

Clinical Article

Multiparametric analysis of cerebral substrates and nitric oxide delivery in cerebrospinal fluid in patients with intracerebral haemorrhage: correlation with hemodynamics and outcome

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Summary

Background. There is no information regarding the possible role of cerebral substrates in the pathogenesis of neuronal injury in intracerebral haemorrhages (ICHs). Purposes of this prospective study were to clarify whether changes in substrates are the consequence of the initial brain damage in ICH and to elucidate the relationship among the biochemical mechanisms and clinical course of patients with ICH.

Method. During a period of two years, patients (GCS ≤ 8) who had ICH secondary to an aneurysm (SAH), stroke (sICH), or trauma (tICH) and underwent ventriculostomy with ICP monitoring and/or underwent cranial surgery were randomly enrolled in this study. Extracellular concentrations of glutamate, aspartate, glycine, GABA, lactate, lactate/pyruvate ratio, and glucose in the CSF were measured by use of high-performance liquid chromatography (HPLC). The nitric oxide (NO) concentration in the CSF was analyzed by chemiluminescence.

Findings. There were 75 patients (38 women and 37 men) with ICH included in this study. Twenty-one patients had SAH, 28 sICH, and 26 tICH. In tICH patients, there was a 30-fold increase in glutamate and a 10-fold in aspartate over reference values. The levels of glutamate, aspartate, GABA, lactate, glucose, and NO differed significantly among the three groups ($p < 0.001$). There were no significant differences in glycine and L/P ratio among the groups. The initial GCS, the mean CPP and outcome six months after the insult were all significantly correlated with the concentration of substrates ($p < 0.01$), both within groups and among the total sample. The CSF levels of glutamate, lactate, NO and glucose correlated significantly with outcome ($p < 0.005$).

Conclusions. This study confirms the correlation between the level of EAAs and the outcome of ICHs, suggesting that neurochem-

ical monitoring of these substances may have a role in caring for patients.

Keywords: Cerebral substrates; excitatory amino acid (EAA); intracerebral haemorrhage (ICH); microdialysis; nitric oxide (NO); subarachnoid haemorrhage (SAH).

Introduction

Although the role of excitotoxic amino acids (EAAs), particularly glutamate, has been described in ischemic stroke and head trauma, there is no information regarding their possible contribution to the pathogenesis of neuronal injury in intracerebral haemorrhage (ICH) [1–3, 15, 29, 30]. Both experimentally and in clinical studies, cerebral ischemia and traumatic brain injury are associated with excess liberation of EAA, enhancement of anaerobic metabolism, and release of nitric oxide (NO) byproducts [1–3, 8, 22, 26–30].

The three most frequent types of intracerebral haemorrhage (ICH) are subarachnoid haemorrhage (SAH), spontaneous intracerebral haemorrhage (sICH), and traumatic intracerebral contusion haemorrhage (tICH). Secondary brain damage after these acute cerebral insults is of increasing interest because it may be as important to the outcome as is the initial lesion [24]. Secondary brain

damage after ICH has been attributed to vasospasm following SAH, ischemia following sICH or SAH, and diffuse brain swelling following tICH. The search for early indicators of secondary deterioration has been enhanced by several new invasive and noninvasive techniques for monitoring patients with ICH [24].

This study was performed to measure the extracellular concentrations of various cerebral substrates and nitric oxide in the acute period after ICH. We sought to clarify whether changes in these substrates vary depending on the type of ICH, whether the levels are predictors of subsequent neurological damage, and to assess the biochemical mechanisms and clinical course of patients with different types of ICH.

Materials and methods

Consent

This study was approved by the medical ethics committee of the hospital. Written informed consent was obtained from the patients or their next of kin.

Patient characteristics

From January 2003 to December 2004, patients admitted to the Department of Neurosurgery at Mackay Memorial Hospital with a diagnosis of SAH, sICH, or tICH and whose initial Glasgow Coma Scale (GCS) on arrival at the emergency department was ≤ 8 were considered for enrolment in this prospective study. Of those, only patients who underwent ventriculostomy with monitoring of intracranial pressure (ICP) and/or cranial surgery were selected. The insertion of an external ventricular drain was determined by conventional clinical indications unrelated to the purpose of the study. Patient outcome was graded on the Glasgow Outcome Scale (GOS) at 6 months after injury. This assessment was made by an independent neurologist (P. T.) who was unaware of the study data.

The ICP monitoring was measured hourly through the ventriculostomy, along while simultaneous measurement of mean arterial pressure (MAP) and cerebral perfusion pressure (CPP). CSF samples (2 to 3 ml) were collected at daily intervals as long as the external ventricular drain remained in place and patent.

Amino acid analysis by HPLC

The CSF samples were immediately frozen at -80°C in liquid nitrogen until analysis. Analysis of the concentrations of glutamate, aspartate, glycine, GABA, lactate, and glucose, and the lactate/pyruvate ratio, were performed by high-performance liquid chromatography (HPLC) (HPLC9, BAS, U.S.A.). The amino acids were derivitized by o-phthalaldehyde (OPA; Sigma, Saint Louis, U.S.A.) before automatic injection into the HPLC column. Fluorometric detection for quantitation of amino acids was performed as previously described [5, 10, 15, 23].

NO assay by chemiluminescence

The NO concentration in the CSF specimens was analyzed by chemiluminescence using a Sievers 280 NO analyzer (Sievers Inc., Boulder,

CO) according to the manufacturer's instructions. We chose to integrate NO release over 4 minutes because this period accounted for $>90\%$ of the NO peak. The protocols for NO measurement were adapted from published procedures [6, 13].

Statistical analysis

All data are presented as the mean \pm standard deviation (SD). We compared the substrate levels we measured with the reference values in the literature [2, 9, 13, 23, 24, 25]. Statistical analysis was performed on a personal computer using Statistical Package for Social Science software (SPSS Inc. Chicago, U.S.A.), version 10.0. Analysis of variance (ANOVA) was used to compare the means among three groups and a post hoc Tukey's honestly significant difference (HSD) test was used to test for differences in the means of two groups. The initial GCS on admission, the GOS six months after insult, the mean CPP during the period of substrates measurement were examined for correlation with the mean substrate concentrations by using Pearson correlation coefficients. Differences were considered statistically significant at a p of <0.05 .

Results

Patient profiles (Table 1)

A total of 75 patients (38 women and 37 men) with ICH were included in this study. Their ages ranged from 21 to 79 years, with a mean of 46 years. Twenty-one patients had SAH, 28 sICH, and 26 tICH. The GCS, CPP and GOS were all lower in the tICH group than in the other two. However, the difference among the three groups was not significant ($p > 0.05$).

CSF collection

CSF was collected on a daily basis over a period ranging from 3 to 14 days (mean, 8.6 ± 3.0 d).

Concentration of substrates (Table 2)

Excitatory amino acids (EAA)

Glutamate/aspartate. The glutamate concentration was 30-fold higher than the reference value in patients with

Table 1. Demographic and clinical data of 75 patients with ICH

	SAH	sICH	tICH	P value
Number	N = 21	N = 28	N = 26	
Gender	M = 9 F = 12	M = 14 F = 14	M = 14 F = 12	
Age (years)	47 ± 6	56 ± 7	34 ± 5	0.176
GCS	5.9	6	5.4	0.827
CPP (mmHg)	84.6 ± 8.9	86.4 ± 7.6	82.3 ± 10.5	0.659
GOS	3.6	3.8	3.4	0.277

Table 2. Mean concentration of CSF substrates in patients with ICH

	Ref. values [#]	sICH (n = 28)	SAH (n = 21)	tICH (n = 26)
Glutamate*	3.2 ± 0.4	78.6 ± 5.3 ^{†,‡}	71.1 ± 4.0 ^{†,§}	95.7 ± 6.3 ^{†,§}
Aspartate*	1.7 ± 0.2	13.7 ± 0.7 ^{†,‡}	13.1 ± 0.6 ^{†,§}	15.8 ± 1.2 ^{†,§}
Glycine	16.8 ± 3.5	57.1 ± 3.1 ^{†,‡}	62.9 ± 3.3 [†]	65.0 ± 3.9 [†]
GABA*	9.1 ± 1.7	38.6 ± 2.1 ^{†,‡}	30.4 ± 1.4 ^{†,§}	26.6 ± 1.5 ^{†,§}
Lactate*	100–200	1330.5 ± 74.3 ^{†,‡}	1411.8 ± 72.4 ^{†,§}	1572.9 ± 116.1 ^{†,§}
L/P	23 ± 4	22.7 ± 1.8 [†]	21.6 ± 1.6 [†]	49.4 ± 2.9 ^{†,‡}
Glucose*	50	80.0 ± 4.8 ^{†,‡}	86.5 ± 4.8 ^{†,§}	94.7 ± 5.9 ^{†,§}
NO*	2.6–5.2	21.4 ± 1.1 ^{†,‡}	26.2 ± 1.3 ^{†,§}	28.2 ± 1.2 ^{†,§}

* p < 0.001; difference among all three groups by ANOVA.

^{†,‡,§} p < 0.05; difference between two groups by post hoc Tukey HSD test Mean ± SD (μmol/L), # reference values [2, 13, 20, 23, 24, 25].

tICH. The difference in glutamate values among three groups was statistically significant (p < 0.001); it was highest in the tICH group, followed by sICH and then SAH. A significant difference existed also between the SAH and the sICH groups and between the SAH and tICH groups (p < 0.05). Aspartate was also highest in patients with tICH, nearly 10 times higher than the reference value. It was significantly higher in the tICH group compared with SAH (p < 0.05) and sICH (p < 0.05), and between the SAH and the sICH groups (p < 0.05).

Inhibitory amino acids (IAA)

Glycine/GABA. The glycine concentration in patients with tICH was 4 times higher than the reference value, and it was highest in the tICH group, followed by SAH and then sICH. A significant difference also existed between the tICH and the sICH groups and between the SAH and sICH groups (p < 0.05). The concentration of γ-aminobutyric acid (GABA) in the sICH group was 4 times higher than the reference level, and the levels in the three groups differ significantly.

Energy-related substrates

Lactate, lactate/pyruvate ratio (L/P ratio), glucose. The concentration of lactate in the tICH group was 10 times higher than the reference level. The difference of lactate value among three groups was statistically significant (p < 0.001), and the level in the tICH group was significantly higher than in the sICH group (p < 0.05). The L/P ratio was 2-fold higher than the reference value in patients with tICH. The ratios were significantly higher in the tICH group compared with the SAH (p < 0.05) and the sICH (p < 0.05), and between the SAH and the sICH groups (p < 0.05). The glucose level was higher in patients with tICH. The glucose values differed significantly among all three groups (p < 0.001).

Nitric oxide

The NO concentration was 26.2 ± 1.3 μmol/L in patients with SAH, 21.4 ± 1.1 μmol/L in the sICH group, and 28.2 ± 1.2 μmol/L in the tICH group. It was highest in patients with tICH, a 10-fold increase over the reference value. The difference among the three groups was statistically significant.

Substrates, GCS, and GOS

In the tICH group (n = 26), the GCS gradually improved over 10 days, simultaneously with decreases in the average levels of substrates (Fig. 1). In each group, the initial GCS, mean CPP, and the GOS six months after the insult all correlated significantly with the mean concentration of substrates except for GABA and L/P (Table 3). The tICH group had higher levels of glutamate, aspartate, lactate and L/P ratio, all of which correlated to a lower GCS, CPP, and GOS (Tables 2

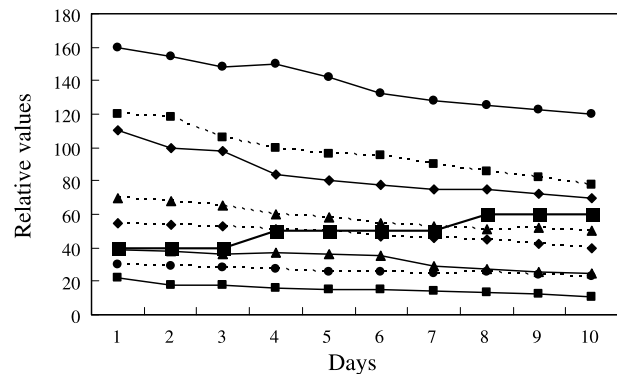


Fig. 1. Changes in the average substrate levels and the mean GCS over 10 days in the tICH group (n = 26). For clarity, the mean GCS was multiplied by 10 and the mean lactate level by 0.1. —■— GCS (×10), —●— Glutamate, —●— Lactate (×0.1), —▲— NO, —◆— Glucose, —■— Aspartate, —▲— Glycine, —●— GABA, —◆— L/P

Table 3. Correlation of outcome, initial GCS, and mean CPP with CSF substrates in patients with ICH (n = 75)

Pearson correlation	GOS	GCS	CPP
Glutamate	-0.442**	-0.432**	-0.443**
Lactate	-0.441**	-0.461**	-0.446**
NO	-0.374**	-0.356**	-0.388**
Glucose	-0.304**	-0.264*	-0.368**
Asparate	-0.282*	-0.293*	-0.332**
Glycine	-0.279*	-0.289*	-0.315**
GABA	-0.222	-0.232*	-0.204
LP ratio	-0.161	-0.165	-0.176

** Correlation is significant at the 0.01 level (2-tailed).
 * Correlation is significant at the 0.05 level (2-tailed).

and 3). On linear regression analysis, the GOS correlated most significantly to CSF levels of glutamate, lactate, NO and glucose (Table 3 and Fig. 2).

Discussion

Spontaneous intracerebral haemorrhage accounts for 10% to 15% of all cases of stroke and is associated with high mortality and morbidity [15, 16]. Vasospasm and ischemia continue to be the major causes of neurological complications after SAH [9]. In traumatic brain injury, EAA have a pivotal role in neuronal death [11, 15, 20,

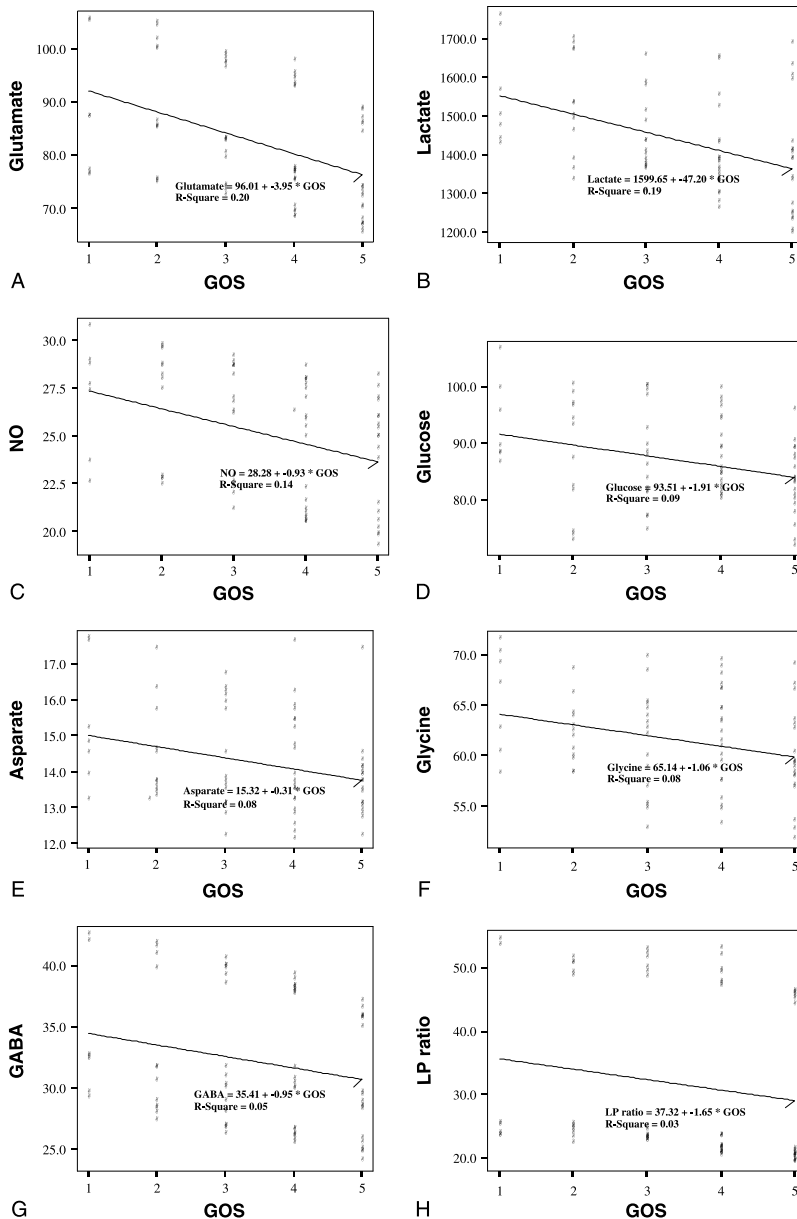


Fig. 2. Linear regression analysis of Glasgow Outcome Scale (GOS) and substrates levels. Glutamate, lactate, NO, and glucose correlated most significantly with GOS. (A) Glutamate, (B) lactate, (C) NO, (D) glucose, (E) aspartate, (F) glycine, (G) GABA, (H) L/P

30]. In addition to mass effect, the presence of a hematoma induces three early pathophysiological changes in the surrounding parenchyma: neuronal and glial cell death, vasogenic edema, and breakdown of the blood–brain barrier [15, 16]. Doppenberg *et al.* reported the devastating effects of space-occupying traumatic intracranial hematomas on substrate delivery [4]. Qureshi *et al.* showed that glutamate and other amino acids accumulate in extracellular fluids in the perihematoma region [15]. Our study showed significant elevation of the levels of cerebral substrates in the CSF of patients after ICH.

Each neuron contains approximately 10 mmol/L glutamate [11, 15]. Mechanical injury from pressure by a hematoma on the surrounding tissue may lead to release of intracellular stores of glutamate into the extracellular space. In addition, injury to astrocytes actively involved in the removal of glutamate may potentiate the extracellular accumulation of glutamate. Excessive concentrations of glutamate lead to activation of both NMDA and non-NMDA receptors and cell death [1, 8]. In both our study and that of other investigators, there was 30-fold increase in CSF glutamate and a 10-fold increase in aspartate in patients with tICH [30], suggesting that glutamate and aspartate are markers of cellular degradation [1].

Glycine is an amino acid which induces a long-lasting inhibitory postsynaptic response. It is a neurotransmitter with cortical hypothalamic projection. It is also produced by glycinergic neurons in the spinal cord, accounting for its presence in CSF obtained by lumbar puncture [23]. We observed a 4-fold increase of glycine in CSF from ventriculostomy in patients with all types of ICH. This suggests glycinergic neuron activation at the brain level with hypothalamic involvement. GABA is the most important inhibitory amino acid in the brain. During and Spencer demonstrated concomitant rises in the level of glutamate, aspartate, and GABA during seizures [5]. We did not find any significant differences in glycine and GABA values in the different types of ICH.

We found increases in lactate up to 10 times and in glutamate level up to 30 times over the standard values. Glutamate clearly influences the release of lactate following traumatic brain injury. Glutamate may drive lactate production to provide lactate as an energy substrate to neurons [1]. In patients with ICH, a sudden increase in lactate, the L/P ratio, and glutamate may serve as a warning signal [9, 21]. The strong positive correlation between glutamate and glucose may indicate an effect of glutamate on glucose uptake by cells which differs

according to the type of injury. We found that the CSF levels of lactate, and glucose and the L/P ratio differed significantly among the three groups. These substrates may be useful for monitoring patients for secondary damage after traumatic brain injury [12].

Nitride oxide is frequently called a double-edged sword in cerebral ischemia. It is a powerful dilator of cerebral vessels and has been reported to have both neuroprotective and cytotoxic effects [13, 17]. The elevated NO level after SAH may be caused by cerebral ischemia [9, 15, 18, 22, 24–26]. Overall, NO correlates with glucose, lactate, and glutamate [19]. The higher NO level in patients with a poorer clinical score at presentation may be a result of greater induction of NO synthase activity as a result of a greater cerebral insult [13]. In our study, the NO level was increased 10 times over the reference value in the tICH group, but the concentration did not differ significantly among the three groups.

Vespa *et al.* reported that the levels of glutamate, aspartate and glycine released into the extracellular fluid at a CCP of less than 70 mmHg were statistically significantly higher than at higher levels of CPP ($p < 0.001$) [28]. In our study, change in CPP also significantly affected the release of cerebral substrates after ICH ($p < 0.01$). Yamamoto *et al.* reported that glutamate concentration was not correlated with initial GCS score [29]. No significant correlation has been found for the concentration of NO metabolites with the patient's preoperative condition, the presence of symptomatic vasospasm, or prognosis [25]. In our study, cerebral substrate levels correlated significantly with the patient's initial GCS ($p < 0.01$). Concentrations of glutamate, aspartate and GABA have been found to correlate closely with outcomes of SAH ($P < 0.05$) [13, 14, 24, 29]. Brain lactate, pyruvate and the lactate/pyruvate ratio have also been recommended for use in estimating the severity of stroke and for predicting the outcome [3]. In a study of SAH, the NO level correlated with clinical severity but not with the neurological outcome [13]. Our study demonstrated a significant correlation between the concentration of CSF substrates in patients with ICHs and the outcome at 6 months, as assessed by the GOS ($p < 0.01$).

In this study, several methodological limitations must be noted. Because our study was limited to severely injured patients with ventriculostomies in place, our results are not necessarily generalizable to less severely injured patients. Laboratory analysis of microdialysis samples performed using HPLC is regarded as the gold standard [7]. However, this technique is time consuming

and not practical for clinical application in the intensive care unit or operating theatre. An on-line bedside analyzer would be preferable for real-time monitoring. The reference values we used were extracted from the literature, so we could not control for any differences in laboratory techniques. Also, we analyzed the levels of cerebral substrates in ventriculostomy CSF without using microdialysis, a method which allows assessment of regional differences within the brain. It may be that there are local effects that do not correlate well with global physiological changes, such as CPP or outcome. Besides, our study could not define the role of the blood-brain and blood-colloid-CSF barriers in the transport of substrates from the blood to the CSF. Because glutamate, aspartate, glycine, and GABA are secreted directly by neurons in the brain itself; the cellular energy metabolites lactate, pyruvate, and glucose may come from neurons or from systemic metabolism; and NO is the product of NO synthases, enzymes which are expressed in the brain [1, 9, 15, 21, 24, 28].

The success of treatment for delayed cerebral ischemia after ICH is time-dependent, and neuronal monitoring methods which can detect early subclinical levels of cerebral ischemia may improve overall treatment results [13, 15]. The aim is to characterize patterns of neuronal injury (using glutamate and aspartate, glycine) and markers of energy metabolism (using glucose, pyruvate and lactate) in patients with ICH [21]. It is hoped that the development of neuroprotective therapy based on monitoring of these substrates may reduce the incidence of secondary brain damage [30]. For example, glutamate antagonists have been shown to reduce the extent of infarction in animal models. Several of these agents have been investigated in phase II or III clinical trials for efficacy in acute stroke [15].

Conclusion

This study confirms the correlation between EAAs and lactate in the outcome of ICH, suggesting that neurochemical monitoring of these substances may have a role in caring for patients. Monitoring of these substrates may also help expand our knowledge of pathophysiological processes in ICH. Knowledge of the mechanisms underlying these processes seems to be necessary for the development of specific treatment strategies.

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Comments

This is an excellent paper. This represents an extremely large body of work, with multiple neurochemical analytes being measured in 75 patients with intracerebral haematomas of different types. This is the only such study of its kind in the world to date, as far as I am aware, in such large numbers of patients and it certainly makes a useful contribution to the literature.

Ross Bullock
Richmond

The current study compares ventricular concentrations of several amino acids (glutamate, aspartate, etc.), lactate, glucose, and NO in patients with intracerebral haemorrhage with acute (GCS, CPP) and chronic (GOS) clinical evaluation parameters. The main finding of the study is that the concentration of the biochemical markers correlates well with the clinical situation of patients.

The basic design of the study and the methodology used by the authors is sound, the manuscript is well written, and the data are well discussed. It is also interesting to see that measurements in the CSF, which are easily performed, result in accurate values with such a small error and do not seem to be inferior to data obtained by microdialysis.

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