Prostacyclin Treatment in Severe Traumatic Brain Injury: A Microdialysis and Outcome Study

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Abstract

Prostacyclin (PGI₂) is a potent vasodilator, inhibitor of leukocyte adhesion, and platelet aggregation. In trauma the balance between PGI₂ and thromboxane A₂ (TXA₂) is shifted towards TXA₂. Externally provided PGI₂ would, from a theoretical and experimental point of view, improve the microcirculation in injured brain tissue. This study is a prospective consecutive double-blinded randomized study on the effect of PGI₂ versus placebo in severe traumatic brain injury (sTBI). All patients with sTBI were eligible. Inclusion criteria: verified sTBI, Glasgow Coma Score (GCS) at intubation and sedation of ≤ 8 , age 15–70 years, a first-recorded cerebral perfusion pressure (CPP) of ≥ 10 mm Hg, and arrival within 24 h of trauma. All subjects received an intracranial pressure (ICP) measuring device, bilateral intracerebral microdialysis catheters, and a microdialysis catheter in the abdominal subcutaneous adipose tissue. Subjects were treated according to an ICP-targeted therapy based on the Lund concept. 48 patients (mean age of 35.5 years and a median GCS of 6 [3–8]) were included. We found no significant effect of prostacyclin (epoprostenol, Flolan) on either the lactate-pyruvate ratio (L/P) at 24 h or the brain glucose levels. There was no significant difference in clinical outcome between the two groups. The median Glasgow Outcome Score (GOS) at 3 months was 4, and mortality was 12.5%. The favorable outcome (GOS 4–5) was 52%. The initial L/P did not prognosticate for outcome. Thus our results indicate that there is no effect of PGI₂ at a dose of 0.5 ng/kg/min on brain L/P, brain glucose levels, or outcome at 3 months.

Key words: lactate-pyruvate ratio; microdialysis; prostacyclin; severe traumatic brain injury

Introduction

SEVERE TRAUMATIC BRAIN INJURY (sTBI) is a major cause of morbidity and mortality in the population, especially in younger age groups. Since the introduction of an intracranial pressure (ICP) guided treatment protocol based on the Lund concept for sTBI (Asgeirsson et al., 1994; Grande et al., 1997, 2002) in our department we have reported favourable outcome, GOS 4–5, in the range of 60 to 70% and a mortality in the range of 10 to 15% (Naredi et al., 2001; Wahlstrom et al., 2005; Olivecrona et al., 2007).

PGI₂ produced in the vascular endothelium is a potent vasodilator and inhibitor of leukocyte activation and adhesion to the vascular endothelium and platelet aggregation (Moncada et al., 1976; Moncada and Amezcua, 1979; Moncada and Vane, 1979b). The equilibrium between PGI₂ and TXA₂ seen in the healthy subject is in the traumatized subject altered and shifted towards TXA₂ (Gryglewski et al., 1978; Moncada and Amezcua, 1979). This relative decrease in PGI₂ levels would lead to decreased vasodilation, increased leu-

kocyte adhesion and platelet aggregation, and thus an impaired microcirculation. Beneficial effects of PGI₂ in experimental models of traumatic brain injury and microvascular permeability have been reported (Moller and Grande, 1997, 1999a, 1999b; Bentzer et al., 1999, 2001a, 2001b). In a study of experimental brain trauma, cerebral cortical blood flow and cortical lesion volume were studied in mice with and without the prostacyclin receptor gene (IP^{+/+} and IP^{-/-}). The study showed that the blood flow returned earlier to normal values and that the lesion volume was less in the IP^{+/+} mice (Lundblad et al., 2008). Low-dose PGI₂ given to five subjects with sTBI seemed to have some effects on energy metabolism related substances measured by brain microdialysis (Grande et al., 2000). We showed in 2001 (Naredi et al.) that epoprostenol given to subjects with sTBI did not induce side effects and had a tendency to improve outcome.

The present study is a prospective consecutive double-blinded randomized study of the effect of epoprostenol versus placebo in subjects with sTBI conducted at a Level 1 trauma center. The specific aim of the study was to establish whether

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the treatment with low-dose epoprostenol would reduce a L/P as measured by cerebral microdialysis after 24 h of treatment. The L/P is considered the most reliable marker for the redox state of the brain (Granhölm and Siesjö, 1969; Persson and Hillered, 1992; Enblad et al., 1996). Secondly, the changes in microdialysis parameters over time in the epoprostenol and placebo groups were studied. Thirdly, the aim was to compare outcome, as measured by GOS, between the two treatment groups at 3 months, and fourthly, to study if the initial L/P can prognosticate outcome estimated as GOS at 3 months.

Material and Methods

Subjects

All subjects with sTBI admitted to the department between January 1, 2002 and December 31, 2005 were eligible to be recruited into the study. The inclusion criteria were as follows: verified severe traumatic head injury, GCS at intubation and sedation of 8 or less, age between 15 and 70 years, a first-measured CPP of 10 mm Hg or more, and arrival in our department within 24 h of trauma. Exclusion criteria were: pregnant or lactating woman, known bleeding diathesis, known allergy to epoprostenol, and penetrating head injury. Thus, the study allowed for inclusion of subjects with GCS 3 and dilated fixed pupils as long as the initial CPP was 10 mm Hg or above.

The treating physician enrolled subjects fulfilling the criteria into the study and a written informed consent was obtained from their relatives. At the follow-up examination the subjects were asked to accept the inclusion into the study and to the follow-up. The subject had to be in need of cerebral intensive care for more than 72 h. Subjects who were extubated and/or discharged alive from the ICU within this time were excluded from the study as their brain injuries were not regarded as severe enough. All subjects dead within the first 3 months of inclusion to the study were regarded as due to the sTBI and thus as mortality in the study.

Monitoring

Intracranial pressure. All subjects received an intraparenchymal ICP measuring device (Codman MicroSensor, Johnson & Johnson Professional, Raynham, MA). The MicroSensor was placed through a burr hole preferable right frontal, approximately at the point of Kocher. The MicroSensor was used for ICP monitoring even if the subject later during the treatment received a ventriculostomy. This ICP sensor has been shown to be reliable with very low drift under clinical conditions (Koskinen and Olivecrona, 2005). If a ventriculostomy was placed, it was mainly used as drainage to treat ICP elevations. The drain was kept closed and opened only for drainage of minimal amounts of fluid.

Other physiological parameters. Arterial blood pressure was invasively continuously monitored with reference level at heart level. Systolic and diastolic blood pressures were registered and mean arterial blood pressure (MAP) and CPP were calculated by the monitoring equipment (Marquette Solar, General Electric Medical Systems, Milwaukee, WI). As all patients were positioned flat, the reference point for ICP and MAP is at the same level, resulting in a negligible hydrostatic

difference between the measuring sites. Thus no correction is needed for the CPP calculation. Further physiological parameters were monitored continuously.

Microdialysis

Two microdialysis catheters with gold tip were inserted into the brain (CMA 70, CMA Microdialysis, Solna, Sweden). They were placed in a standardized fashion frontally on each side approximately at the point of Kocher. The catheter placed in the most injured hemisphere, as judged from the primary CT scans, was designated A, and the other catheter designated B. A third microdialysis catheter (CMA 60, CMA Microdialysis) was placed in the subcutaneous adipose tissue of the upper part of the abdomen and designated C. The CMA 106 or 107 microdialysis pumps (CMA Microdialysis) were used and all three catheters perfused at a flow rate of 0.3 μ L/min. The "perfusion fluid CNS" (CMA Microdialysis) was used for the brain catheters, and "perfusion fluid T1" (CMA Microdialysis) was used for the subcutaneous catheter. The first dialysate was discharged. The sampling was started (0.5–2.5 h after the start of the microdialysis) and the sampling time for each sample was 2 h. The first sample of microdialysis was collected as 0-sample (zero sample, baseline). The vials were stored in a freezer (-20°C) for not more than 24 h and then frozen to -70°C , if not directly analyzed. The microdialysis samples were analyzed as soon as possible using a CMA 600 analyzer (CMA Microdialysis). Absolute values are reported for glucose levels and for L/P calculations.

Injury severity

For describing the severity of the injuries, ISS (Injury Severity Score) was used. As a measure of severity of illness, the subjects were scored according to APACHE II (Acute Physiology and Chronic Health Evaluation II).

Test drug

Epoprostenol (Flolan, GlaxoSmithKline, Brentford, United Kingdom) was used as active and saline as placebo test drug. The hospital pharmacy prepared the active drug and placebo in individually identifiable and numbered containers. The investigators, the treating physicians, and the ICU nurses, as well as the subjects and their relatives, were blinded through the follow-up time. The test drug was started at the same time as the microdialysis 0 sample was changed and given at an infusion rate corresponding to a dose of 0.5 ng/kg/min for 72 h. The test drug was then tapered over 24 h.

Treatment

An ICP-targeted treatment protocol was used. This has thoroughly been described elsewhere (Olivecrona et al., 2007), and is based on the Lund concept (Asgeirsson et al., 1994; Grande et al., 1997, 2002). In short, all subjects were continuously sedated, using midazolam and fentanyl, and artificially ventilated ($P_{\text{a}}\text{O}_2 \geq 12 \text{ kPa}$; $P_{\text{a}}\text{CO}_2$ 4.5–5.5 kPa). Normovolemia and normal colloid osmotic pressure were aggressively maintained, using infusion of packed red blood cells, albumin, Ringer's acetate, and glucose solutions. A neutral fluid balance was targeted, if necessary with help of furosemide. In order to normalize the blood pressure, after the establishment of normovolemia, and so to reduce the capillary

hydrostatic pressure and the general level of stress in the subjects, continuous infusions of clonidine and metoprolol were used. Further treatments steps to control the ICP were low-dose thiopental infusion, aiming at continuous delta activity on the EEG; ventriculostomy for drainage of CSF; and uni- or bilateral craniectomy. The subjects were all treated in the supine position without head elevation.

Randomization

Subjects were randomized to epoprostenol or placebo treatment by means of the random number method. With an effect size of 0.4 and a power of 0.8, the required total sample size needed was calculated to be 50.

Outcome

The subjects were followed-up at 3 months after injury. The interview followed the guidelines of the extended GOS and was performed by independent staff. The outcome is reported as GOS.

Data storage and statistics

All the collected data were digitally stored. Physiological and respiratory parameters from the ICU system (Marquette Solar, General Electric Medical Systems) were stored on a computer using the LABpilot software (CMA Microdialysis). The data were simultaneously stored in the patient's case file in the ICU system (Picis, Inc., Wakefield, MA). The microdialysis data were transferred from the CMA 600 analyzer to a personal computer and further processed with the LABpilot software. Statistical analysis and further processing of the data were performed with commercial statistical packages. Every case file was scrutinized for outliers due to, for example, calibration periods and mechanical disturbances. Further, the correlation in time of the sampling values was controlled.

Results are reported as means $\pm$ standard error of the mean (SEM) for continuous variables and in cases of discrete variables as median and range. When applicable, two-sided paired or un-paired Student's *t*-test was used for continuous data. Wilcoxon rank sum test was used for discrete variables. χ^2 -test was applied for proportions. Correlations were used as indicated, and Receiver Operator Characteristics (ROC) analyses were used to test predictability. A *p* of ≤ 0.05 was considered statistically significant.

Ethics and approval

The study was approved (Dnr 00-175) by the local ethics committee at Umeå University Hospital. The study was classified as a drug study and appropriate approval was given by Läkemedelsverket (Medical Products Agency, 151:633/01).

Results

Forty-nine subjects were randomized into the study. The relatives of one patient refused participation after randomization. Thus, 48 subjects (17 females, 31 males) participated in the study. Figure 1 depicts the progress through the study. In the epoprostenol group (PG-G) 23 subjects were included and in the placebo group (Plac-G) 25 subjects. The mean age was 35.5 ± 2.2 years. The median GCS on intubation and sedation was 6 (3–8). Figure 2 shows the GCS distribution. There were

no statistically significant differences in age, gender, GCS, ISS, or APACHE II between the two groups (unpaired Student's *t*-test [age], χ^2 test [gender], and Wilcoxon rank sum test) (Table 1). The ISS and the APACHE II indicate that the subjects were severely traumatized. Thirty-three (68.7%) were multi-traumatized. There was no significant difference in the time from accident until implantation of microdialysis catheters between the two groups (unpaired Student's *t*-test, see Table 1).

In Figure 3, the ICP, MAP, and CPP over time are depicted. There was no statistically significant difference between the two groups. It is clear that both ICP and CPP were well within the preset treatment goals. An ICP of above 20 mm Hg was recorded in less than 3% of all the registered ICP values during the first 120 h of treatment (once a minute). The same figure was found for CPP values lower than 50 mm Hg. In Figure 4, the ICP, MAP and CPP between survivors and deceased subjects are illustrated. ICP in the survivors was significantly lower than that in the deceased subjects.

The initial L/P was clearly elevated as compared to supposed normal values. We found a statistically significant reduction in L/P in the A catheter comparing the 0-sample with the sample after 24 h of treatment (65.9 ± 7.3 and 41.8 ± 5.1 , $p \leq 0.01$, paired Student's *t*-test). Similar results were found in the B catheter even though the difference was not statistically significant (63.9 ± 13.4 and 37.3 ± 2.8 , $p = 0.09$; paired Student's *t*-test). In the B catheter there was one subject with an abnormally high baseline L/P of 470.6 and a L/P of 63.8 at 24 h. Excluding this subject, the baseline L/P in the B catheter would be 52.3 ± 6.9 , and after 24 h of treatment 36.7 ± 2.8 ($p \leq 0.02$). Table 2 illustrates the L/P in the 0-sample and at 24 h of treatment for both groups and divided by the different catheters. There was no statistically significant difference between the PG-G and Plac-G comparing the L/P at 24 h of treatment or comparing the relative decrease in the L/P. Figure 5A shows the temporal profile of L/P in the A and B catheters for the whole material, and in Figure 5B the corresponding profile in the treatment groups. There was no statistical significant difference in the temporal profile of the L/P comparing catheters A and B or between the groups. The L/P was in both of the catheters and in both the treatment groups clearly elevated at the start of the treatment and did gradually decrease over time. We did not find any significant difference between the treatment groups at 24 h, even if we only considered the subjects with an initial L/P of >40 .

The initial L/P (A catheter) differs between survivors and non-survivors, 62.5 ± 8.2 and 86.2 ± 18.6 , respectively. Due to great variation in the L/P in the deceased subjects, the difference was not statistically significant. If postulating that the subjects deceased at 3 months should have a higher L/P than the survivors due to a more severe brain injury, one could apply a one-sided un-paired Student's *t*-test. In this situation the difference between 0-samples L/P in survivors and deceased is statistically significant at $p < 0.05$. Furthermore, in ten subjects, the 0-sample L/P exceeded 100 in one or both of the microdialysis catheters (mean 194.2 ± 37.2).

The cerebral glucose levels in the 0-sample and at 24 h of treatment did not show a statistically significant difference. They were in the A catheter 2.0 ± 0.3 mmol/L and 1.9 ± 0.2 mmol/L, respectively, and in the B catheter 2.0 ± 0.2 mmol/L and 1.9 ± 0.2 mmol/L, respectively. In Table 3 the glucose levels in both catheters separated by treatment at baseline and

FIG. 1. Flow through the study.

at 24 h of treatment are depicted. There was a tendency toward a gradual decrease in the CNS glucose level over time (Fig. 6). There was no significant difference in glucose levels between the PG-G and Plac-G. The baseline subcutaneous glucose concentration was 3.6 ± 0.4 mmol/L and after 24 h the

value was 4.9 ± 0.3 mmol/L ($p < 0.0001$ paired Student's *t*-test). As shown in Figure 6, there was a slight increase in the subcutaneous glucose concentration during the first few hours, which levelled out at a stable concentration. The ratio between the glucose concentration in the A catheter and the

FIG. 2. Distribution of GCS in the total material on sedation and intubation.

C catheter is constant over time (Fig. 6). The same result was seen when the ratio between the B and C catheter was calculated.

At the 3-month follow-up, the favorable outcome (GOS 4–5) was 52% and the mortality 12.5%. Figure 7 depicts the GOS distribution. There was no statistically significant difference in outcome between the treatment groups (χ^2 -test). Even if the outcome is divided into in three groups, favorable (GOS 4–5), unfavorable (GOS 2–3), and death (GOS 1), no statistically significant difference can be proven between the treatment groups (χ^2 -test). If excluding subjects with an initial GCS of 3 and/or bilateral dilated and fixed pupils, the favorable outcome would be 61% and the mortality 0%. Furthermore, of the deceased subjects, only two died during the ICU time. An uncontrollable high ICP was the direct cause of death in these subjects. They both presented with GCS of 3 and fixed dilated pupils. One of the subjects had had a circulatory arrest at the scene of the accident. The causes of death found among the subjects deceased outside the ICU were pulmonary artery embolism, pneumonia, and the decision to stop further active treatment (GOS 2).

Figure 8 depicts the initial L/P in the A catheter in relation to the GOS at 3 months. In neither the A nor the B catheter was there a statistically significant correlation between L/P in the 0-samples and GOS at 3 months nor the dichotomized GOS at 3 months (GOS 4–5 and GOS 1–3). ROC curve analysis showed no useable predictive value of the 0-sample L/P (A catheter) for dichotomized GOS (area under the curve 0.4867). However, when using deceased and alive in the dichotomization, the area under the curve was 0.701 (95% CI 0.556–0.835) and a L/P cut-off level of 110 (sensitivity 60%, specificity 87.5%). If the aim would be to use the result of the first sample L/P for prediction whether to treat or not, that is, to predict death due to the sTBI, the cut-off value would be 137. This analysis is based on the presumption that the test has to have a specificity of 95%; one of 20 subjects that would have been salvageable were left to die. The sensitivity of the test would then be 20%. Similar results were found when analyzing the B catheter.

Complications

No complications related to the test drug were observed. There were no bleeding complications due to the insertion of either the ICP sensor or the microdialysis catheters. Although leakage of CSF around the catheters occasionally was seen, no infections, superficial or deep, were identified.

Discussion

The primary hypothesis of the study was that supplemental treatment with epoprostenol in the dosage of 0.5 ng/kg/min would significantly reduce the L/P compared with the placebo group at 24 h of treatment. This was not met and thus we have to accept the 0-hypothesis that there is no difference between the two groups.

PGI₂ is synthesized in the vascular endothelium. PGI₂ affects the microvascular permeability, inhibits platelet and leukocyte aggregation, and has vasodilating properties (Moncada and Vane, 1979a, 1979b). Further PGI₂ has been shown to decrease leukocyte activation and adhesion to vascular endothelium, and thus possess anti-inflammatory

TABLE 1. BASAL DATA, GENDER, AGE, GCS, INJURY SEVERITY, TREATMENT TIMES, AND GOS AT 3 MONTHS

	All n = 48	Prostacyclin group n = 23	Placebo group n = 25
Mean age $\pm$ SEM	35.5 $\pm$ 2.2	35.8 $\pm$ 3.6	35.2 $\pm$ 2.6
GCS			
Median (range)	6 (3–8)	5 (3–8)	6 (3–8)
Mean $\pm$ SEM	5.3 $\pm$ 0.2	5.1 $\pm$ 0.3	5.5 $\pm$ 0.4
ISS			
Median (range)	29 (9–50)	29 (9–50)	29 (9–43)
Mean $\pm$ SEM	28.8 $\pm$ 1.4	29.7 $\pm$ 2.0	28.0 $\pm$ 2.0
APACHE II			
Median (range)	20.5 (12–32)	20.0 (12–29)	21.0 (14–32)
Mean $\pm$ SEM	20.8 $\pm$ 0.7	20.6 $\pm$ 1.1	21.0 $\pm$ 1.0
Time from accident until admission			
Mean $\pm$ SEM (hours)	6.2 $\pm$ 0.7	6.5 $\pm$ 1.2	6.0 $\pm$ 0.8
Time from accident until implantation of catheters			
Mean $\pm$ SEM (hours)	12.2 $\pm$ 0.7	12.8 $\pm$ 1.1	11.6 $\pm$ 0.9
GOS 3 months			
Median (range)	4 (1–5)	4 (1–5)	3 (1–5)
Mean $\pm$ SEM	3.5 $\pm$ 3.2	3.7 $\pm$ 0.2	3.4 $\pm$ 0.3

FIG. 3. Mean arterial blood pressure (MAP), cerebral perfusion pressure (CPP), intracranial pressure (ICP) over time in the total material. Values are mean $\pm$ sem.

FIG. 4. Mean arterial blood pressure (MAP), cerebral perfusion pressure (CPP), intracranial pressure (ICP) over time in survivors and deceased at 3 months. Values are mean.

TABLE 2. L/P IN 0-SAMPLE AND AFTER 24 H OF TREATMENT WITH TEST DRUG

Time	Prostacyclin group (n = 23)		Placebo group (n = 25)		Significance
	A catheter	B catheter	A catheter	B catheter	
0-sample	61.9 ± 8.1	82.1 ± 28.8	69.6 ± 12.0	49.3 ± 6.7	A to A n.s.
Mean ± SEM					B to B n.s.
24 h	43.1 ± 5.0	38.7 ± 3.9	49.7 ± 11.0	36.2 ± 3.9	A to A n.s.
Mean ± SEM					B to B n.s.
Significance	$p < 0.005$	n.s.	n.s.	n.s.	

A catheter brain (worse side); B catheter brain (better side); n.s., non-significant (unpaired Student's *t*-test between groups, paired Student's *t*-test within groups).

properties (Jones and Hurley, 1984; Erlansson et al., 1991). PGI₂ has also been shown to reduce an increased microvascular permeability caused by inflammation (Morel et al., 1990). The reduction of permeability positively affects the injured blood brain barrier, making it less permeable to fluids and macromolecules and thus decreasing the risk for extracellular edema (Mizuno-Yagyu et al., 1987; Blebea et al., 1990; Jahr and Grande, 1996). There is experimental and clinical evidence that PGI₂ may reduce vasospasm in nontraumatic subarachnoid hemorrhage (SAH) (Boullin et al., 1979; Brandt et al., 1981). We have shown that nimodipine resistant vasospasm in aneurysmal SAH can be reduced by infusion of low-dose epoprostenol (Koskinen et al., 2009). TXA₂ is, on the other hand, a powerful vasoconstrictor and promoter of platelet aggregation (Moncada et al., 1976). An imbalance of PGI₂ and TXA₂ in the injured brain may result in vasoconstriction, and microembolism and thus impaired microcirculation. The imbalance may also cause vasospasm (Seifert et al., 1987). The endogenous normal production of PGI₂ is estimated to be in the range of 0.08 to 0.1 ng/kg/min (Lewis and Dollery, 1983; Ritter et al., 1983; Davies and Hagen, 1993). The synthesis of PGI₂ and TXA₂ is altered immediately after trauma, and thus the balance between the two substances is dislocated toward the effect of TXA₂. A relative ischemia in the injured brain would be the consequences of such an impairment of the microcirculation. Thus, from a theoretical and experimental point of view, PGI₂ can have beneficial effects in conditions with a compromised microcirculation.

We report a mean ICP over time in this study of below 20 mm Hg and a mean CPP of around 60 mm Hg. This shows that we have managed to follow the intentions of the treatment protocol—ICP below 20 mm Hg and CPP not below 50 mm Hg. There have been discussions about whether this relatively low accepted CPP would lead to an increase of secondary brain injuries and thus a worse outcome (Andrews et al., 2002; Clifton et al., 2002). The initially decreasing and then stable and low L/P and stable brain glucose levels would indicate that the accepted CPP level does not give rise to ischemia defined as a pathological L/P. This observation is also in accordance with the recommendations in the guidelines for treatment of sTBI from the Brain Trauma Foundation (2007) where a CPP of 50–70 mm Hg is acceptable. The high number of favorable outcomes and low mortality indicate that an ICP targeted therapy based on the Lund concept contributes to avoidance of secondary injuries.

The elevation of the L/P in the brain is considered a reliable indicator of cerebral ischemia (Granhölm and Siesjö, 1969; Persson and Hillered, 1992; Enblad et al., 1996). The L/P is elevated, at least in the early course of sTBI, as an

indicator of brain injury and a compromised microcirculation. One can speculate that the amount of PGI₂ infused in the present study was too low to affect the L/P. However, the PGI₂ dose administered (0.5 ng/kg/min) was close to the estimated endogenous production of PGI₂. The used dose of PGI₂ was also in correspondence with the doses (0.5–1 ng/kg/min) used by Grande and colleagues (2000), eliciting some effects on energy metabolism related substances in five patients treated for sTBI. Our present findings do not support the findings by Grande et al. (2000). However, that study was a pilot and there was no control group. Thus, it is impossible to judge whether the effects were elicited by epoprostenol or were a part of the natural course of the brain trauma. We have earlier published a pilot and safety study using PGI₂ at a dose of 0.5 ng/kg/min (Naredi et al., 2001). The choice of dose thus relied on the report from Grande and colleagues and our own safety study.

The L/P in the total material and in the two groups follows each other closely over time. As an indicator of a severe brain injury, the L/P was elevated to values well above the presumed normal value of 20 (Reinstrup et al., 2000; Schulz et al., 2000). The L/P decreased over time and had significantly decreased at 24 h of treatment without significant difference between the PG-G and Plac-G. Still at 120 h after start of treatment, the L/P was clearly higher than the above stated normal value. The changes we report in L/P over time in subjects with sTBI are well in accordance with the findings of several other authors, that is, a starting point in high values and a decrease towards normal levels over time (Persson and Hillered, 1992; Stahl et al., 2001b). The initial elevation of the L/P would suggest a relative lack of oxygen compromising the mitochondrial function with an effect on the redox-state. The L/P are elevated, but in the majority of the subjects far from the values attributed to a truly ischemic brain, when values in the hundreds would be expected (Schulz et al., 2000). However, the normal value is derived from a low number of subjects (Reinstrup et al., *n* = 5; Schulz et al., *n* = 14) and have been questioned (Vespa et al., 2005). Our results show that the L/P levelled out around 40. Whether this actually is the normal value or not cannot be judged from our series. There are indications from other authors that a cut-off level of L/P of approximately 40 is more appropriate (Vespa et al., 2005, 2007). Furthermore, a publication from our department on microdialysis data in awake patients suffering from idiopathic normal pressure hydrocephalus reported a L/P of about 40 (Agren-Wilsson et al., 2005). We therefore consider that a L/P in the range of 40 may be normal. Based on this assumption that a L/P >40 is abnormal, and only considering subjects with an initial L/P exceeding this

FIG. 5. (A) The temporal profile of the L/P ratio for the whole material divided by brain microdialysis catheters (A and B). Values are mean $\pm$ sem. (B) The temporal profile of the L/P for the two treatment groups (PG-G and Plac-G) divided by brain microdialysis catheters (A and B). Values are mean.

TABLE 3. GLUCOSE (MMOL/L) IN 0-SAMPLE AND AFTER 24 H OF TREATMENT WITH TEST DRUG

Time	Prostacyclin group (n = 23)			Placebo group (n = 25)			Significance
	A catheter	B catheter	C catheter	A catheter	B catheter	C catheter	
0-sample							
Mean $\pm$ SEM	2.5 $\pm$ 0.6	2.3 $\pm$ 0.4	4.3 $\pm$ 0.6	1.7 $\pm$ 0.2	1.8 $\pm$ 0.3	3.1 $\pm$ 0.4	A to A n.s. B to B n.s. C to C n.s.
24 h							
Mean $\pm$ SEM	2.1 $\pm$ 0.4	2.3 $\pm$ 0.4	5.0 $\pm$ 0.5	1.7 $\pm$ 0.3	1.6 $\pm$ 0.2	4.9 $\pm$ 0.5	A to A n.s. B to B n.s. C to C n.s.
Significance	n.s.	n.s.	n.s.	n.s.	n.s.	$p < 0.0005$	

A catheter brain (worse side); B catheter brain (better side); C catheter adipose subcutaneous tissue; n.s., non-significant (unpaired Student's *t*-test between groups, paired Student's *t*-test within groups).

did not change our results concerning the effect of epoprostenol.

Interestingly, we did not observe a statistically significant difference in the results between the A catheter and the B catheter. It has been shown that there is a difference in the microdialysis results, depending on the catheter placement and in particular in relation to the most- or least-injured hemisphere (Stahl et al., 2001a, 2003; Bellander et al., 2004; Engstrom et al., 2005). The difficulty of designating better and worse sides is well illustrated with the subject in which the baseline L/P (470.6) in the B catheter (better side) by far exceeded the value on the worse side (59.1 in the A catheter). At 24 h the L/P had decreased to 63.8 and 40.0, respectively.

There was no significant difference in the brain glucose concentrations, neither between PG-G and Plac-G, nor between the baseline sample and the samples at 24 h. The

glucose levels were within the range considered normal, that is, about 2 mmol/L (Reinstrup et al., 2000; Schulz et al., 2000). The changes in glucose levels over time show the same pattern as that reported by other authors (Stahl et al., 2001a, 2001b; Lourido et al., 2002). There was no difference in the temporal profile of brain glucose in the PG-G and Plac-G or the catheters (A and B). Thus, no indirect signs of hypoperfusion or ischemia were observed. The observations of normal glucose levels and a L/P that, during the first 24 h, declines and stabilizes in a normal range indicate that the treatment given seems to protect the subjects from secondary brain injuries. The elevated L/P cannot be taken as a sign of an irreversible ischemia but could perhaps be interpreted as a warning sign. This is supported by the normal glucose levels. The stable subcutaneous glucose concentration gives an indirect sign of a stable blood glucose concentration in the normoglycemic range.

FIG. 6. The temporal profile of glucose concentration (mmol/L) for the two brain catheters (A and B), the subcutaneous catheter (C), and the ratio between the catheters A and C. Values are mean $\pm$ sem.

FIG. 7. Outcome as GOS in the total material.

The initial L/P has been suggested to be a surrogate endpoint for clinical outcome. We show that there is no statistically significant correlation between the initial L/P and the clinical outcome measured as GOS at 3 months. Further, our data show that the initial L/P is not a useable prognostic measure for the clinical outcome (GOS) in this cohort of subjects. The results also show the difficulty in using the primary L/P as a divider between when to treat or not to treat.

Caution should be taken in interpreting microdialysis data, as the microtrauma after the insertion of the microdialysis probe can induce erroneous values. This is a problem that all microdialysis reports share, but few deal with. Unfortunately, many publications do not even mention when the first microdialysis sample is collected in relation to insertion. The consensus meeting on microdialysis in neurointensive care (Bellander et al., 2004) recommends an equilibration period of at least one hour. As our 0-sample is collected in the majority of cases between the second and fourth hours, we consider our equilibration period to be appropriate.

It has been argued that the placement of the catheters play a crucial role in the microdialysis results demonstrated. Indeed, a consensus on the placement of microdialysis catheters in the

brain has been published (Hutchinson, 2002; Bellande et al., 2004). We have chosen to place the brain catheters in a standardized fashion, bilaterally and frontally. This is to our knowledge the most practical way of handling the insertion of microdialysis catheters in these severely ill subjects. If the method is to be used as a standard monitoring technique, it has to be reliable and easy to use, and the location of the catheters should not be the issue of reliability. One can argue that the measuring point should be in the so-called penumbra zone (Engstrom et al., 2005). There is no common definition of the penumbra zone, and the difficulty of defining the penumbra zone is seldom addressed. Further, it is important that the sampling site is not located in a contusion, as a large portion of this tissue is not salvageable. Furthermore, the pathophysiology of sTBI is a dynamic process; an area judged to be normal can over time turn into a area with a radiological lesion and an injured area can turn into a non-salvageable region. The only way to place the microdialysis catheters with high accuracy and in a pre-targeted volume is to do it by means of a stereotactical or neuronavigation supported procedure. This is, in our opinion, not practically feasible in these severely ill subjects and in a daily clinical practice.

Indeed, one can argue that the localization of the microdialysis catheters in the present study was not optimal in relation to the penumbra zone. We cannot exclude this possibility; the microdialysis results might be different with another catheter placement. This is a problem shared with many other publications and will be part of future studies.

We have earlier reported favorable outcomes in our sTBI subjects in the range of 60 to 70% and a mortality of approximately 15% (Naredi et al., 2001; Wahlstrom et al., 2005; Olivecrona et al., 2007). In the present study we found favorable outcomes, assessed at 3 months after the trauma, in 52% of the subjects, with a mortality of 12.5%. Two subjects died during treatment in the ICU, corresponding to an in-house mortality of 4.2%. Subjects were treated in the ICU until they had a stable ICP of below 20 mm Hg, or died. Thus, 95.8% of the subjects with sTBI were discharged alive. The treatment used seems to prevent death in sTBI due to therapy-resistant ICP, as only two of the subjects died due to this reason. The treatment seems to yield a high number of subjects with favorable outcome after sTBI. The favorable outcome we report is lower as compared to our earlier reports. This is most probably due to the fact that the earlier published outcomes are reported after a later follow-up time. Furthermore, the patients in the present study were more severely injured as judged from the GCS at intubation and sedation compared to those in our previous studies (Naredi et al., 2001; Wahlstrom et al., 2005; Olivecrona et al., 2007). The high ISS and APACHE II scores also illustrate the severity of the injuries in the subjects. In most outcome studies of sTBI, patients with dilated non-reactive pupils and/or GCS of 3 are excluded. We have chosen to accept all subjects irrespective of clinical status into the study as long as the subject presented an initial CPP of 10 mm Hg or higher. If we exclude the subjects, with fixed, dilated pupils and/or GCS of 3 from the study, the mortality would be 0%. An independent study from the Umeå University Hospital using the local trauma bank confirmed that the mortality in subjects primarily admitted alive with a sTBI ($n = 10$) to our hospital during one year (2001) was 0% (Styrke et al., 2007). Most important is that a low mortality rate does not result in surviving vegetative subjects. We show that the

FIG. 8. Initial L/P in the A catheter to GOS at 3 months. Values are mean $\pm$ sem.

number of vegetative subjects, in spite of the low mortality, is low. Irrespective of dichotomization, there was no difference in the outcome assessed as GOS between the PG-G and the Plac-G. We realize that it might be difficult to show any improvement in clinical outcome using a single new element, such as prostacyclin, as the outcome in the control group already is very good.

Conclusions

Epoprostenol at the dose of 0.5 ng/kg/min had no influence on the brain L/P or brain glucose levels measured by microdialysis. In addition, no effect of prostacyclin on the clinical outcome was identified at 3 months. The present study does not exclude the possibility that prostacyclin in higher doses may be beneficial. The treatment protocol was followed and did not result in ischemic episodes in the brain. The initial L/P did not prognosticate for clinical outcome. Microdialysis remains a valuable research tool, but in our opinion the findings are difficult to interpret in a clinical setting. Accordingly, to base treatment decisions on the findings is even more difficult.

Author Disclosure Statement

This study was supported by GlaxoSmithKline, Stockholm, Sweden, which supplied the hospital pharmacy with the active test drug.

Acknowledgments

We thank the staff at the neurosurgical department and intensive care unit of Umeå University, without whose kindness, interest, and dedicated work the study could not have been performed. A special thanks to our research nurses, Kristin Nyman and Anna-Lena Östlund, for their invaluable help and support in making this study possible. This study was financially supported by the Department of Clinical Neurosciences University Hospital Research Fund, Tore Nilsson Fund, Kempe Fund, Capio Research Fund, and Umeå University.

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