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MILD HYPOTHERMIA PREVENTS CEREBRAL EDEMA AND CSF LACTATE ACCUMULATION IN ACUTE LIVER FAILURE

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ABSTRACT

Evidence from both clinical and experimental studies demonstrates that mild hypothermia prevents encephalopathy and brain edema in acute liver failure (ALF). As part of a series of studies to elucidate the mechanism(s) involved in this protective effect, groups of rats with ALF resulting from hepatic devascularization were maintained at either 37°C (normothermic) or 35°C (hypothermic), and neurological status was monitored in relation to cerebrospinal fluid (CSF) concentrations of ammonia and lactate. CSF was removed via implanted cisterna magna catheters. Mild hypothermia resulted in a delay in onset of encephalopathy and prevention of brain edema; CSF concentrations of ammonia and lactate were concomitantly decreased. Blood ammonia concentrations, on the other hand, were not affected by hypothermia in ALF rats. These findings suggest that brain edema and encephalopathy in ALF are the consequence of ammonia-induced impairment of brain energy metabolism and open the way for magnetic resonance spectroscopic monitoring of cerebral function in ALF. Mild hypothermia could be beneficial in the prevention of severe encephalopathy and brain edema in patients with ALF awaiting liver transplantation.

Key words: Hypothermia; acute liver failure; lactate; brain edema; hepatic encephalopathy; brain energy metabolism.

INTRODUCTION

Hyperammonemia is a consistent finding in human acute liver failure (ALF) as well as in experimental animal models of ALF resulting from hepatectomy (Holmin et al., 1983), hepatic devascularization (Mans et al., 1994; Swain et al., 1992a,b), or toxic liver injury (Swain et al., 1992b), and in all cases severe encephalopathy and brain edema are observed. More recently, a positive correlation was reported between arterial ammonia

concentrations and the incidence of brain herniation in patients with ALF (Clemmensen et al., 1999). Brain ammonia may reach concentrations in excess of 1 mM in ALF (Swain et al., 1992a), and reduction of brain ammonia using agents such as L-ornithine-L-aspartate are effective in the prevention of brain edema and encephalopathy in experimental ALF (Rose et al., 2000).

The mechanisms responsible for the deleterious effects of ammonia on brain function are not completely understood. However, two general mechanisms are currently the subject of intensive investigation, namely a compromise in brain energy metabolism and alterations of neurotransmitter function. In the case of energy metabolism, ammonia may potentially interfere with ATP synthesis by inhibition of the tricarboxylic acid cycle enzyme α-ketoglutarate dehydrogenase (Lai and Cooper, 1986) or by an NMDA receptor–mediated mechanism (Kosenko et al., 1994).

Hypothermia has been shown to extend the survival time and prevent brain edema in rats with acute hyperammonemia with or without liver failure (Rose et al., 1999; Schenker and Warren, 1961; Traber et al., 1989). Moreover, mild hypothermia is protective in experimental models of brain injury and stroke (Busto et al., 1987; Dietrich et al., 1994). A previous report demonstrated a significant effect of mild hypothermia on blood–brain ammonia transfer, leading to prevention of brain edema in rats with experimental ALF (Rose et al., 1999) and in patients with ALF awaiting liver transplantation (Jalan et al., 1999). The aim of the present study was to evaluate the effects of mild hypothermia in this context in relation to its effects on brain energy metabolism.

Hepatic Devascularization

Adult male Sprague–Dawley rats weighing 175–200 g were anesthesized with halothane and an end-to-side portacaval anastomosis (PCA) was performed according to the guidelines of Lee and Fisher (1961). In brief, rats underwent a laparotomy and the inferior vena cava and portal vein were isolated, allowing the inferior vena cava to be clamped (anastomosis clamp, Roboz Instruments Inc., Washington, D.C.) and an elliptical piece of vein 1.5 times the portal vein diameter to be removed. The portal vein was then ligated and cut, and an end-to-side anastomosis was performed under a dissecting microscope. Total surgery time was under 15 min. Overall mortality for shunted rats was less than 10%. Sham-operated control rats were matched for weight, anesthesized with halothane and a laparotomy was performed. The inferior vena cava and portal vein were clamped for 15 min, and then released. Following surgery, all animals were housed individually with free access to standard laboratory chow and water under controlled conditions of temperature, humidity, and light cycles. Forty-eight hours after PCA surgery, animals were reanesthesized with halothane and subjected to hepatic artery ligation (HAL) or laparotomy (controls), and E-50 arterial catheters were inserted in the aorta to allow for blood sampling throughout the experiment.

CSF Removal

Cisterna magna catheters were installed in groups of animals, as previously described (Swain et al., 1992b), 24 h after PCA or sham operation. In brief, the animal's head was mounted with the skull in a horizontal position in a stereotaxic apparatus. A 3-cm incision was made in the skin from the back of the head and the overlying connective tissue was removed to expose the skull. A small hole was drilled in the skull using a dental burr (009) on the midline immediately rostral to the interparietal–occipital bone suture. The hole was drilled in such a way that the occipital bone could be used as guideline while inserting the cannula (PE-10 tubing, Clay Adams, Parsipanny, NJ). The catheter was inserted into the cisterna magna. Correctness of placement was accompanied by a spontaneous flow of clear CSF. When successful implantation had been confirmed, the skull was cleaned and dried and dental acrylic cement (Yates and Bird, Chicago, IL) was applied to anchor the cannula.

Body Temperature and Blood Glucose Monitoring

Following the insertion of the cisterna magna catheter, body temperatures were monitored and maintained at 37°C by means of heating lamps (the ALF-37°C group). In the ALF-35 group hypothermia occurred spontaneously in the absence of external heating. Body temperatures in this group of animals were maintained at 35°C by intermittent warming with heating lamps as necessary. Arterial blood glucose levels were monitored and glucose was administered subcutaneously as needed to maintain normoglycemia.

Neurological Evaluation

Animals were assessed neurologically every 30 min during the progression of ALF. Animals that could no longer right themselves after being placed on their backs were considered to have lost their righting ability (defined as the precoma stage); animals in which both righting ability and a corneal reflex could not be elicited were considered to be in coma. Samples for lactate analysis in cisternal CSF were taken before HAL, 5 h after HAL, and at precoma and coma stages of encephalopathy. At the end of the experimental (1 h in coma stage), animals were sacrificed and the brains were removed. Half the brain was frozen and stained with cresyl violet for neuropathological verification of probe placement. The frontal cortex of the other half of the brain was used for brain water measurement. CSF and blood ammonia samples were taken at baseline (following HAL) and at precoma and coma stages. Animals were sacrificed at the end of the experiment. All animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication 6–23 (revised), 1985).

Brain Water Measurement

One cerebral hemisphere was kept at 4°C and brain water measured at coma stages of encephalopathy. The brain was cut into 2-mm slices, and 1-mm punch biopsy specimens were obtained from the gray matter of the cerebral cortex. Water content of each specimen was measured gravimetrically using a density gradient of bromobenzene–kerosene precalibrated with K_2SO_4 as previously described (Swain et al., 1992b). The

cortical samples were placed onto the fluid column, and the equilibration point was measured within 2 min. The specific gravity of the tissue was calculated and results were expressed as percentage of water content. Eight measurements were made per animal, and values were arithmetically averaged.

Ammonia

Ammonia concentrations were estimated in plasma and CSF using a commercial ammonia test kit.

Statistical Analysis

Data are expressed as mean ± SEM from eight animals per treatment group. Differences between groups (ALF-35, ALF-37, Sham) at the same time point were compared by one-way ANOVA (Bonferoni's correction), and differences within groups at different time points were compared by means of one-way ANOVA with repeated measures (Bonferoni's correction). Values of p less than 0.05 were considered to be significant.

RESULTS

Following PCA and HAL, normothermic rats (ALF-37 group) developed progressive encephalopathy consisting of loss of activity, loss of righting ability (precoma stage) progressing to loss of corneal reflex (coma sage). ALF-37 rats developed precoma at 8.1±0.8 h and coma at 13.4 ± 1.1 h. Mild hypothermia (35°C) afforded complete protection against hepatic encephalopathy in rats with ALF (ALF-35 group) as previously described (Rose et al., 2000). Thus at times at which the ALF-37 rats were comatose, none of the ALF-35 rats had developed significant neurological symptoms. Hypothermic rats eventually became comatose at 18.3 ±1.1 h post HAL. This protective effect was observed in 8/8 rats in the ALF-35 group. No rats in the sham-operated groups developed neurological symptoms.

Both ALF-37 and ALF-35 groups of rats developed hyperammonemia. However, plasma ammonia levels were not significantly different between the ALF-37 and ALF- 35 groups at any time point studied (Table 1). CSF ammonia concentrations, on the other hand, were significantly less (by 34%, $p < 0.01$) in rats from the ALF-35 group (672 ± 73 g/dL) compared to those in ALF-37 group at coma stages of encephalopathy (1007 ± 78 g/dL) (Table 1). There was also a significant difference ($p < 0.01$) in CSF ammonia between the two groups at pre-coma stages: ALF-35, 557 ± 40 g/dL, versus ALF-37, 838 ± 32 g/dL. This data was previously published (Rose et al., 2000).

Table 1. Plasma and CSF Ammonia in ALF

	Ammonia concentration (μg/dL)		
Treatment group	Baseline	Precoma	Coma
Plasma			
Sham	62.0 ± 10.9	41.4 ± 11.3	64.7 ± 9.5
ALF-37	129.1 ± 10.9*	511.7 ± 27.6**	640.4 ± 33.8**
ALF-35	161.8 ± 28.1*	455.4 ± 41.5**	585.7 ± 34.2**
CSF			
Sham	49.6 ± 10.2	64.4 ± 11.3	52.4 ± 9.5
ALF-37	73.6 ± 25.3	837 ± 31.5**	1007.3 ±77.8 **
ALF-35	77.2 ± 28	556.1 ± 39.6**†	671 ± 72.8**†

Note: Values significantly different from sham-operated controls at each time point are indicated by $^{*}p < 0.05$ and $^{**}p < 0.001$ by ANOVA. Values significantly different from ALF-37 at a given time point are indicated by $^{\dagger}p < 0.05$.

Normothermic rats with ALF manifested an increase in brain water content whereas mild hypothermia led to a significant attenuation of this increase (sham, 80.22%; ALF-37, 81.74%; ALF-35, 80.48%, p < 0.01 compared to ALF-37 group) (Table 2).

Table 2. Brain Water Content

Parameter	Sham ($n = 8$)	ALF-37 ($n = 8$)	ALF-35 ($n = 8$)
% Brain water	80.22 ± 0.12	81.74 ± 0.13*	80.48 ± 0.15†

Note: Values significantly different from sham-operated controls are indicated by $^{*}p < 0.001$ and values significantly different from the normothermic group are indicated by $^{\dagger}p < 0.001$, by ANOVA.

Mild hypothermia significantly reduced the lactate accumulation in CSF of ALF rats (Fig. 1).

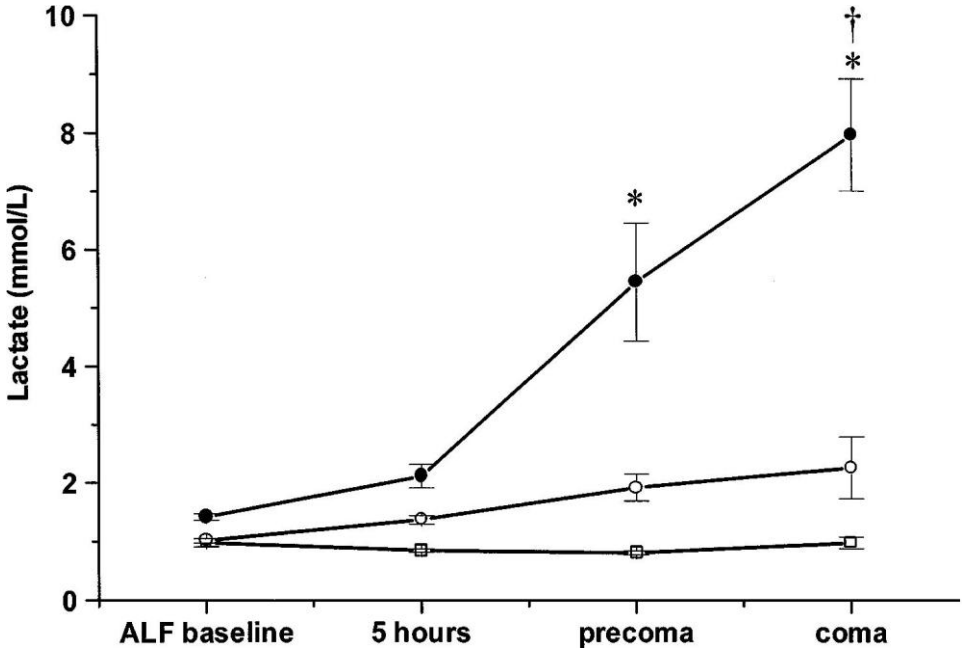

Figure 1. Prevention of the increase in CSF lactate by mild hypothermia in rats with ALF due to hepatic devascularization. Groups of ALF rats were maintained at 37°C (normothermic) (•) or 35°C (hypothermic) (o) and lactate concentrations were measured in cisternal CSF at various stages during the development of severe encephalopathy. Precoma stage was defined as time at which animals lost their righting ability; coma stage: loss of corneal reflex. Values significantly different from sham-operated controls (□) indicated by *$p < 0.01$ significantly different from ALF-37 and, †$p < 0.01$ significantly different from ALF-35, by analysis of variance and post hoc Tukey test. Symbols indicated mean values ± SE.

DISCUSSION

Results of the present study confirm and extend previous studies (Traber et al., 1989; Rose et al., 2000) demonstrating that mild hypothermia delays the time of onset of hepatic encephalopathy and prevents brain edema in rats with ALF.

Following hepatic devascularization, blood ammonia levels rise to attain levels in the 200–500 g/dL range (Swain et al., 1992a). Similar blood ammonia increases have been reported in patients with acute liver failure who developed brain herniation (Clemmensen et al., 1999). Hypothermia was also previously shown to prevent the central nervous system consequences of pure hyperammonemia in the absence of liver disease (Schenker and Warren, 1962).

Within hours of hepatic devascularization in the rat, brain ammonia concentrations are increased and these increases parallel the severity of encephalopathy and appearance of

brain edema (Swain et al., 1992a). It has been proposed that ammonia toxicity is (directly or indirectly) the cause of brain edema in this model of ALF (Cordoba and Blei, 1996). However, findings from the present study reveal that the beneficial effects of hypothermia are not mediated by a direct effect on blood ammonia. On the other hand, CSF ammonia concentrations were significantly reduced in hypothermic rats at times following hepatic devascularization when blood ammonia concentrations were unchanged. These findings suggest that one of the mechanisms responsible for the protective action of mild hypothermia in ALF is reduced blood–brain ammonia transfer.

The finding, in the present study, of a significant prevention of the increased lactate in CSF due to mild hypothermia, suggests a role for impaired brain energy metabolism in the pathogenesis of HE and brain edema in ALF. Increases in brain lactate are a consistent finding in acute liver failure (Hawkins et al., 1973; Holmin et al., 1983; Therrien et al., 1991). Preliminary studies from our unit suggest that the increased lactate production in brain in ALF is a consequence of increased expression of lactate dehydrogenase (unpublished data). Furthermore, EEG changes were reported to parallel increases in brain lactate in ALF (Deutz et al., 1988), suggesting that impaired brain energy metabolism underlies the pathogenesis of encephalopathy. Results of the present study are consistent with this possibility since hypothermia prevented the rise in CSF lactate and caused a significant delay in onset of encephalopathy in ALF.

It is unlikely that the increase in CSF lactate in ALF is the result of increased blood levels of the metabolite since Alexander et al. (1962) were unable to observe any significant increase in CSF lactate following its intravenous administration. Furthermore, Posner and Plum (1961) reported an independence of blood and CSF lactate in a range of neurological disorders. It is therefore likely that the increase in CSF lactate observed in the present study in ALF is the consequence of intrinsic cellular metabolic changes. In this regard, it is well established that both ammonia toxicity and liver failure are associated with decreased cerebral oxygen consumption (Fazekas et al., 1956; McKhann and Tower, 1961). Moreover, ammonia inhibits cerebral oxidative metabolism in vitro by an inhibitory action on the tricarboxylic acid cycle enzyme α-ketoglutarate dehydrogenase (Lai and Cooper, 1986). More recently, it was proposed that ammonia exposure also results in impaired cerebral energy metabolism via a mechanism involving NMDA-receptor activation (Kosenko et al., 1994). Whatever the mechanism, the rise in brain lactate in ALF probably reflects an impairment of brain glucose oxidation caused by exposure to ammonia, a phenomenon which has been recognised for over 40 years (McKhann and Tower, 1961; Schenker and Warren, 1962).

It is possible that increases in brain lactate may contribute to the pathogenesis of brain edema in ALF. Exposure of cultured astrocytes to lactate results in significant cell swelling (Staub et al., 1990), and results of the present study reveal that CSF lactate increases are maximal at coma stages of encephalopathy in ALF, a time at which brain edema is maximal. Furthermore, mild hypothermia prevents both brain edema and the accumulation of lactate in CSF in ALF. On the other hand, increased brain lactate is a feature that is common to both acute (Hawkins et al., 1973; Holmin et al., 1983) and

chronic (Hindfelt et al., 1977; Therrien et al., 1991; Yao et al., 1987) liver failure and brain edema is generally confined to the acute condition. It is conceivable that compensatory mechanisms occur in chronic liver failure, which render the astrocytes less vulnerable to lactate-induced swelling. Further studies are required in order to assess this possibility.

In conclusion, mild hypothermia prevents brain edema and delays the onset of severe encephalopathy in experimental ALF. This protective effect of mild hypothermia is accompanied by a decrease in CSF ammonia and a concomitant reduction in CSF lactate. These findings are consistent with an effect of mild hypothermia on cerebral energy metabolism. Further studies using ^{1}H magnetic resonance spectroscopy would be useful in assessing the relationship between brain lactate accumulation and severity of encephalopathy and brain edema in patients with ALF.

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