cv\text{-}a)CO_2 = pcvCO_2 - paCO_2$. In addition, cerebral venous blood samples were drawn for analysis of protein S-100B. S-100B samples were immediately centrifuged at 1600*g* for 15 min, and the supernatants were stored in aliquots at −76 °C until analysis. Concentrations of S-100B were measured in duplicate using a commercially available immunoluminometric assay kit (Sangtec® 100 ELISA, DiaSorin Inc., Stillwater, Minnesota, USA), and the averages were reported. According to the manufacturer's instructions, this assay is a monoclonal two-site immunoassay with a detection limit of $0.02\,\mu g \cdot l^{-1}$ for S-100B in humans, and this test has been found to be cross-reactive with pig serum.

Experimental protocol

The experiment was started with an incision (width, 12 cm; depth, 3 cm) across the right liver lobe to simulate uncontrolled haemorrhage. Haemodynamic decompensation was defined as a MAP of less than 25 mm Hg, or a heart rate of less than 20% of its peak value. At that point, FiO_2 was raised to 1.0 and all animals received a hypertonic-hyperoncotic hydroxyethyl starch solution (HyperHAES®; Fresenius, Bad Homburg, Germany, $4\,ml \cdot kg^{-1}$ over 2 min) and either norepinephrine (Aventis Pharma, Frankfurt am Main, Germany; bolus of $12.5\,\mu g \cdot kg^{-1}$ followed by a continuous infusion of $30\,\mu g \cdot kg^{-1} \cdot h^{-1}$; 6 animals), norepinephrine (bolus of $25\,\mu g \cdot kg^{-1}$ followed by a continuous infusion of $60\,\mu g \cdot kg^{-1} \cdot h^{-1}$; 6 animals) or arginine vasopressin (Pitressin®, Parke-Davis, Karlsruhe, Germany; bolus of $0.2\,IU \cdot kg^{-1}$ followed by a continuous infusion of $2\,IU \cdot kg^{-1} \cdot h^{-1}$; 6 animals). Bleeding was controlled by manual compression of the liver 30 min after drug administration, and FiO_2 adjusted to 0.5. Crystalloid (Ringer's solution, $10\,ml \cdot kg^{-1} \cdot h^{-1}$) and colloid (hydroxyethyl starch 130/0.4, $10\,ml \cdot kg^{-1} \cdot h^{-1}$) solutions were administered continuously. Haemodynamic parameters, blood gases, cerebral venous protein S-100B, and microdialysis samples were taken during stable conditions, at haemodynamic decompensation, and subsequently 30 and 90 min after drug administration. At the

end of the experimental protocol, the animals were euthanised with an overdose of propofol, sufentanil, and potassium chloride, and subjected to necropsy to check for correct positioning of the catheters.

Statistical analysis

Statistics were performed using commercially available statistics software (GraphPad Prism version 4.03 for Windows, GraphPad Software, San Diego, CA). A Kolmogorov–Smirnov test was used to test for Gaussian distribution. Variables were analysed with one way repeated measures analysis of variance with Bonferroni correction for multiple comparisons; haemodynamic values are expressed as mean ± standard deviation (SD), blood gas, S-100B and microdialysis values are expressed as mean ± standard error of mean (SEM). Correlations between Gly, La and the La/Py ratio were analysed with Spearman's rank correlation. Statistical significance was considered at $p<0.05$.

Results

Haemodynamic data, blood gases, protein S-100B and microdialysis values at the different experimental stages are presented in Tables 1, 2 and 3, respectively. Following liver trauma, CPP and CO decreased continuously. Criteria for haemodynamic decompensation were reached after 32 ± 5 min, and the total blood loss was $38 \pm 5\,ml \cdot kg^{-1}$. Haemorrhage to a CPP of 16 ± 5 mm Hg increased heart rate by 103%, and reduced CO by 65% ($p<0.001$ vs. baseline). Microdialysis measurements showed an increase of La, Gly, and the La/Py ratio compared to baseline (mean ± SEM; La 2.4 ± 0.2 vs. $1.4 \pm 0.2\,mmol \cdot l^{-1}$, $p<0.01$; Gly 37 ± 7 vs. $27 \pm 6\,\mu mol \cdot l^{-1}$, n.s.; La/Py ratio 50 ± 8 vs. 30 ± 4, $p<0.01$). Cerebral venous protein S-100B increased during systemic haemorrhage. After drug administration, CPP and CO increased all along the experimental procedure, reaching 68 and 115% of baseline values 90 min after initiation of therapy ($p<0.01$ to $p<0.001$ values before therapy). La, Gly, and the La/Py Py ratio further increased (La $3.4 \pm 0.3\,mmol \cdot l^{-1}$; Gly $69 \pm 10\,\mu mol \cdot l^{-1}$; La/Py ratio 58 ± 8; 30 min after initiation of therapy, $p<0.001$), and remained increased 90 min following drug administration, respectively. Protein S-100B remained increased after 30 min of therapy, but decreased close to baseline values after 90 min of therapy. PaO_2 values closely reflected changes in FiO_2. P(cv-a)CO_2 significantly increased with haemorrhage ($p<0.001$ vs. baseline). After drug administration, both arterial and cerebral venous lactate levels

Table 1. *Haemodynamic variables at baseline, haemorrhage and during therapy*

	Baseline $n=18$	Before therapy $n=18$	+10 min $n=17$	+30 min $n=17$	+90 min $n=12$
HR, beats · min^{-1}	99 ± 22	193 ± 27*	194 ± 30*	202 ± 32*	200 ± 35*
MAP, mm Hg	85 ± 15	25 ± 3*	43 ± 18*†	52 ± 16*†	58 ± 18*†
CPP, mm Hg	70 ± 16	16 ± 5*	34 ± 16*†	43 ± 16*†	46 ± 20*†
CO, l · min^{-1}	5.8 ± 1.3	2.0 ± 0.5*	2.9 ± 0.7*	3.6 ± 1.3*‡	6.2 ± 2.0‡
ICP, mm Hg	14 ± 4	9 ± 3*	9 ± 3*	11 ± 2	13 ± 2†

Values are mean ± SD. *HR* Heart rate; *CPP* cerebral perfusion pressure; *CO* cardiac output; *ICP* intracranial pressure; *MAP* mean arterial blood pressure; *Time points* baseline before induction of haemorrhage, baseline before therapy; *+10 min* 10 min of therapy; *+30 min* 30 min of therapy; *+90 min* 90 min of therapy. * $p<0.001$ vs. baseline; † $p<0.01$ vs. before therapy; ‡ $p<0.001$ vs. before therapy. Six animals died due to ventricular fibrillation during therapy; from that point, data of these animals were excluded from further analysis

Table 2. *Cerebral microdialysis at baseline, haemorrhage and during therapy*

	Baseline $n=18$	Before therapy $n=18$	+30 min $n=17$	+90 min $n=12$
Glucose, mmol · l^{-1}	1.0 ± 0.2	0.8 ± 0.2	1.5 ± 0.3§	1.6 ± 0.4§
Lactate, mmol · l^{-1}	1.4 ± 0.2	2.4 ± 0.2*	3.4 ± 0.3†	2.7 ± 0.4*
Pyruvate, μmol · l^{-1}	52 ± 7	63 ± 8	78 ± 10*	49 ± 6
Lactate/pyruvate ratio	30 ± 4	50 ± 8*	58 ± 8#	58 ± 11#
Glycerol, μmol · l^{-1}	27 ± 6	37 ± 7	69 ± 10‡	64 ± 12‡

Values are mean ± SEM. *Time points* baseline before induction of haemorrhage, baseline before therapy; *+30 min* 30 min of therapy; *+90 min* 90 min of therapy. * $p<0.01$ vs. baseline; # $p<0.001$ vs. baseline; † $p<0.05$ vs. before therapy; ‡ $p<0.01$ vs. before therapy; § $p<0.001$ vs. before therapy. Six animals died due to ventricular fibrillation during therapy; from that point, data of these animals were excluded from further analysis

Table 3. *Arterial and cerebral venous blood gases, and cerebral venous protein S-100B at baseline, haemorrhage and during therapy*

		Baseline $n=18$	Before therapy $n=18$	+30 min $n=17$	+90 min $n=12$
pH, Units	art	7.49 ± 0.01	7.43 ± 0.02	$7.24 \pm 0.02^{\#\S}$	$7.28 \pm 0.02^{\dagger}$
	cv	7.40 ± 0.01	7.29 ± 0.02	$7.13 \pm 0.02^{\dagger}$	$7.20 \pm 0.02^{\#}$
BE, $mmol \cdot l^{-1}$	art	7.7 ± 0.6	$0.5 \pm 1.2^{*}$	$-5.7 \pm 1.1^{\#}$	$-3.8 \pm 1.2^{\#}$
	cv	8.4 ± 0.6	$1.5 \pm 1.4^{*}$	$-6.1 \pm 1.1^{\dagger}$	$-4.1 \pm 1.2^{\#}$
Glucose, $mg \cdot dl^{-1}$	art	118 ± 5	190 ± 19	198 ± 34	184 ± 32
	cv	107 ± 5	174 ± 17	187 ± 33	179 ± 27
Hb, $g \cdot dl^{-1}$	art	7.8 ± 0.2	7.5 ± 0.3	$5.6 \pm 0.2^{\dagger}$	$4.3 \pm 0.2^{\ddagger}$
	cv	7.8 ± 0.1	7.5 ± 0.3	$5.7 \pm 0.2^{*\dagger}$	$4.3 \pm 0.2^{\#\S}$
pCO_2, mm Hg	art	41 ± 1	35 ± 1	$47 \pm 1^{\dagger}$	$48 \pm 1^{\dagger}$
	cv	54 ± 1	58 ± 1	$67 \pm 1^{*}$	60 ± 1
pO_2, mm Hg	art	153 ± 8	143 ± 4	$398 \pm 17^{\dagger}$	232 ± 6
	cv	36 ± 2	$25 \pm 1^{*}$	$44 \pm 1^{\dagger}$	$51 \pm 2^{\dagger}$
SO_2, %	art	100 ± 0	100 ± 0	100 ± 0	100 ± 0
	cv	46 ± 2	$22 \pm 2^{\#}$	46 ± 3	$61 \pm 3^{\dagger}$
p(cv-a)CO_2, mm Hg		13 ± 1	$24 \pm 1_{\#}$	$19 \pm 1^{\dagger}$	$13 \pm 1^{\S}$
S-100B, $\mu g \cdot dl^{-1}$	cv	0.65 ± 0.08	$0.83 \pm 0.14^{*}$	$0.97 \pm 0.14^{\#}$	$0.68 \pm 0.11^{\dagger}$

Values are mean ± SEM. *BE* base excess; *Hb* haemoglobin content; *pCO_2* partial pressure of carbon dioxide; *pO_2* partial pressure of oxygen; *SO_2* oxygen saturation; *p (cv-a) CO_2* cerebral venous – arterial partial pressure difference of carbon dioxide; *Time points* baseline before induction of haemorrhage, baseline before therapy; *+30 min* 30 min of therapy; *+90 min* 90 min of therapy. * $p<0.01$ vs. baseline; # $p<0.001$ vs. baseline; † $p<0.05$ vs. before therapy; ‡ $p<0.01$ vs. before therapy; § $p<0.001$ vs. before therapy. Six animals died due to ventricular fibrillation during therapy; from that point, data of these animals were excluded from further analysis

further increased significantly (Fig. 1, $p<0.001$ vs. baseline), and pH and BE decreased significantly ($p<0.05$ to $p<0.001$ vs. before therapy). Gly was significantly correlated with La and the La/Py ratio, respectively (Figs. 2 and 3, $p<0.0001$). Overall, 12 out of 18 animals survived throughout the study period. Six animals died due to ventricular fibrillation during therapy and, from that point, the data of these animals were excluded from further analysis.

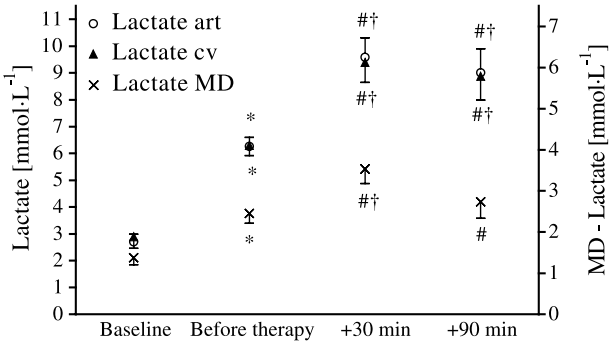

Fig. 1. Lactate levels ($mmol \cdot l^{-1}$) of arterial (circle) and cerebral venous (triangle) blood, and intracerebral microdialysis samples (cross) (mean ± SEM) at baseline before induction of haemorrhage, baseline before therapy, and following therapy after 30 min (+30 min) and 90 min (+90 min). *Art* arterial blood; *cv* cerebral venous blood; *MD* microdialysis; *Time Points* baseline before induction of haemorrhage, before therapy; *+30 min* 30 min of therapy; *+90 min* 90 min of therapy. *$p<0.01$ vs. baseline; #$p<0.001$ vs. baseline; †$p<0.05$ vs. before therapy. Six animals died due to ventricular fibrillation during therapy; from that point, data of these animals were excluded from further analysis

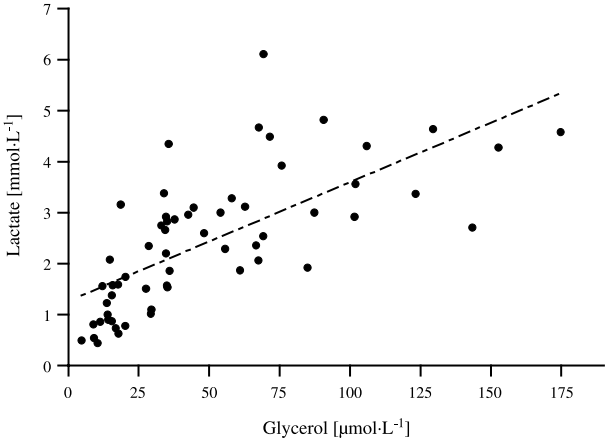

Fig. 2. Correlation between glycerol and lactate assessed by intracerebral microdialysis during the study period ($r=0.80$; $p<0.0001$; $n=60$ measurements in 18 animals). Six animals died due to ventricular fibrillation during therapy; from that point, data of these animals were excluded from further analysis

Discussion

The main findings of the present experimental study are as follows: (1) systemic haemorrhage to a CPP of 16 mm Hg resulted in increased lactate values and acidaemia in both systemic and cerebral venous blood gases; (2) haemorrhage caused increased cerebral venous protein S-100B levels indicating astroglial cell damage; (3) intracerebral microdialysis revealed anaerobic metabolism and intracerebral cell membrane degradation;

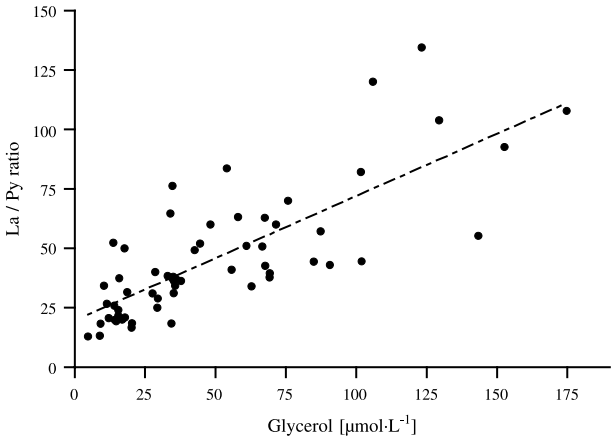

Fig. 3. Correlation between glycerol and the lactate/pyruvate ratio (La/Py ratio) assessed by intracerebral microdialysis during the study period ($r = 0.77$; $p < 0.0001$; $n = 60$ measurements in 18 animals). Six animals died due to ventricular fibrillation during therapy; from that point, data of these animals were excluded from further analysis

(4) Gly was significantly correlated with La and the La/Py ratio.

During past decades, reliable monitoring of cerebral oxygenation and metabolism during systemic haemorrhage has been a challenging task. General parameters, like peripheral oxygen saturation and blood pressure are not sufficient to provide information in this respect, since brain circulation is controlled by autoregulation, and cerebral pathology can impair oxygen supply to the brain despite an intact systemic circulation [22].

Trauma-induced acidaemia

Both during trauma and following therapy systemic and cerebral venous lactate increased, and acidaemia developed, as indicated by decreased pH and BE. Accumulation of lactate has been traditionally explained as a result of tissue hypoperfusion, hypoxia, anaerobic metabolism, or inadequate hepatic clearance [17, 18]. Further, systemic and cerebral venous haemoglobin levels were reduced after initiation of therapy. Moreover, decreased haemoglobin, increased lactate levels and acidaemia were persistent throughout the study period. In contrast, as both $pcvO_2$ and $p(cv\text{-}a)CO_2$ may serve as indirect parameters of global cerebral oxygenation supply and demand [19, 25], the decreased $pcvO_2$ and increased $p(cv\text{-}a)CO_2$ indicated reduced cerebral oxygenation at haemodynamic decompensation, but both parameters reached baseline values 30 and 90 min after drug administration, theoretically providing optimal conditions for ongoing aerobic cerebral metabolism sufficient for the integrity of neuronal structures.

Cerebral venous blood gases and microdialysis

Contrarily, both cerebral venous blood gases and intracerebral microdialysis revealed a mismatch between brain energy metabolism and supply of oxygen and glucose throughout the experimental procedure, thus refuting the primary endpoint hypothesis. However, the dural sinuses of the pig drain blood predominantly from cerebral veins, but also partially from extracranial tissue [9]. As such, it cannot completely be excluded that blood obtained from the sagittal catheter contained blood of non-cerebral origin. In contrast, intracerebral microdialysis is an established technique to analyse brain tissue biochemistry specifically [28]. In this respect, microdialysis La, Gly, and the La/Py ratio further increased significantly 30 min after initiation of therapy, and remained elevated 90 min following drug administration. The extracellular cerebral La/Py ratio and Gly are most sensitive and specific in indicating cerebral ischaemia [23, 26]. Therefore, the consensus statement on microdialysis recommends the use of the La/Py ratio as the primary marker, and Gly as an additional one, at least for secondary ischaemia in traumatic brain injury [2]. Furthermore, different information regarding substrate availability (Glu), redox state of the tissue (La/Py ratio), and cell membrane damage (Gly) can be obtained from microdialysis [6]. The observed microdialysis values at baseline in our study are comparable with the interstitial concentrations in normal piglet brain tissue that have been published previously [1]. Accordingly, changes of intracerebral microdialysis values that we found during our study period may reflect a pathophysiological range of ongoing brain metabolism indicating cerebral ischaemia and intracerebral cell membrane damage. As a secondary outcome of our study, the impairment of cerebral aerobic metabolism was significantly correlated with an increase in cerebral glycerol level reflecting degradation of phospholipid membranes [10, 27].

Microdialysis glycerol

Although blood gases indicated ongoing lactataemia, acidaemia and anaemia throughout the experimental procedure reflecting changes of systemic and cerebral metabolism continuously at the bedside in contrast to blood gases, intracerebral microdialysis further revealed elevated glycerol levels that may indicate cerebral cell membrane degradation during systemic haemorrhage and after initiation of therapy. The concept of monitoring cerebral interstitial glycerol levels by microdialysis has received considerable attention, and substantial evidence

can be found that microdialysis glycerol reflects membrane phospholipid degradation in stroke [8], subarachnoid haemorrhage [26] and traumatic brain injury [10]. However, there is still some concern about other sources of increased cerebral glycerol, such as spill over from the systemic pool of glycerol and formation of glycerol from glucose [11]. Nevertheless, intracerebral microdialysis provided real-time, continuous information on brain-specific metabolic monitoring and degradation of cellular membranes, sequentially.

Cerebral venous S-100B

To further elucidate the issue of potentially neuronal cell damage, we analysed cerebral venous protein S-100B. Our data confirm the results of studies that demonstrated increased serum S-100B levels during systemic haemorrhage in the absence of brain trauma [21]. These authors, however, have questioned a cerebral origin of increased S-100B levels, though they did not report on either cerebral venous material for protein S-100B analysis or other cerebral parameters. In contrast, increased systemic S-100B has previously been suggested to at least in part indicate blood-brain barrier dysfunction and damage of astroglial cells after various cerebral ischaemic conditions [3, 7, 20].

Intracranial pressure

ICP decreased with haemorrhage, and was not significant different to baseline value after 30 and 90 min of therapy. Thus, administration of hypertonic solutions has been reported to prevent an increase of ICP and to improve CPP and cerebral oxygen delivery compared to crystalloid solutions after haemorrhagic shock [24]. However, comparison between different resuscitation strategies with respect to cerebral haemodynamics, microdialysis variables and S-100B levels was not the primary issue of this study.

Limitations

Several limitations to this study should be noted. Since we were unable to measure direct cerebral blood flow using radioactive microspheres due to limitations posed by government regulations, we cannot comment on cerebral metabolic rate of oxygen. Furthermore, we could not make conclusions regarding the effects of anaesthesia, which may in fact be neuroprotective. A cerebral oxygenation parameter, such as $pcvO_2$ is dependent on FiO_2 and was changed twice in this study; however, the study protocol was designed to reflect a realistic model of out-of-hospital systemic haemorrhage trauma management. We did not compare each microdialysis value with systemic blood gas measurements as blood gas parameters reflect single time points, while microdialysis samples were collected at intervals of 30 min. Since we did not provide intensive care including transfusion of red blood cells or fresh frozen plasma because of limitations in laboratory and haematology resources, we stopped the experiment after 90 min of drug administration, and did not await recovery of measured parameters to baseline values. Finally, measurement of cerebral metabolism with a catheter placed in the cortex is a local method and may, therefore, underestimate global mismatch of cerebral oxygen demand and supply due to heterogeneity of the brain.

Conclusions

Intracerebral microdialysis was able to reflect changes of cerebral energy metabolism, and indicated anaerobic glycolysis and degradation of cellular membranes during systemic haemorrhage and after initiation of therapy. In addition, in our study, increased protein S-100B may suggest blood-brain barrier dysfunction and damaged astroglial cells.

Authors' contributions

PM performed data acquisition and analysis of the microdialysis derived data and drafted the manuscript; EC carried out anaesthesia and instrumentation of the animals and was responsible for haemodynamic data; BB performed withdrawal of blood samples; MS had a significant contribution on drafting the manuscript (Discussion section); BW performed the laparotomy; JS participated in the study design and helped to draft the manuscript; VD conceived of the study and helped to draft the manuscript (Methods section). All authors read and approved the final manuscript.

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