Investigation of the effect of chlormethiazole on cerebral chemistry in neurosurgical patients: a combined study of microdialysis and a neuroprotective agent

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Aims Promising pre-clinical results from laboratory studies of neuro-protective drugs for the treatment of patients with stroke and head injury have not been translated into benefit during clinical trials. The objective of the study was to assess the feasibility of administering a potential neuro-protective drug (chlormethiazole) in conjunction with multimodality monitoring (including microdialysis) to patients with severe head injury in order to determine the effect of the agent on surrogate endpoints and penetration into the brain.

Methods Multimodality monitoring including cerebral and peripheral microdialysis was applied to five head-injured patients on the neuro-intensive care unit. Chlormethiazole (0.8%) was administered as a rapid (10 ml min⁻¹) intravenous loading infusion for 5 min followed by a slow (1 ml min⁻¹) continuous infusion for 60 min. The following parameters were monitored: heart rate, mean arterial blood pressure, intracranial pressure, cerebral perfusion pressure, peripheral oxygen saturation, continuous arterial oxygen partial pressure, arterial carbon dioxide partial pressure, arterial pH, arterial temperature, cerebral tissue oxygen pressure, cerebral tissue carbon dioxide pressure, cerebral pH, cerebral temperature, electroencephalograph (EEG), bispectral index, plasma glucose, plasma chlormethiazole, and cerebral and peripheral microdialysis assay for chlormethiazole, glucose, lactate, pyruvate and amino acids.

Results Despite achieving adequate plasma concentrations, chlormethiazole was not detected in the peripheral or cerebral microdialysis samples. The drug was well tolerated and did not induce hypotension, hyperglycaemia or withdrawal seizures. The drug did not change the values of the physiological or chemical parameters including levels of GABA, lactate/pyruvate ratio and glutamate. The drug did, however, induce EEG changes, including burst suppression in two patients.

Conclusions Chlormethiazole can be safely given to ventilated patients with severe head injury. There was no evidence of hypotension or withdrawal seizures. Combining a pilot clinical study of a neuro-protective agent with multimodality monitoring is feasible and, despite the lack of effect on physiological and chemical parameters in this study, may be a useful adjunct to the development of neuro-protective drugs in the future. Further investigation of the capability of microdialysis in this setting is required. By investigating the effect of a drug on surrogate end-points, it may be possible to identify promising agents from small pilot clinical studies before embarking on large phase III clinical trials.

Keywords: cerebral metabolism, chlormethiazole, head injury, microdialysis, neuroprotection

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Introduction

Following acute brain injury, cascades of biochemical processes are activated which contribute to cellular damage

and death [1–6]. These processes include excitatory amino acid activation, lactic acidosis, ion pump failure, free radical formation and lipid peroxidation [7–14]. Drugs have therefore been developed in order to intercept these processes with the aim of reducing secondary injury [15]. Encouraging laboratory investigations with glutamate antagonists, free radical scavengers and steroids have progressed to clinical trials but to date no proven efficacy has been demonstrated [12, 16–19]. Reasons for the failure of trials of clinical protection may include mechanisms of action which are not applicable in humans, differential pharmacokinetics and biodistribution in clinical subjects compared with experimental animals, and insensitive outcome measures which have focused on clinical outcome as the primary efficacy variable [16–19].

Understanding of the pathophysiology of acute brain injury has increased due to the application of cerebral microdialysis in the clinical setting. Monitoring of cerebral extracellular chemistry in patients with head injury and stroke is now possible and has demonstrated the importance of the role of excitatory amino acids, lactate and free radicals [20–27]. The principle is based on the insertion of a fine tube lined with a dialysis membrane into the cerebral parenchyma and perfused with a physiological solution [20, 28, 29]. Molecules diffuse from the extracellular space across the membrane into the solution which is then collected for analysis.

Recently, the sedative agent chlormethiazole, licensed for use in seizures and alcohol withdrawal, has been reported to have neuro-protective properties [30–37] and is undergoing clinical trials in stroke patients [38, 39]. Chlormethiazole is thought to activate the GABA_A receptor resulting in membrane hyperpolarization [40–42]. The aim of this study was to apply chlormethiazole in conjunction with microdialysis to a small sample of patients with severe head injury with the following objectives: (1) to determine the feasibility and safety of using multimodality monitoring to investigate the effect of a neuro-protective drug in patients with acute brain injury, (2) to determine the effect of chlormethiazole on surrogate end point measures including cerebral chemistry, oxygenation and intracranial pressure and (3) to determine whether microdialysis can be used to assess the blood brain barrier penetration of chlormethiazole.

Methods

Patient selection

The study was approved by the Cambridge Local Research Ethics Committee and written consent obtained from the next of kin after a full explanation of the study. Inclusion criteria were patients with head injury requiring ventilation and intracranial pressure monitoring.

Exclusion criteria were patients under the age of 16 years and those with deranged coagulation parameters and/or a low platelet count. Patients were managed according to standard protocols including sedation with propofol and fentanyl, and paralysis with atracurium [43].

Multimodality monitoring

Standard intensive care monitoring

Routine intensive care monitoring (blood pressure – arterial line, central venous pressure – subclavian line, heart rate and rhythm – ECG, oxygen saturation – pulse oximetry) was applied.

Specific multimodality monitoring

Three intracerebral probes were inserted via a cranial access device [44] into the superficial frontal cortex of the brain:

- (i) Intracranial pressure transducer (Codman, Raynham, MA, USA)
- (ii) Neurotrend multiparameter sensor (oxygen, carbon dioxide, pH, temperature; Codman, Raynham, MA, USA)
- (iii) Microdialysis catheter (CMA70 10 mm membrane perfused with Ringer's solution K⁺ 4 mm, Na⁺ 147 mm, Ca⁺⁺ 2 mm, Cl[–] 155 mm at 0.3 µl min^{–1} using the CMA106 pump). Vials were changed at 30 min intervals.

The electrical activity of the brain was recorded using bi-spectral index and 19-channel digital electroencephalography (EEG; Profile, Oxford Instruments, UK). EEG recording was commenced 30 min before the chlormethiazole infusion and continued until 60 min after the infusion.

A peripheral CMA60 30 mm membrane microdialysis catheter was inserted into the adipose tissue of the anterior abdominal wall to measure peripheral extracellular chemistry. A Paratrend multiparameter sensor (oxygen, carbon dioxide, pH, temperature; Diametrics, High Wycombe, UK) was inserted into the femoral artery for continuous blood gas analysis. Venous plasma samples were taken at the same time as microdialysis samples and assayed for chlormethiazole and glucose.

Chlormethiazole protocol

- (i) *In vitro study* In order to determine transfer of chlormethiazole across the dialysis membrane and solubility in the Ringer's solution, an *in vitro* study was performed. Two CMA 70 catheters were tested for recovery by immersion in a solution of 1.0 µg ml^{–1} chlormethiazole in Ringer's solution. Unadulterated Ringer's solution was pumped through each catheter at 0.3, 1, 2 or 5 µl min^{–1}, and the resultant dialysate was analysed for chlormethiazole. The concentration of

chlormethiazole within the test solution was determined before and after the recovery study.

(ii) *Clinical study* Chlormethiazole (Chlormethiazole edisylate 0.8% 8 mg ml⁻¹) was infused according to the protocol shown in Table 1. This dose was selected as the standard dose to effect sedation. The drug was administered as an intravenous infusion at 10 ml min⁻¹ for 5 min followed by 1 ml min⁻¹ for 60 min. There is a 17 min delay between microdialysis catheter tip and collecting vial, therefore the second study vial was changed at +17 min.

Microdialysis sample analysis

Microdialysis samples were analysed for basic substrates and metabolites, excitatory, inhibitory and structural amino acids, and chlormethiazole by three methods:

(i) *CMA600 bedside microdialysis analyser (substrates and metabolites)* Levels of glucose, lactate, pyruvate were assayed at the bedside using the CMA600 analyser (enzyme spectrophotometry) and then frozen at -70° C.

(ii) *High performance liquid chromatography (amino acids)* The samples were thawed and a 1 µl aliquot diluted 1:20. 5 µl of this solution was mixed with 3 µl of buffer containing norvaline (internal standard) and 3 µl of orthophthaldialdehyde (OPA)/3-mercaptopropionic acid (MPA). The chromatography system consisted of two high pressure pumps with a gradient controller and mixing chamber (Micro-Tech Scientific, Sunnyvale, CA, USA). The amino acids were separated using a C18 column and detected using a Gilson model 122 fluorometer (Gilson, Middleton, WI, USA) fitted with a 100 nl fluorobooster flow cell.

Table 1 Chlormethiazole infusion administration protocol.

Time (min)	Event	Chlormethiazole (ml min ⁻¹)	Microdialysis/ plasma sample
-13		0	✓
0	Start rapid infusion	10	
5	Start slow infusion	1	
17		1	✓
27		1	
37		1	
47		1	✓
57		1	
67		1	
77	End slow infusion	0	✓
87		0	
97		0	
107		0	✓
117		0	
127		0	
137	End study	0	✓

(iii) *Mass spectrometry (chlormethiazole assay)*. For analysis of microdialysis samples, 5 µl aliquots were injected onto a reverse phase analytical column (50 × 2 mm, 3µ Luna C18(2) Phenomenex, Macclesfield, UK) by an auto-sampler (Model 1100, Agilent Technologies, Bracknell, UK). The mobile phase was composed of 35% acetonitrile in 0.1% formic acid, filtered and degassed before use, and the flow rate of 0.3 ml min⁻¹ gave a retention time for chlormethiazole of 1.8 min. Chlormethiazole, detected using an u.v. detector set at a wavelength of 254 nm, had a limit of detection of 0.05 µg ml⁻¹ (signal to noise=5). The liquid chromatography eluent was also passed into an ion-trap mass spectrometer (Bruker Esquire-LC Ion-Trap, Coventry, UK) for confirmation of peak identity. Using an Electrospray Interface in positive mode, chlormethiazole produced a mass ion at m/z = 162. Limit of detection was 0.01 µg ml⁻¹ (signal to noise=5).

For analysis of blood, plasma samples were mixed with an equal volume of acetonitrile, centrifuged for 15 min at 13 000 rev min⁻¹, and the supernatant was injected onto the liquid chromatography system. Chlormethiazole was identified and quantified by reference to standard solutions. Calibration curves were constructed to cover the range 0.01–10 µg ml⁻¹.

Analysis

Results were expressed as the mean ± 95% confidence intervals for the five patients at baseline (pre-infusion of chlormethiazole), during the infusion and post-infusion.

Results

Patient demography

Five patients with severe head injury were studied. The demography of the study sample and the dose of other drugs are shown in Table 2.

In vitro assay of chlormethiazole

The *in vitro* relative recovery of chlormethiazole at different flow rates for CMA70 catheters in unstirred, room temperature (22° C) Ringer's solution containing 1.0 µg ml⁻¹ chlormethiazole is shown in Table 3. This indicated that for a flow rate of 0.3 µl min⁻¹ chlormethiazole crossed the dialysis membrane with a relative recovery rate of 64% (i.e. the concentration of chlormethiazole in the dialysate was 64% of the true concentration in the surrounding solution).

In vivo assay of chlormethiazole

Chlormethiazole was assayed in the plasma, in the peripheral adipose tissue microdialysate and in the cerebral

Table 2 (a) Patient demography.

Patient	Age (years)	Sex	Initial GCS	CT	Time injury-drug (h)
1	64	F	11	Contusions	59
2	29	M	8	Extradural contusions	32
3	31	M	6	Diffuse injury	59
4	55	M	11	Contusions	96
5	52	M	13	Traumatic SAH	55

GCS, Glasgow Coma Score; CT, computed tomography.

(b) Drug dosages.

Patient	Propofol 2% (ml h ⁻¹)	Fentanyl (µg h ⁻¹)	Atracurium (mg h ⁻¹)
1	12	50	70
2	6–8	100	70
3	7–10	150	70
4	23	200	70
5	20	50	70

Table 3 *In vitro* relative recovery of chlormethiazole at different flow rates for CMA70 catheters in unstirred, room temperature (22° C) Ringer's solution containing 1.0 µg ml⁻¹ chlormethiazole. Values are mean ± 95% confidence intervals for three consecutive samples collected after equilibration following a change in flow rate.

Flow rate (µl min ⁻¹)	Catheter A (%)	Catheter B (%)
0.3	64.5 ± 2.7	64.0 ± 2.5
1.0	56.5 ± 13.4	54.3 ± 3.2
2.0	40.9 ± 5.5	42.7 ± 7.5
5.0	21.5 ± 7.0	21.4 ± 6.5

Table 4 Mass spectrometry assay for plasma concentrations of chlormethiazole (µg ml⁻¹).

Time (min)	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
-13	0	0	0	0	0
17	1.78	1.82	1.13	0.94	1.4
47	1.78	1.6	1.09	0.72	0.96
77	1.98	1.76	0.69	0.72	0.9
107	0.56	0.58	0.09	0.15	0.27
137	*	0.38	0.1	0.11	0.22

(* sample not taken).

microdialysate. The chlormethiazole concentrations in the plasma are shown in Table 4 and Figure 1. Chlormethiazole was readily detected in the plasma with a rapid increase followed by steady state levels. However, assay of both peripheral and cerebral microdialysis vials was unable to detect chlormethiazole *in vivo*. We calculated that a steady state plasma concentration of 1.2 µg ml⁻¹

Figure 1 *In vivo* assay of chlormethiazole. The chlormethiazole infusion is shown on the left axis. The infusion commenced at time 0 at a rate of 10 ml min⁻¹ for 5 min and then 1 ml min⁻¹ for 60 min. The mean plasma concentrations are shown on the right axis (○). There was a rapid rise in the plasma concentration to approximately 1.5 µg ml⁻¹ followed by a steady state which declined rapidly following cessation of the infusion.

would equate to a free unbound fraction of $1.2 \times 0.35 = 0.42 \mu\text{g ml}^{-1}$ and therefore a total body water concentration of $3/(3 + 11 + 28) \times 0.42 = 0.03 \mu\text{g ml}^{-1}$ assuming a plasma volume of 3 l, interstitial fluid of 11 l and intracellular fluid of 28 l and equal distribution throughout body compartments. Taking the microdialysis relative recovery rate to be 64%, this equates to $0.03 \times 0.64 = 0.02 \mu\text{g ml}^{-1}$ potentially recoverable in the microdialysate. The assay sensitivity was within this limit of detection.

Effect of chlormethiazole on physiological parameters

The effect of chlormethiazole on the physiological monitoring parameters is shown in Table 5 and Figure 2.

Table 5 Effect of chlormethiazole on physiological variables. Results are expressed as mean ($\pm$ 95% confidence intervals).

Variable	Baseline (pre-rapid infusion)	End of rapid infusion	End of slow infusion	End of study
HR	75 $\pm$ 19	78 $\pm$ 20	81 $\pm$ 28	79 $\pm$ 27
MAP (mmHg)	93 $\pm$ 13	92 $\pm$ 14	95 $\pm$ 14	90 $\pm$ 9.4
ICP (mmHg)	15 $\pm$ 3.2	14 $\pm$ 2.3	15 $\pm$ 4.6	16 $\pm$ 8.3
CPP (mmHg)	76 $\pm$ 12	79 $\pm$ 12	81 $\pm$ 11	74 $\pm$ 8.7
SaO ₂	99 $\pm$ 1.1	99 $\pm$ 1.0	99 $\pm$ 0.72	99 $\pm$ 1.8
pO _{2a} (kPa)	15.9 $\pm$ 4.91	13.5 $\pm$ 4.53	11.0 $\pm$ 4.95	12.2 $\pm$ 4.59
pCO _{2a} (kPa)	4.82 $\pm$ 0.83	4.83 $\pm$ 0.82	4.88 $\pm$ 0.53	4.89 $\pm$ 0.55
pHa	7.41 $\pm$ 0.110	7.41 $\pm$ 0.112	7.41 $\pm$ 0.083	7.41 $\pm$ 0.111
Ta (°C)	36.9 $\pm$ 1.30	36.8 $\pm$ 1.27	36.8 $\pm$ 1.49	36.7 $\pm$ 1.60
pO _{2b} (kPa)	2.4 $\pm$ 2.0	2.5 $\pm$ 2.0	2.8 $\pm$ 2.5	2.6 $\pm$ 2.3
pCO _{2b} (kPa)	6.52 $\pm$ 1.46	6.29 $\pm$ 1.65	6.53 $\pm$ 1.36	6.69 $\pm$ 1.70
pHb	7.12 $\pm$ 0.067	7.12 $\pm$ 0.065	7.12 $\pm$ 0.076	7.11 $\pm$ 0.084
Tb (°C)	37.1 $\pm$ 1.21	37.1 $\pm$ 1.23	37.0 $\pm$ 1.40	37.0 $\pm$ 1.50

HR- heart rate, MAP- mean arterial blood pressure, ICP- intracranial pressure, CPP- cerebral perfusion pressure, SaO₂- peripheral oxygen saturation, pO_{2a}- arterial oxygen partial pressure, pCO_{2a}- arterial carbon dioxide partial pressure, pHa- arterial pH, Ta- arterial temperature, pO_{2b}-brain tissue oxygen, pCO_{2b}- brain tissue carbon dioxide, pHb- brain tissue pH, Tb- brain temperature.

Figure 2 Effect of chlormethiazole infusion (left axis, commencing at time 0) 10 ml min⁻¹ for 5 min followed by 1 ml min⁻¹ for 60 min on mean arterial blood pressure (○) and intracranial pressure (●) (right axis). The infusion was well tolerated and did not induce hypotension. There were no effects on intracranial pressure.

The results demonstrated that the physiological variables were unchanged as a result of the chlormethiazole infusion. The drug was well tolerated and did not cause significant hypotension or reduction in cerebral perfusion pressure.

Effect of chlormethiazole on chemical parameters

The effect of chlormethiazole on cerebral and peripheral chemistry is shown in Tables 6 and 7. The baseline plasma glucose was 6.9 $\pm$ 2.6 mM pre-infusion, 7.6 $\pm$ 1.4 mM during the infusion and 7.1 $\pm$ 1.7 mM post-infusion. There was no significant change in the levels of substrates, metabolites or amino acids during the chlormethiazole infusion.

Effect of chlormethiazole on neurophysiology

Overall, there was no change in the bispectral index as a result of the chlormethiazole infusion (baseline (pre-rapid infusion) 43 $\pm$ 9.8, end of rapid infusion 43 $\pm$ 5.5, end of slow infusion 45 $\pm$ 8.3, end of study 45 $\pm$ 7.0). The EEG showed for patients 1, 3, 4 and 5 a background of slow theta and delta frequencies with periods of superimposed fast activity. Patient 2, in contrast, showed a background of predominantly alpha frequencies with a fronto-central distribution. Episodes of burst suppression were detected in two of the patients studied (4 and 5). This pattern of electrical activity increased during the chlormethiazole infusion period and decreased after the end of the infusion. No epileptiform activity was seen prior to, during or on withdrawal of the chlormethiazole infusion.

Discussion

There are a number of postulates which should be satisfied before a potential neuro-protective drug undergoes phase III studies [19]. These include that the pathophysiological processes must be demonstrated in both animal models and humans, that the process must be reversed by the agent in animal models, and that the agent is safe and penetrates the injured human brain in therapeutic doses. Application of imaging, e.g. positron emission tomography and probe, e.g. microdialysis technology has the potential to yield important information about the pharmacokinetics and pharmacodynamics of putative neuro-protective agents. Furthermore, surrogate endpoints, e.g. extracellular brain glutamate levels

Table 6 Effect of chlormethiazole infusion on chemical substrates and metabolites (mean $\pm$ 95% confidence intervals).

Site	Chemical	Baseline	Chlormethiazole infusion	End of study
Peripheral	Glucose (mM)	4.0 $\pm$ 2.7	3.9 $\pm$ 3.1	4.3 $\pm$ 4.1
	Lactate (mM)	2.3 $\pm$ 3.1	2.2 $\pm$ 3.2	2.1 $\pm$ 3.0
	Pyruvate (μ M)	109 $\pm$ 114	111 $\pm$ 96	116 $\pm$ 112
	Lactate/pyruvate	22 $\pm$ 14	18 $\pm$ 13	24 $\pm$ 19
Cerebral	Glucose (mM)	1.7 $\pm$ 0.37	2.0 $\pm$ 0.55	1.7 $\pm$ 0.44
	Lactate (mM)	3.5 $\pm$ 2.36	3.4 $\pm$ 2.03	3.4 $\pm$ 1.86
	Pyruvate (μ M)	146 $\pm$ 84	148 $\pm$ 83	151 $\pm$ 68
	Lactate/pyruvate	24 $\pm$ 12	23 $\pm$ 9.7	22 $\pm$ 5.7

Table 7 Effect of chlormethiazole infusion on extracellular amino acid concentrations μ M (mean $\pm$ 95% confidence intervals).

Chemical	Baseline	Chlormethiazole infusion	End of study
Aspartate	6.8 $\pm$ 4.1	4.9 $\pm$ 3.1	8.3 $\pm$ 11
Glutamate	8.1 $\pm$ 5.2	7.3 $\pm$ 5.6	7.7 $\pm$ 5.8
GABA	0.078 $\pm$ 0.057	0.079 $\pm$ 0.070	0.063 $\pm$ 0.043
Taurine	14 $\pm$ 18	15 $\pm$ 22	14 $\pm$ 19
Glycine	49 $\pm$ 32	44 $\pm$ 45	50 $\pm$ 41
Asparagine	11 $\pm$ 6.3	11 $\pm$ 9.0	11 $\pm$ 8.8
Serine	45 $\pm$ 11	35 $\pm$ 8.2	47 $\pm$ 43
Glutamine	624 $\pm$ 200	564 $\pm$ 268	620 $\pm$ 394
Histidine	24 $\pm$ 15	23 $\pm$ 19	23 $\pm$ 18
Threonine	24 $\pm$ 15	23 $\pm$ 20	25 $\pm$ 21
Citrulline	3.9 $\pm$ 2.3	3.6 $\pm$ 3.2	3.6 $\pm$ 2.9
Arginine	18 $\pm$ 12	18 $\pm$ 17	18 $\pm$ 17
Alanine	52 $\pm$ 40	50 $\pm$ 52	54 $\pm$ 48
Tyrosine	21 $\pm$ 13	20 $\pm$ 16	21 $\pm$ 18
Valine	64 $\pm$ 48	63 $\pm$ 60	63 $\pm$ 56
Methionine	13 $\pm$ 7.1	12 $\pm$ 9.3	12 $\pm$ 9.5

become available to improve the specificity of outcome detection.

The objective of this feasibility study was to combine multimodality monitoring (including cerebral microdialysis) of head injured patients with the administration of a putative neuro-protective agent, chlormethiazole. Head injured patients, compared with stroke patients, are already routinely monitored with intraparenchymal probes and are therefore a suitable population to be studied with this technique. Extensive microdialysis studies have been performed in head injury and the technique has been shown to be safe [22–24, 45, 46], Chlormethiazole was selected as the neuro-protective agent for this study as it has already been widely administered to patients for other indications and has been extensively investigated in animal models [30–37, 47], In fact, one of the major drawbacks of the use of chlormethiazole in clinical practice, that of respiratory depression, is overcome in mechanically ventilated patients. As the aim was that of a pilot feasibility study as opposed to that of a definitive efficacy study, the drug was administered during relatively stable periods and not

early after the injury or during conditions of energy perturbation.

The results demonstrate that combining microdialysis with the application of a neuro-protective agent in the clinical setting can be safely achieved and that there were no adverse side effects due to chlormethiazole. Concerns that the glucose content of the chlormethiazole vehicle solution would result in hyperglycaemia and an increased glucose load to the brain were not realized. Also, there was no evidence of seizure activity following withdrawal of the drug. The venous sampling confirmed that satisfactory levels of chlormethiazole were achieved in the plasma. Our hypothesis that microdialysis would detect blood brain barrier penetration of chlormethiazole was not proven. Microdialysis has been shown to be effective at determining the cerebral penetration of other drugs, e.g. phenytoin by Scheyer *et al.* [48] and in other patients in our own unit (personal observations). There are three possible reasons for failure to detect chlormethiazole in the microdialysate. Firstly, that the dose was insufficient to penetrate the brain. However, satisfactory plasma levels were achieved and this dose has been shown to be effective in patients with status epilepticus. Secondly, that the drug binds to the microdialysis catheter membrane and/or is insoluble in the dialysis fluid. This explanation is refuted by the *in vitro* study. Thirdly, the drug enters neurones directly, e.g. from capillaries via the podocyte processes into astrocytes and then neurones with minimal passage through the extracellular space. An adequate brain concentration may have been achieved but the drug rapidly partitioned into the cells due to its lipid solubility. Green *et al.* have demonstrated that in gerbils administration of 600 μ mol kg⁻¹ of chlormethiazole results in a peak total brain concentration 40% higher than that of plasma [47]. Further animal studies may help to elucidate the reasons for the failure of the detection of chlormethiazole in the microdialysate.

Chlormethiazole is thought to exert its action by enhancing GABA_A receptor activity [40–42] with a possible secondary reduction in the excitatory amino acid levels. Potentiation of GABA activity has been shown to

be neuro-protective in animal studies [49–55] and we have demonstrated large elevations in the concentration of cerebral extracellular levels of GABA in patients with head injury and subarachnoid haemorrhage during episodes of cerebral ischaemia [56]. These increases in endogenous GABA levels may be neuro-protective in humans. We hypothesized that the chlormethiazole infusion may have a beneficial effect on cerebral chemistry but the results showed that there was no effect on amino acid, substrate or metabolites levels in these patients under basal conditions. This suggests that a specific action on particular amino acids is unlikely. Baldwin *et al.* have demonstrated that chlormethiazole attenuated the rise in concentration of all amino acids in a model of focal ischaemia in the rat [30]. Although there appeared to be no change in levels in our clinical study, it may be that chlormethiazole will affect the concentration of extracellular amino acids under conditions of extreme ischaemia in unstable head injured patients or during temporary clipping in aneurysm surgery. It is also possible that longer infusions of chlormethiazole may show an effect. Also, the propofol may have attenuated a change in the concentration of GABA. There is evidence that part of the action of propofol is via the GABA axis [57–62].

In conclusion, this study demonstrates the feasibility of performing microdialysis in conjunction with the administration of a neuro-protective agent, and that chlormethiazole can be safely given to ventilated patients with severe head injury. The study indicates that the drug can be safely used in present and future clinical trials of neuro-protection. This initial data collected under basal conditions indicates that the drug administered at 10 ml min^{-1} for 5 min and then 1 ml min^{-1} for 60 min has no effect on the delivery of substrate (oxygen and glucose) or production of metabolite (pyruvate and lactate) or extracellular amino acid production. The absence of chlormethiazole from the microdialysate suggests that it is rapidly taken up by cells. Study designs of this type using surrogate endpoints may have the potential for screening future neuro-protective drugs in small number of patients prior to committing to large phase III studies. However, as this was essentially a negative study, further investigation of the capability of monitoring including microdialysis in this setting is required. In particular, the factors determining drug detection by microdialysis need to be assessed.

Addendum

Astra Zeneca announced at the end of 2000 that the study of chlormethiazole in the treatment of 1200 patients suffering major stroke was stopped because no efficacy benefits were demonstrated. The results of our investigation support this decision and the use of preliminary studies to determine the effects of potential neuro-protective

agents on physiological and biochemical markers in selected patients, prior to embarking on larger studies.

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