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BRIEF ARTICLE

Ciclosporin does not attenuate intracranial hypertension in rats with acute hyperammonaemia

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Abstract

AIM: To investigate the neuroprotective potential of ciclosporin during acute liver failure. We evaluated the effect of intrathecally administered ciclosporin on intracranial pressure, brain water content and aquaporin-4 expression in a rat model with acute hyperammonaemia.

METHODS: Twenty-four male Wistar rats with portacaval anastomosis were randomised into four groups receiving ciclosporin or vehicle and ammonia or saline infusion. Ciclosporin or vehicle was given intrathecally prior to the ammonia or saline infusion. The ammonia or saline infusion was given intravenously for 4 h, while intracranial pressure and arterial pressure was recorded. At the end of the experiment, cerebral cortex and cerebellar brain tissue was analysed for water and aquaporin-4 content.

RESULTS: The following intracranial pressures were found at the end of the experiment: ammonia + ciclosporin: 10.0 ± 1.7 mmHg, ammonia + vehicle: 6.8 ± 1.0 mmHg, saline + ciclosporin: 3.1 ± 0.5 mmHg, saline + vehicle: 3.3 ± 0.6 mmHg. Ammonia infusion had a significant effect on intracranial pressure and brain water content, which both were higher in the groups receiving ammonia ($P < 0.001$, two-way analysis of variance). Treatment with ciclosporin resulted in relevant tissue concentrations of ciclosporin (> 0.2 micromolar) but did not reduce intracranial pressure after 4 h. Furthermore, ciclosporin did not attenuate the increase in cerebral water content, and did not affect aquaporin-4 expression.

CONCLUSION: Intrathecal administration of ciclosporin does not attenuate intracranial hypertension or brain oedema in rats with portacaval anastomosis and 4 h of ammonia infusion.

Key words: Brain oedema; Acute liver failure; Neuroprotection; Hyperammonaemia; Ciclosporin

Core tip: Acute liver failure and hyperammonaemia can lead to severe brain oedema. Preserving mitochondrial function by treatment with ciclosporin has shown potential in *in vitro* studies. In this study we evaluated the effect of ciclosporin in a rat model of acute hyperammonaemia on intracranial pressure, brain water content and expression of the water channel aquaporin-4. We did not find a beneficial effect of ciclosporin on intracranial pressure, brain water content or aquaporin-4 expression.

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INTRODUCTION

Development of grade Ⅲ/Ⅳ hepatic encephalopathy in

patients with acute liver failure (ALF) signifies risk of cerebral oedema. Cerebral oedema constitutes a potentially life-threatening complication of ALF if it leads to increased intracranial pressure (ICP). Emergence of intracranial hypertension (ICH) is a poor prognostic sign, and renders the patient at risk of fatal cerebral herniation.

Ammonia is considered to play a pivotal role in the generation of the cerebral oedema, and high arterial ammonia levels have been demonstrated to be predictive of ICH[1-3].

The cerebral oedema in ALF is characterised by cerebral hyperperfusion and loss of autoregulation of the cerebral blood flow (CBF), and these events are both considered fundamental in the pathogenesis of ICH[4]. Additional factors have also been implicated in the cerebral oedema associated with ALF, including infection[5], inflammation[6] and hyponatraemia[7].

The cerebral oedema seen during hyperammonaemia is predominantly cytotoxic, and involves astrocyte swelling[8,9]. The cellular effects of hyperammonaemia are thought to include oxidative stress (OS) and nitrosative stress (NS)[10-14], induction of the mitochondrial permeability transition (MPT)[15-17] and activation of mitogen-activated protein kinases (MAPKs)[18]. Astrocytes are characterised by an abundant presence of water channels, especially aquaporin-4 (AQP4), and ammonia-mediated upregulation of AQP4 has been demonstrated in recent studies of cultured astrocytes and in animal models of ALF[19-21]. This line of events is thought to lead to mitochondrial dysfunction, reduced oxidative adenosine triphosphate (ATP) production, astrocyte swelling and cerebral dysmetabolism.

Recent studies employing cultured astrocytes have demonstrated suppression of ammonia-induced astrocyte swelling by ciclosporin (Cs) - a widely used immunosuppressant drug belonging to the group of calcineurine inhibitors[16]. The neuroprotective effects of Cs are most likely attributable to inhibition of the MPT and attenuation of AQP4 upregulation rather than immunosuppression[16]. The MPT is a calcium-dependent reaction, characterised by a sudden increase in the permeability of the inner mitochondrial membrane mediated by opening of the permeability transition pore (PTP), which causes collapse of the electric potential across the inner mitochondrial membrane. This results in compromised ATP production by oxidative phosphorylation, and subsequently energy failure[22-25]. Cs blocks the MPT by inhibiting opening of the PTP, thus preserving mitochondrial phosphorylation efficiency and function[17]. Induction of the MPT in central nervous tissue is strongly implicated in the pathogenesis of astrocyte swelling, although the extent to which the MPT contributes to ICH in ALF is yet to be determined.

Animal models of other disease states such as traumatic brain injury (TBI) have also demonstrated neuroprotective effects of Cs. Findings in such studies show attenuation of axonal damage[26], and most importantly inhibition of the MPT securing mitochondrial function[27]. However, Cs itself also has neurotoxic effects in healthy brain tissue where it reduces mitochondrial function and thereby increases lactate levels and reduces the glutamate and glutamine concentrations in the brains of rats[28]. This could however be a beneficial feature during hyperammonaemia where both glutamate-induced cytotoxicity and astrocytic glutamine accumulation play pivotal roles[29]. However, case reports have been published where post-transplant immunosuppression with calcineurine inhibitors has triggered severe hyperammonaemia, mostly in patients with inborn deficiencies of the urea cycle[30] and we find that the use of Cs under hyperammonaemic conditions needs experimental evaluation before clinical use can be justified.

The use of Cs as a neuroprotectant in animal models of ALF has not yet been evaluated. This might be due in part to the fact that Cs does not cross the blood-brain barrier (BBB) very well[31] and the BBB is considered to remain largely intact in ALF[32,33] in contrast to TBI. The challenge of cerebral bioavailability can be overcome by administering Cs intrathecally (*ith*) where the brain tissue concentration of Cs can reach relevant levels for neuroprotection without systemic toxicity[26]. In the present study, we aimed to examine the effect of Cs administered *ith* on ICP, brain water content and cerebral AQP4 expression in rats with acute hyperammonaemia. We also measured the cortical concentration of glutamate and glutamine. We used a well-established rat model of hepatic encephalopathy, brain oedema, and cerebral hyperperfusion induced by acute hyperammonaemia after construction of a portocaval anastomosis (PCA)[34].

We hypothesised that Cs would attenuate ICH, preserve normal brain water content and reduce upregulation of AQP4.

MATERIALS AND METHODS

All procedures involving laboratory animals were conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and approved by Danish Animal Inspectorate. The experiments were performed in the animal facilities associated with the Hepatology Laboratory, Rigshospitalet, Copenhagen, Denmark.

Animals

Male Wistar rats (Charles River, Sulzfeld, Germany) were housed in plastic cages with free access to water and rodent chow, and kept at constant room temperature and humidity with a 12/12-h light per dark cycle.

The study included 24 anaesthetised animals, and they were randomly assigned to one of the following experimental groups: (1) PCA + ammonia infusion *iv* + Cs *ith* (n = 6); (2) PCA + ammonia infusion *iv* + vehicle *ith* (n = 6); and (3) PCA + saline *iv* + Cs *ith* (n = 6); (4) PCA + saline *iv* + vehicle *ith* (n = 6)

Surgical procedure

The PCA was done as an end-to-side anastomosis. In isoflurane anaesthesia, the rats underwent laparotomy. The portal vein and vena cava were isolated, and after the portal vein was ligated and cut, the distal part was sutured onto a hole in the side of the vena cava. The anastomosis was completed in less than 15 min, and the abdomen was sutured in two layers. The animals were returned to their housing, and the experiment started 24 h later.

Experimental procedure

After induction of anaesthesia with isoflurane, 0.2 to 0.3 mL pentobarbital (50 mg/mL) was administered in a tail vein. Every 10 min, the reaction to claw pinching was checked, and supplementary pentobarbital given when necessary. Arterial and venous catheters (PE-50) were inserted into femoral vessels for monitoring blood pressure, *iv* drug administration and blood sampling. The arterial catheters were flushed with 500 IU heparin and one connected to a pressure transducer. The rats were then tracheotomised and mechanically ventilated (Hallowell EMV, E-vet, Haderslev, Denmark) with a respiratory frequency of 65 breaths/min and a tidal volume of 5 to 10 mL with a mixture of atmospheric air and oxygen adjusted to maintain a normal arterial partial pressure of carbon dioxide and avoid hypoxia during the whole experiment. Arterial blood samples were taken every 30 min. for analysis of pO_2 and pCO_2 (ABL 505; Radiometer, Copenhagen, Denmark). The animals were placed in a stereotactic frame and, with the head fixated, a midline scalp incision was made and one small borehole was drilled in the skull. The borehole was used for the placement of a catheter (PE-10) in the cisterna magna, and was used for *ith* administration of Cs/vehicle, and ICP monitoring by connection to a pressure transducer. Continuous measurements of mean arterial pressure (MAP) and ICP were recorded on a computer using the software Perisoft (Perimed, Stockholm, Sweden). During the experiment, body temperature was monitored with an intra-abdominal thermistor and maintained at 37 ℃ with a ventral heading pad. After an initialisation period, stable baseline values were recorded. Then 150 μL of cerebrospinal fluid was aspirated through the intracranial catheter. In the drug-treated groups, 150 μL of Cs (Sandimmune®, Novartis, Basel, Switzerland) diluted with isotonic saline was infused over 30 min into the cisterna magna using a microdialysis pump (CMA/102) corresponding to a Cs dosage of 10 mg/kg and followed by a flush of saline over 3 min. The vehicle-treated groups were given a vehicle composed of ethanol, ricinus oil and saline [according to the manufacturers information (Novartis)] in an equivalent volume following the same procedure. The catheter was subsequently reconnected to a pressure transducer. In appropriate groups either an *iv* ammonium acetate infusion of 55 μmol/kg per minute or saline infusion 2 mL/h (0.9 mg/mL) was initiated (t = 0). The experiment was terminated at t = 4 h, and arterial blood samples were taken to determine plasma levels of alanine aminotransferase, coagulation factors Ⅱ + Ⅶ + Ⅹ and ammonia. Animals were sacrificed while anaesthetised, and underwent decapitation. The brain was removed from the skull, and dissected into two halves by a saggital cut. Half of the cerebral cortex and half of the cerebellum were immediately frozen in liquid nitrogen and stored at -80 ℃ for later analysis of Cs content and AQP4 expression.

Ciclosporin dosage

Based on studies by other groups[26,27] we chose to administer 10 mg/kg of Cs *ith*, which is sufficient to achieve tissue concentrations able to inhibit MPT (> 0.2 μmol/L) and thus conserve mitochondrial function[35]. We measured the Cs concentration in the cerebral cortex by liquid chromotography and mass spectrometry (Waters Micromass, Waters Corporation, Milford, MA, United States) after tissue homogenisation and protein precipitation in acetonitrile.

Analysis of brain water content by wet/dry method

The remaining halves of the cerebral cortex and cerebellum were used for determination of brain water content by the wet/dry weight method. The brain tissue was transferred onto pre-weighed glass scales, and weighed on a high-precision scale determining "wet weight". The specimens were subsequently dried for 24 h in an oven at 110 ℃. The dried brain tissue acclimatised in an exicator, and was afterwards weighed determining "dry weight". The percentage water content of the cortex and the cerebellum was calculated according to the following formula: [(wetweight - dryweight)/wetweight] × 100.

Analysis of AQP4 protein expression by Western blot

Protein analysis was done as previously described[36]. In brief, samples of frozen cerebral cortex and cerebellar tissue were homogenised and centrifuged. The resultant pellets were resuspended in an acidic buffer and after measuring the protein concentrations, the samples were loaded onto an Invitrogen mini-cell-system (Invitrogen Taastrup, Denmark) with 3 μg protein per lane. Protein was transferred to a polyvinylidene fluoride membrane (Invitrogen) by electroelution and incubated overnight with the primary antibody. The membrane was subsequently washed and then incubated with horseradish-peroxidase-conjugated secondary antibody (SC2020, 1:5000; Santa Cruz Biotechnology). Finally, detection of bound antibody was performed using the enhanced chemiluminescence system (PerkinElmer, Waltham, MA, United States) and camera detecting system LAS 9000 with software ImageGauge 2006 Software (FujiFilm, Stockholm, Sweden).

Analysis of cortical glutamate and glutamine content

The cerebral cortical tissue was weighed and homogenised in a six-fold amount of ice-cold 1 mol/L $HClO_4$. The homogenate was centrifuged and the supernatant neutralised by ice-cold 1.6 mol/L KOH containing 0.4 mol/L K_2CO_3. The concentrations of glutamate

Table 1 Baseline values

Group	Weight (g)	MAP (mmHg)	ICP (mmHg)
PCA + ammonia infusion + Cs	295.5 ± 11.8	110 ± 4.2	1.0 ± 0.2
PCA + ammonia infusion + vehicle	326.2 ± 15.3	103 ± 4.0	0.8 ± 0.2
PCA + saline infusion + Cs	320.8 ± 12.4	116 ± 2.0	0.6 ± 0.2
PCA + saline infusion + vehicle	308.3 ± 14.7	106 ± 4.2	0.8 ± 0.3

PCA: Portacaval anastomosis; Cs: Ciclosporin; MAP: Mean arterial pressure; ICP: Increased intracranial pressure.

Table 2 Baseline biochemistry

Group	Arterial pH	Arterial pCO_2 (mmHg)	Arterial pO_2 (mmHg)	Haemoglobin (mmol/L)	Blood glucose (mmol/L)
PCA + ammonia infusion + Cs	7.46 ± 0.008	37.9 ± 1.2	136.6 ± 7.7	9.0 ± 0.19	6.4 ± 0.29
PCA + ammonia infusion + vehicle	7.47 ± 0.013	38.2 ± 0.4	130.2 ± 3.8	9.4 ± 0.26	5.9 ± 0.52
PCA + saline infusion + Cs	7.49 ± 0.005	35.7 ± 0.5	144.6 ± 1.7	8.6 ± 0.19	6.3 ± 0.21
PCA + saline infusion + vehicle	7.46 ± 0.002	37.9 ± 1.1	129.7 ± 6.7	9.2 ± 0.40	5.3 ± 0.12

PCA: Portacaval anastomosis; Cs: Ciclosporin.

and glutamine were then measured in the supernatant by an enzymatic method using a YSI 2700 (YSI, OH, United States) and the actual cortical concentration in units of mmol/100 g could then be calculated.

Statistical analysis

All results are presented as mean ± SE of the mean. Two-way analysis of variance (ANOVA) was used to evaluate the individual effects of ammonia infusion and Cs on ICP, MAP, brain water and biochemical parameters at the end of the experiment. Normal distribution of data was confirmed by the use of Shapiro-Wilk test of normality. One-way ANOVA was used to evaluate differences in baseline characteristics. An unpaired student's *t* test was used to compare AQP4 levels between groups and correction for multiple comparisons was done by the Holm-Bonferroni method. *P* values below 0.05 were considered significant.

RESULTS

We found no significant differences at baseline between the experimental groups regarding MAP, ICP, body weight, arterial pH, pCO_2, pO_2, blood glucose and Hgb (Tables 1-2). As expected, we found a highly significant effect of ammonia infusion on plasma levels of ammonia at $t = 4$ h ($F = 201.9$, $P < 0.001$). At $t = 4$ h, we found no effect of ammonia or Cs on MAP (data not shown), alanine aminotransferase levels or coagulation factors Ⅱ, Ⅶ and Ⅹ (Table 3).

At $t = 4$ h, two-way ANOVA demonstrated a highly significant effect of ammonia infusion on ICP (after logarithmic transformation, $F = 19.41$, $P < 0.001$), but

Table 3 Biochemistry at $t = 4$ h

Group	ALT (U/L)	Arterial ammonia (μmol/L)	PP (arb. units)
PCA + ammonia infusion + Cs	525 ± 185	1165.5 ± 87.2	0.32 ± 0.01
PCA + ammonia infusion + vehicle	600 ± 266	900.5 ± 70	0.37 ± 0.02
PCA + saline infusion + Cs	470 ± 221	108.3 ± 5.9	0.40 ± 0.03
PCA + saline infusion + vehicle	830 ± 468	140.3 ± 15	0.33 ± 0.03

PCA: Portacaval anastomosis; Cs: Ciclosporin; ALT: Alanine transferase; PP: Coagulation factor Ⅱ + Ⅶ + Ⅹ.

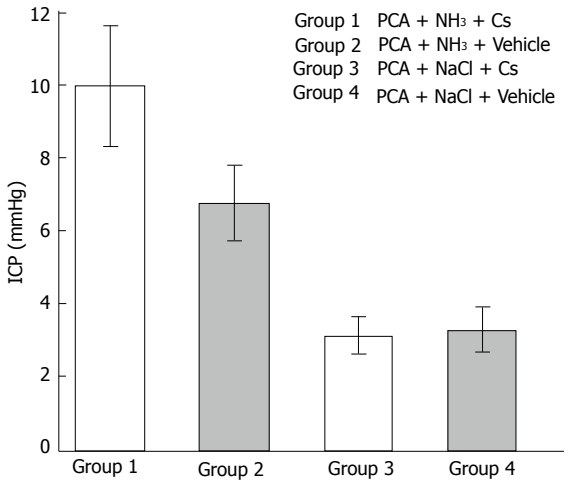

Figure 1 Intracranial pressure. Bar plot of mean ICP in the experimental groups after 4 h of ammonia infusion. Error bars represent SE of the mean. A highly significant effect of ammonia infusion on ICP was found (two-way analysis of variance, $F = 22.1$, $P < 0.001$). We found no effect of ciclosporin. ICP: Intracranial pressure; PCA: Portacaval anastomosis; Cs: Ciclosporin.

no significant effect of Cs (Figure 1). No interaction was found between ammonia and Cs on ICP. Looking at changes in ICP from baseline to $t = 4$ h, we observed a highly significant effect of ammonia ($F = 20$, $P < 0.001$), but not of Cs. In group 1, ICP increased from 1.0 ± 0.2 to 10.0 ± 1.7 mmHg, in group 2 from 0.8 ± 0.2 to 6.8 ± 1.0 mmHg, in group 3 from 0.6 ± 0.2 to 3.1 ± 0.5 and from 0.8 ± 0.3 to 3.3 ± 0.6 mmHg in group 4. Regarding changes in MAP from baseline to $t = 4$ h, we found no effect of ammonia or Cs, but in all groups MAP decreased during the experiment (110 ± 4.2 to 92 ± 10.9 mmHg in group 1, 104 ± 4.0 to 86 ± 5.0 mmHg in group 2, 116 ± 2.0 to 103 ± 4.2 mmHg in group 3 and 106 ± 4.2 to 88 ± 4.5 mmHg in group 4).

Brain water content

Two-way ANOVA demonstrated no significant effect of Cs on cortical or cerebellar water content, but ammonia significantly increased water content in the cerebral cortex ($F = 7.8$, $P < 0.05$) and in the cerebellum ($F = 16.2$, $P < 0.001$) (Figure 2A and B). No interaction was found between ammonia and Cs on cortical or cerebellar water content. There was a significant correlation between ICP and cortical and cerebellar water content ($r^2 = 0.20$, $P < 0.05$ and $r^2 = 0.45$, $P < 0.001$ respectively).

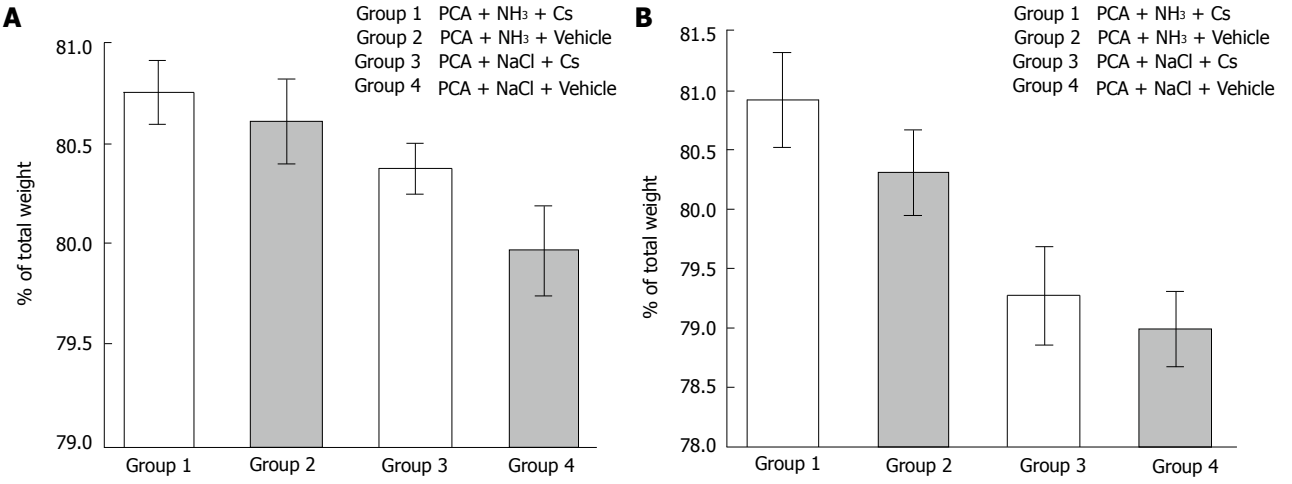

Figure 2 Brain water content. A: Bar plot of mean cortical water content in the experimental groups after 4 h of ammonia infusion. Error bars represent standard error of the mean. A significant effect of ammonia infusion on cortical water content was found (two-way analysis of variance, $F = 7.8$, $P < 0.05$). We found no effect of Cs; B: Bar plot of mean cerebellar water content in the experimental groups after 4 h of ammonia infusion. Error bars represent standard error of the mean. A highly significant effect of ammonia on increased cerebellar water content was found (two-way analysis of variance, $F = 16.2$, $P < 0.001$). We found no effect of Cs. PCA: Portacaval anastomosis; Cs: Ciclosporin.

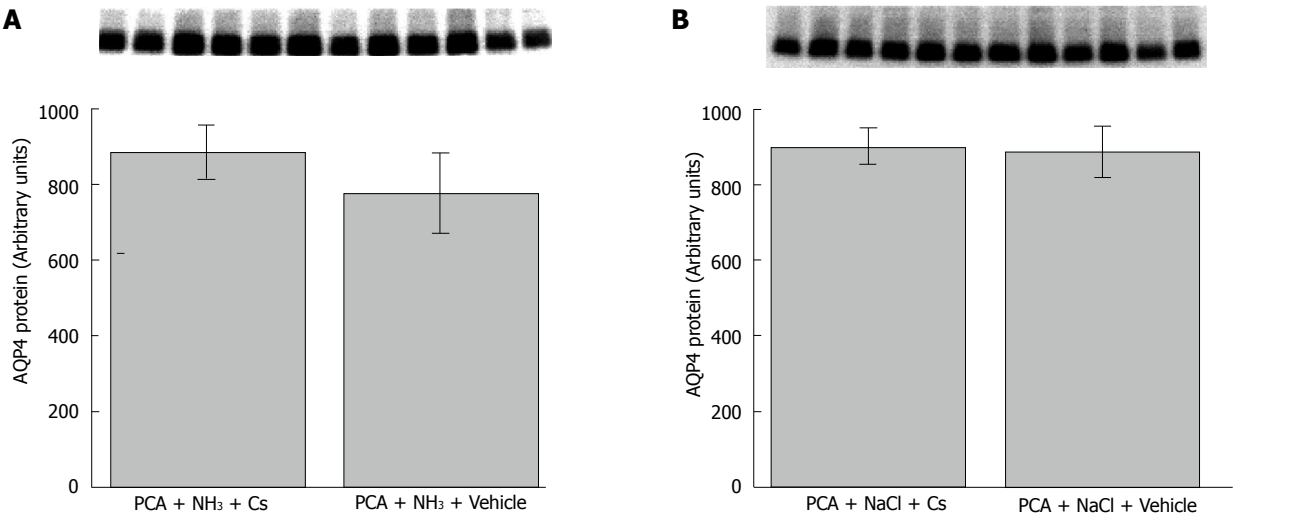

Figure 3 Brain aquaporin-4 expression. A: Bar plot and Western blot of AQP4 content of cerebral cortex in group 1 (six leftmost bands) and group 2 (six rightmost bands). No statistical significant difference was found between groups (unpaired student's *t* test on densitometric values); B: Bar plot and Western blot of AQP4 content of cerebral cortex in group 3 (six leftmost bands) and group 4 (six rightmost bands). No statistical significant difference was found between groups (unpaired student's *t* test on densitometric values). AQP: Aquaporin; PCA: Portacaval anastomosis; Cs: Ciclosporin.

Aquaporin-4

We found no significant differences in the mean AQP4 content in the cerebral cortex between group 1 *vs* 2 and group 3 *vs* 4 (Figure 3). This indicates that there was no effect of Cs on AQP4 expression.

Brain tissue concentration of Cs

In the two groups receiving Cs intrathecally, we found a brain cortex concentration of 2.14 ± 0.74 μmol/L. The concentrations were not significantly different between the two groups and we did not find a significant correlation between cerebral cortex or cerebellar brain water content and the tissue concentration of Cs (data not shown).

Brain tissue concentration of glutamate and glutamine

In the two groups receiving ammonia, the cortical glutamine concentration was 2.8 ± 0.09 mmol/100 g (group 1) and 2.8 ± 0.14 mmol/100 g (group 2). In the saline groups the glutamine concentration was 1.2 ± 0.06 mmol/100 g (group 3) and 1.0 ± 0.07 mmol/100 g (group 4). There was a significant effect of ammonia ($F = 308$, $P < 0.001$) but not of Cs. The glutamate concentration was 1.0 ± 0.05 mmol/100 g (group 1), 1.0 ± 0.07 mmol/100 g (group 2), 0.9 ± 0.11 mmol/100 g (group 3) and 0.9 ± 0.07 mmol/100 g (group 4). There was no significant effect of either ammonia or Cs on the glutamate levels.

DISCUSSION

We employed a well-characterised rat model with PCA and acute hyperammonaemia, and found that hyperammonaemia resulted in ICH and increased cortical and cerebellar water content. Furthermore, we found a significant correlation between ICP and brain water. However, our results did not support the hypothesis of a neuroprotective effect of Cs in ALF. Cs did not attenuate ICH and did not preserve normal brain water content. Furthermore, Cs did not affect AQP4 expression or glutamine levels. We also observed an insignificant tendency towards higher ICP in rats treated with ammonia and Cs (group 1) compared with the group receiving ammonia and vehicle (group 2). This speaks against the risk of a type Ⅱ error causing our negative result and raises the concern that Cs actually might aggravate the cerebral oedema due to intrinsic neurotoxic features. A higher ICP, although within the normal range, has also been observed in TBI patients treated with Cs compared to placebo[37]. The theoretical background supporting the neuroprotective features of Cs in ALF is expressed in the "Trojan Horse Hypothesis" proposed by Albrecht and Norenberg[38]. This thesis suggests that astrocytic glutamine is transported into the mitochondria and hydrolysed by phosphate-activated glutaminase. This process yields very high levels of ammonia in this cellular compartment, which are thought to lead to OS and NS formation and induction of the MPT[39]. MPT is believed to produce additional OS, to activate MAPK and to cause upregulation of AQP4[18,40]. Precisely how OS and NS induce the MPT and how these factors act in concert to activate MAPK and cause upregulation of AQP4 is not fully understood. Furthermore, clarification of how this line of events mediates astrocyte swelling is yet to be achieved, and it is not clear whether AQP4 facilitates intracellular water accumulation or elimination[20]. Cs is thought to block the MPT by interacting with Cyclophilin D (CypD)[41-43]. CypD is a protein endowed with peptidyl-prolyl cis-trans isomerase activity, and constitutes the mitochondrial isoform of cyclophilins[35,44,45]. Cs binds CypD, and thereby displaces it from the PTP. This displacement is thought to reveal an inhibitory site where the natural inhibitory agent (organic phosphate) is able to bind, thereby lowering the open probability of the PTP[46,47]. The alleged neuroprotective effects of Cs during hyperammonaemia are thus based on preservation of mitochondrial function by inhibition of the MPT.

Recent studies of cultured astrocytes have demonstrated suppression of ammonia-mediated astrocyte swelling and ammonia-induced AQP4 upregulation by Cs[21], and thereby supported the pathogenic mechanisms outlined above. While studies of brain metabolism in patients with ALF indirectly support the theory of mitochondrial dysfunction[48,49], it is important to stress the fact that the occurrence of the MPT and AQP4 upregulation has not been demonstrated in clinical studies. This challenges the potential of this neuroprotective strategy in clinical practice. Our present study intended to translate the favourable *in vitro* results of Cs as a neuroprotectant to an *in vivo* experiment. The reasons of our conflicting negative results are not easily identified. However, it is likely that astrocytes respond differently to ammonia in an *in vivo* setting compared to cultured astrocytes. Furthermore, additional and mostly undefined factors likely influence brain metabolism, water homeostasis and autoregulation of CBF, which could mask the speculated neuroprotective properties of Cs. The neurotoxic properties of Cs might also have counteracted the beneficial effect observed *in vitro*. On the other hand, animal models of other disease states such as TBI have demonstrated neuroprotective effects of Cs[26,27] at similar doses and administration time. The divergent results might reflect that the mechanisms involved in brain oedema formation during hyperammonaemia are more complex than we currently understand and certainly belong to a different entity than the brain oedema associated with TBI. Further animal studies of other ALF models and other inhibitors of the MPT might clarify the potential of this neuroprotective strategy.

In conclusion, we found that Cs was unable to reduce ICH in a rat model of brain oedema induced by PCA and acute hyperammonaemia. In addition, we showed that Cs did not prevent the increase in brain water content and did not affect AQP4 expression.

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COMMENTS

Background

Acute liver failure and hyperammonaemia can lead to severe brain oedema. Preserving mitochondrial function by treatment with ciclosporin has shown potential in *in vitro* studies.

Research frontiers

Cerebral oedema constitutes a potentially life-threatening complication of acute liver failure if it leads to increased intracranial pressure. Emergence of intracranial hypertension (ICH) is a poor prognostic sign, and renders the patient at risk of fatal cerebral herniation.

Innovations and breakthroughs

In this study authors evaluated the effect of ciclosporin (Cs) in a rat model of acute hyperammonaemia on intracranial pressure, brain water content and expression of the water channel aquaporin-4. Authors did not find a beneficial effect of ciclosporin on intracranial pressure, brain water content or aquaporin-4 expression.

Applications

Authors found that Cs was unable to reduce ICH in a rat model of brain oedema induced by portocaval anastomosis and acute hyperammonaemia. In addition, authors showed that Cs did not prevent the increase in brain water content and did not affect aquaporin-4 expression.

Peer review

The manuscript written by Larsen *et al* analyzed the effect of ciclosporin on intracranial pressure in rats with hyperammonemia. They also examined cerebral water content and aquaporin-4 expression, and found no favourable effect of ciclosporin on those parameters. The data are inconsistent with *in vitro* analyses, but may give important information on daily practice.

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