Brain Lactate by Magnetic Resonance Spectroscopy During Fulminant Hepatic Failure in the Dog

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A noninvasive test is needed to assess the severity of encephalopathy during fulminant hepatic failure. This feasibility study was designed to compare a noninvasive test, brain lactate measurement by magnetic resonance spectroscopy, with intracranial pressure monitoring in a large animal model of fulminant hepatic failure. Five dogs received an intraventricular catheter for intracranial pressure measurement. Liver injury was induced by intravenous bolus of D-galactosamine. Brain lactate concentrations were determined by magnetic resonance spectroscopy for up to 48 hours after D-galactosamine administration (t = 0 hour). A dose of D-galactosamine exceeding 1.5 g/kg resulted in fulminant hepatic failure. Brain lactate levels increased to >10 mmol/L in the two dogs that developed severe intracranial hypertension of >50 mm Hg and sustained cerebral perfusion pressures of <40 mm Hg. Both dogs experienced brain death, 42 and 48 hours after the administration of D-galactosamine. Brain lactate concentrations determined by magnetic resonance spectroscopy were in agreement with brain tissue concentrations of lactate determined by high-performance liquid chromatography at necropsy. Plasma lactate concentrations were only mildly elevated (3.2 and 4.2 mmol/L) at the time of brain death. Elevated levels of brain lactate are associated with intracranial hypertension and poor neurological outcome during fulminant hepatic failure.
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A noninvasive test is needed to assess the severity of encephalopathy in patients with fulminant hepatic failure (FHF), including those awaiting liver transplantation or during treatment with a bioartificial liver. One possibility is the in vivo measurement of brain lactate by magnetic resonance spectroscopy (MRS), a relatively new technology that has shown promise in the evaluation of other neurological disorders, including neonatal asphyxia,[1,2] meningitis,[2] cardiac arrest,[2] head trauma,[2,3] and mitochondrial errors of metabolism.[4-6] In pediatric patients with central nervous system disease, MRS has shown that an elevated concentration of brain lactate correlated strongly with poor neurological outcome (long-term disability or death).[2] MRS has also shown that the level of brain lactate correlated with the duration of brain edema and coma in a patient with acute decompensation of maple syrup urine disease.[7]

Based on these reports with other neurological disorders, brain lactate by MRS was considered to be a marker of advanced encephalopathy during acute liver failure. The current feasibility study was designed to compare in vivo brain lactate measurement by MRS with intracranial pressure (ICP) monitoring by intraventricular catheter in a large animal model of FHF. Cerebral edema, manifest as intracranial hypertension, is an important cause of cerebral ischemia during FHF. Because the blood brain barrier is impermeable to lactate,[8] increased brain lactate is most likely the result of cerebral ischemia. Therefore, both ICP and brain lactate were assumed to reflect the extent of cerebral edema during acute liver failure. Because other brain metabolites, such as glutamine and glutamate, may contribute to the formation of cerebral edema,[9-13] concentrations of glutamine and glutamate were also determined by MRS in this study.

Materials and Methods

All animals were treated humanely and according to a protocol approved by the Animal Care and Use Committee at the University of Minnesota in accordance with guidelines set forth by the National Academy of Sciences. Five male dogs, 1–3 years of age and weighing 15–20 kg,

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were conditioned for 4 weeks before use. All animals were studied under general anesthesia, which included thiopental induction (15 mg/kg) and inhaled halothane (0.5%–1.0%), titrated to maintain systolic blood pressure in a range of 100–130 mm Hg. Intravenous gentamicin (2 mg/kg) and ticarcillin (50 mg/kg) were administered at the time of induction. Each dog received a left external jugular line for administration of maintenance fluid and D-galactosamine (Sigma Chemical Co., St. Louis, MO), which was used to induce acute hepatic failure as described previously by Sielaff et al.[14] The dose of D-galactosamine ranged from 0 to 2.0 g/kg and was administered as a bolus infusion over 10 minutes, starting at t=0 hours. In four dogs (D-galactosamine dose, 0.0, 1.0, 1.7, and 2.0 g/kg), maintenance fluids consisted of lactated Ringer's solution supplemented with 5% dextrose and 10 mEq/L KCl at 50 mL/h. A fifth dog (D-galactosamine dose, 2.0 g/kg) received no dextrose in his intravenous maintenance fluids (lactated Ringer's solution with 10 mEq/L KCl at 50 mL/h). A carotid artery line was placed for measurement of systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) and sampling of arterial blood. A urethral catheter was placed for measurement of urine output throughout each experiment. An intraventricular catheter (Ventrix catheter; Camino NeuroCare, San Diego, CA) was placed in the left cerebral ventricle for measurement of ICP.

MRS data were acquired on each animal before the administration of the dose of D-galactosamine (baseline), at 12, 24, and 36 hours, and at 2–4-hour intervals until brain death or up to 48 hours. Sessions of data acquisition lasted 30 minutes and were divided into 10 3-minute intervals to allow for the correction of small frequency drifts that were assessed to be <5 Hz. MRS data were collected under in vivo conditions using a 9.4 T, 31-cm bore diameter scanner (Varian, Palo Alto, CA). A 12-cm single-turn surface transmit/receive coil soldered from a series of capacitors (American Technical Ceramics, Huntington St., NY) was used.[15] The volume for measurement of brain metabolites was set to 1 cm^3 and was placed in the right temporal lobe adjacent to the lateral ventricle on the side contralateral to the ICP catheter. This site was selected to avoid image signal loss related to hemorrhagic artifact from the insertion path of the ICP catheter. Positioning was verified before each MRS session using fast low-angle shot imaging with a repetition time of 60 milliseconds, an echo time of 6 milliseconds, and a flip angle of approximately 30°.[15] Shimming of the 1 cm^3 volume was performed using Fastmap,[16] which resulted in water line widths between 12 and 20 Hz and creatine line widths between 8 and 16 Hz (i.e., 0.02 and 0.04 ppm). Spectroscopy was based on a modification of stimulated echo acquisition mode using a repetition time of 3 seconds, echo time of 20 milliseconds, and a mixing time (TM) of 36 milliseconds.[17-19] Special care was taken to minimize eddy current effects, which were estimated in phantoms to contribute <3 Hz to the peak width.

Data processing consisted of at least fourfold zero-filling (zero-padding) to ensure proper digitization of the peak shape, 5 Hz exponential multiplication and manual zero-order and first-order phase correction. Peak analysis was performed using Lorentzian peak fitting of lactate (1.33 ppm), *N*-acetyl-aspartate (NAA) (2.02 ppm), glutamate (2.37 ppm), and glutamine (2.46 ppm) in the range from 1.0 to 2.5 ppm. The millimolar concentrations of lactate, glutamine, and glutamate were determined by comparison of peak area with NAA, which was assumed to be stable at 10 mmol/L.[20]

Vital signs (blood pressure, heart rate, ICP, and urine output) were recorded at baseline and then every 2 hours. Arterial blood gases were obtained at 6-hour intervals and 30 minutes after ventilator changes. Ventilation was adjusted to maintain arterial pCO_2 at 35–40 mm Hg. Arterial blood samples were also obtained before the administration of the dose of D-galactosamine (t=0 hours), at t=12 hours, t=24 hours and then at 6-hour intervals. Blood samples were analyzed for electrolytes, aspartate aminotransferase, ammonia, lactate, and international normalization ratio in the clinical laboratory at the University of Minnesota. At the completion of each experiment, animals were killed by pentobarbital-potassium overdose. Necropsy included gross examination of the brain and liver. Liver sections were placed in formalin, processed in the usual fashion, and examined microscopically after staining with hematoxylin-eosin.

Brain tissue from the right cortex was snap-frozen and stored at −80°C for lactate analysis. Briefly, brain tissue was weighed by analytical balance (model HL 52; Mettler Instrument Corp., Highstown, NJ) and transferred to a microhomogenizer (part no. MH-10; Micro-Metric Instruments Co., Tampa, FL) containing 500 µL of ice-cold deionized water. After 60 seconds of homogenization, the contents of the homogenizer were sonicated at room temperature for 6 minutes and then transferred to a 2-mL microfuge tube. Homogenizers were rinsed with two aliquots of 500 µL deionized water to prevent loss of tissue residue. After 2 minutes of microfugation at 12,000 rpm, samples were passed across a 0.20-µm polytetrafluoroethylene filter. Lactate concentrations were determined from filtered samples by a standardized kit (Sigma Chemical Co.).

Results

The extent of liver injury and encephalopathy varied directly with the dose of D-galactosamine (0.0, 1.0, 1.7, and 2.0 g/kg). Liver injury was determined by histological examination of liver tissues obtained at necropsy (Fig. 1), along with two (international normalization ratio and aspartate aminotransferase) biochemical markers of liver

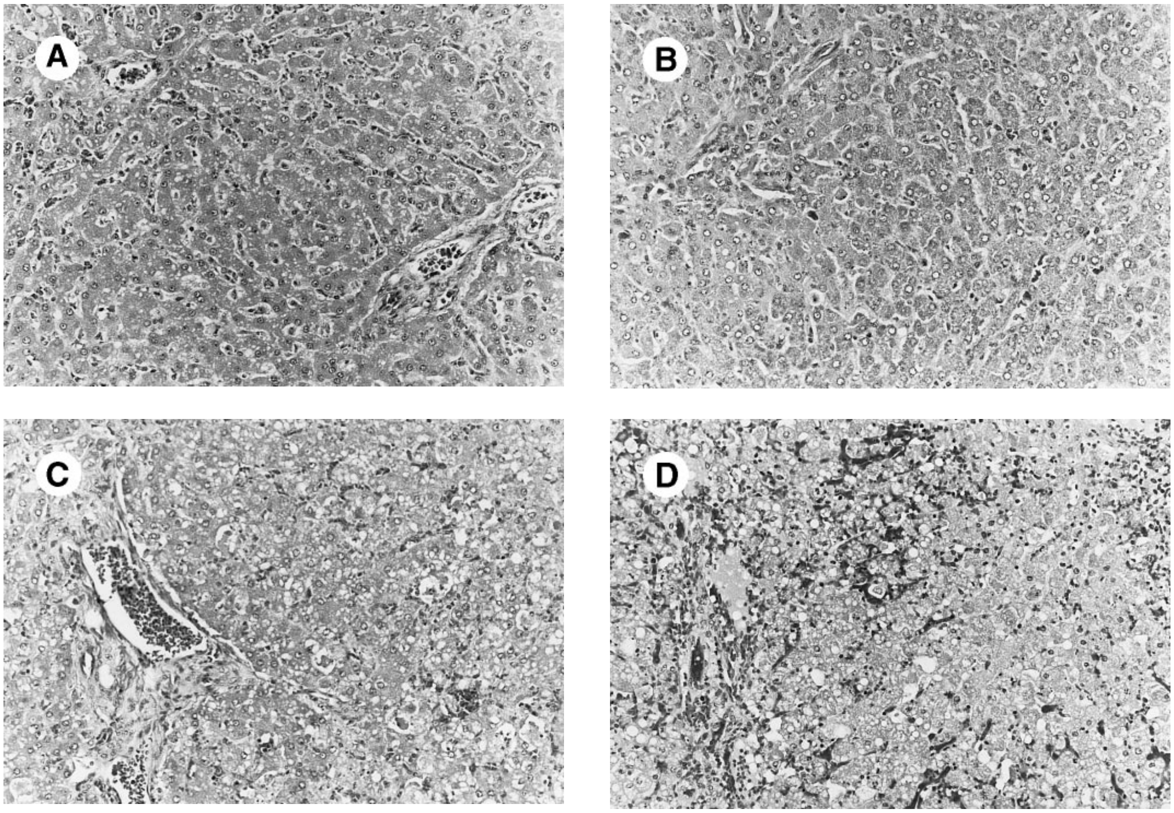

Figure 1. Histologic appearance of liver tissue obtained at necropsy. A dose-dependent extent of liver injury after an intravenous bolus of D-galactosamine. (A) Normal dog liver from control (D-galactosamine, 0.0 g/kg). (B) Rare necrotic hepatocytes and mild lobular disarray 48 hours after administration of 1.0 g/kg D-galactosamine. (C) Scattered foci of necrosis without an acute inflammatory response 46 hours after administration of 2.0 g/kg D-galactosamine. (D) Increased hepatocellular necrosis, hepatocyte drop-out, and nuclear debris at the time of hypoglycemic death. This dog also was administered 2.0 g/kg D-galactosamine, but maintenance fluids contained no supplemental glucose.

function (Table 1). Encephalopathy was determined from intraventricular measurements of ICP and gross examination of the brain at autopsy. A dose of D-galactosamine of 1.7–2.0 g/kg caused sustained intracranial hypertension (ICP of >50 mmHg) and brain death within 48 hours, as summarized in Table 1. Brain death was defined as the simultaneous decrease in SBP and ICP after a progressive increase in ICP. Figure 2 shows that cerebral perfusion pressure (CPP; CPP = MAP − ICP) decreased steadily before brain death in both dogs that were administered lethal doses of D-galactosamine, but CPP remained stable at a lower dose of D-galactosamine (0.0 and 1.0 g/kg).

Table 1. Dose-Dependent Effect of D-Galactosamine on Liver Failure and Encephalopathy

Dose (g/kg)	AST_{max} (U/L)	INR_{max}	ICP_{max} (mm Hg)	Outcome
None	17	1.1	18	Stable survival
1.0	287	1.5	27	Mild liver injury
1.7	5745	>10	60	Brain death at 48 h
2.0	4100	>10	74	Brain death at 42 h

Abbreviations: max, maximum value obtained during each experiment; AST, aspartate aminotransferase; INR, international normalization ratio.

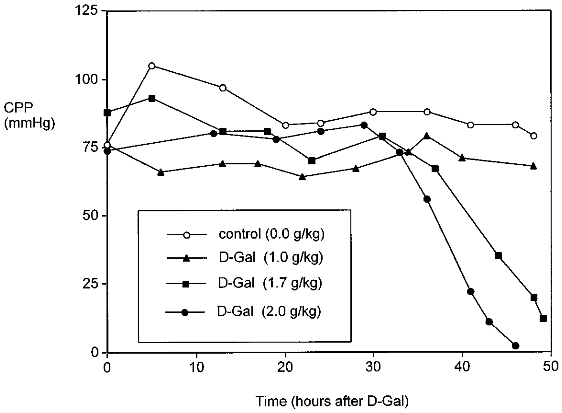

Figure 2. CPP decreased about 30 hours after a lethal dose of D-galactosamine (D-Gal) but remained stable at lower doses. Brain death occurred about 4 hours after CPP decreased to <40 mm Hg in both dogs with advanced FHF. ○, Control (0.0 g/kg); ▲, D-galactosamine (1.0 g/kg); ■, D-galactosamine (1.7 g/kg); ●, D-galactosamine (2.0 g/kg).

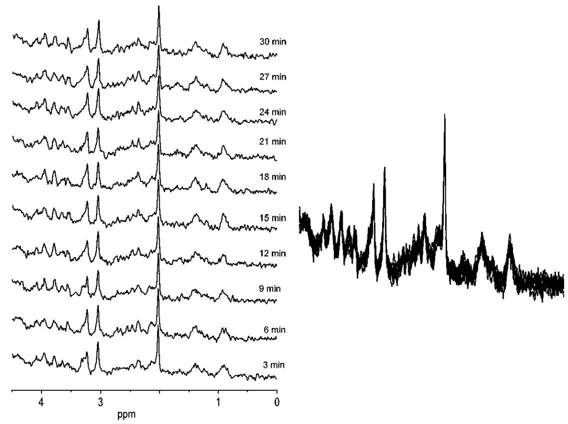

Figure 3. Representative MRS data obtained during one 30-minute session. Ten spectra were collected at 3-minute intervals, (A) individually and (B) superimposed.

The control dog, which was not administered D-galactosamine, showed no signs of cerebral edema. There was mild edema of the brain in the dog that was administered 1.0 g/kg of D-galactosamine. The mild edema may have been the result of trauma during placement of the ICP catheter because the right hemisphere, contralateral to the ICP catheter, appeared less edematous. Both dogs that experienced severe intracranial hypertension showed marked edema and swelling of their brains and died from apparent brain stem herniation.

Thirty-minute sessions, divided into 10 3-minute intervals, were used for the acquistion of MRS data. Only small frequency drifts were observed during each 30-minute session, as shown in Fig. 3. A sharp increase in brain lactate was measured by MRS during the development of FHF induced by D-galactosamine. The increase in brain lactate preceded brain death and coincided with the decrease in CPP. Sequential MRS spectra in Fig. 4 show the increase in brain lactate after a lethal dose of D-galactosamine (2.0 g/kg). Brain lactate levels remained high after brain death, characterized by a sudden and progressive decrease in ICP as shown in Fig. 5. Brain lactate concentrations were also determined from right hemispheric tissue that corresponded to the area of MRS sampling. Table 2 shows that final concentrations of lactate in the brain were similar by MRS and tissue assay. These final concentrations of lactate were much higher than plasma concentrations of lactate obtained immediately before necropsy (Table 2).

Concentrations of glutamine, glutamate, and NAA were determined in the brain by MRS throughout each experiment. There were no sustained or reproducible trends in the concentration of brain glutamine or brain glutamate during the development of FHF (data not shown). The concentration

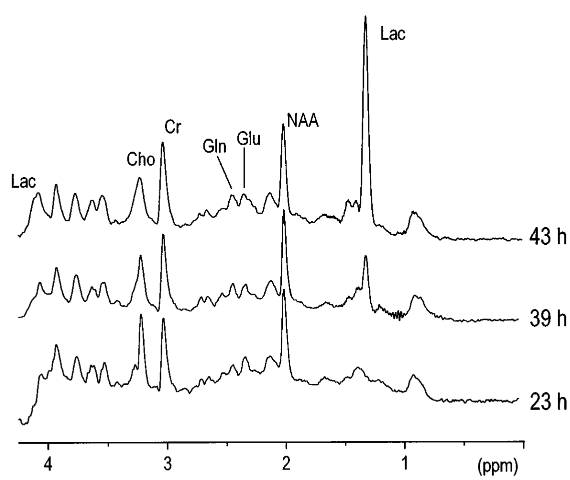

Figure 4. Sequential MRS spectra during the development of FHF in the dog. An increase in brain lactate (Lac) was observed over time (23, 39, and 43 hours) in the same dog after administration of D-galactosamine (2.0 g/kg) at t = 0 hours. Other chemical species that were identified by ^{1}H-proton spectroscopy at a field strength of 9.4 T are labeled and include choline (Cho), creatine (Cr), glutamine (Gln), glutamate (Glu), and NAA. NAA levels were stable until the time of brain death.

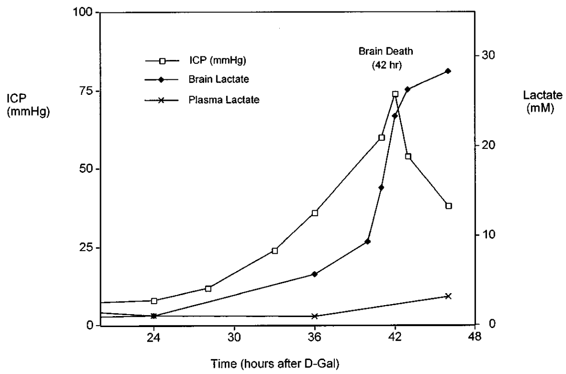

Figure 5. Graph of ICP and brain lactate concentration versus time after infusion of D-galactosamine (D-gal; 2.0 g/kg) and the induction of FHF in the dog. A sharp increase in brain lactate preceded the maximum ICP at 42 hours. An abrupt decrease in SBP coincided with the maximum ICP and was interpreted as the time of brainstem herniation and brain death. Plasma lactate concentrations remained relatively stable at the time of brain death and suggested that lactate production occurred centrally. Elevated brain lactate appears to be a marker of intracranial hypertension and cerebral hypoperfusion. □, ICP (in mm Hg); ◆, brain lactate; X, plasma lactate.

of NAA remained stable with <5% fluctuation in standard deviation of the mean peak area of NAA (2293.3 ± 93.1 arbitrary units) during the development of FHF (dose, 2.0 g/kg).

A fifth dog was administered no supplemental dextrose and experienced severe liver injury (maximum aspartate aminotransferase, 13,782 U/L) and sudden death 44 hours after administration of D-galactosamine (dose, 2.0 g/kg). Of importance, death occurred without intracranial hypertension (ICP of 18 mm Hg) and with a normal brain lactate (<2 mmol/L). This dog was the only subject to develop hypoglycemia and died with a blood glucose of <20 mg/dL.

Table 2. Comparison of Final Lactate Concentrations

Dose of D-Galactosamine (g/kg)	Final Lactate Concentration Plasma (mmol/L)	Brain-MRS (mmol/L)	Brain-Tissue (mmol/L)
None	0.7	<2 mmol/L	1.3
1.0	1.2	<2 mmol/L	1.4
1.7	4.2	11.8	18.0
2.0	3.2	28.3	27.7

Discussion

The diagnosis of hepatic encephalopathy is made from a combination of clinical and laboratory findings, which include neurological deficits and evidence of hepatocyte damage. Unfortunately, these findings are nonspecific predictors of patient survival. Cerebral edema and brainstem herniation, significant contributors to mortality in acute liver failure, are poorly estimated by physical examination or standard liver function tests.[21,22] Computed tomography scan and magnetic resonance imaging of the head are useful to exclude other etiologies of altered mental status.[23] Unfortunately, intracranial changes may occur late in FHF and are difficult to identify by imaging studies.[24] Epidural, subdural, and intraventricular catheters are helpful in guiding therapy to prevent brainstem herniation because they are direct measures of ICP.[24] Intracranial monitoring devices are invasive and problematic during acute liver failure, especially in the setting of coagulopathy.[25] In at least one study, significant morbidity (intracranial bleeding, 22%) and mortality (death, 9%) resulted from ICP monitoring of patients with FHF.[26] A safer, noninvasive method of assessing cerebral edema in FHF is therefore needed.

MRS is a relatively new technology that has great potential in the evaluation of patients with central nervous system disorders, including encephalopathy in liver disease.[10,12,27-34] The concentrations of many cerebral metabolites, such as glutamine, glutamate, and lactate, can be measured in the brain under in vivo conditions with MRS (see Fig. 4).

In the current study, we report a sharp increase in brain lactate that occurred shortly before brain death in dogs with advanced FHF. The sharp increase in brain lactate was associated with sustained intracranial hypertension (Fig. 5) and cerebral hypoperfusion (Fig. 2) and appeared to be prognostic of brainstem herniation. As required by the Animal Care and Use Committee at the University of Minnesota, dogs were under general anesthesia during the development of FHF. As a result, a neurological examination was not possible. Therefore, brain death was defined as the simultaneous decrease in SBP and ICP after a progressive increase in ICP.

Elevated concentrations of brain lactate have already been shown to correlate with outcome in children with a variety of central nervous system disorders. In one study, elevated levels of brain lactate correlated significantly ($P < .001$) with serious long-term disability and death in a group of 36 pediatric patients with acute central nervous system injuries.[2] Mildly elevated concentrations of brain lactate were associated with reversible encephalopathy in a 14-year-old boy with Reye's syndrome[35] and a patient with acute decompensation of maple syrup urine disease.[7]

A proposed explanation for brain lactate production and brain death during FHF induced by D-galactosamine is outlined in Fig. 6. According to this paradigm, acute liver failure triggers both cytotoxic and/or vasogenic changes in the brain that result in the formation of edema.[36] The underlying cause of brain edema in acute liver failure remains unresolved. It has been suggested that glutamine may accumulate in astrocytes during hyperammonemia of liver failure and serve as a primary osmole in causing astrocyte swelling and the formation of brain edema.[37] Evidence in support of glutamine serving as an organic osmolyte was obtained in hyperammonemic animals[38] or animals after total hepatectomy[39] or portocaval anastomosis.[13] In our study of drug-induced FHF, the concentration of brain glutamine increased to 8.3 mmol/L (see Fig. 4), which is within 100% variation of the basal concentration of brain glutamine. The less than expected increase in brain glutamine may highlight potential differences between the D-galactosamine model and other models of hepatic encephalopathy.

Our results suggest that lactate production is increased in the brain after D-galactosamine administration and the development of FHF. In fact, brain lactate concentrations in the range of 10–25 mmol/L were detected by MRS before brain death. These concentrations of brain lactate might be expected

Action		Reaction	Comment
Acute Liver Injury *	→	Cerebral Edema	Cytotoxic and/or Vasogenic mechanism
Cerebral Edema	→	↑ ICP	Brain enlargement occurring in a closed space
↑ ICP	→	↓ CPP	CPP = MAP - ICP CPP=cerebral perfusion pressure MAP=mean arterial pressure
↓ CPP	→	↑ Anaerobic Metabolism	Microvascular hypoperfusion, Cellular hypoxia, ↓ Oxygenation utilization
↑ Anaerobic Metabolism	→	↑ Brain Lactate	↑↑ Production in brain,
↑ Brain Lactate	→	↑↑ Cerebral Edema	Pathogenic effect of lactate (*i.e.*, secondary osmole)
↑↑ Cerebral Edema	→	Brainstem Herniation	Brain Death

Figure 6. Proposed explanation of brain death in D-galactosamine–induced FHF.

to have a pathogenic or osmotic effect on further formation of cerebral edema.[40] Therefore, along with serving as a marker of anaerobic metabolism, lactate may serve as a secondary osmole and contribute to the formation of cerebral edema and brainstem herniation. In support of this hypothesis is the observation that ICP and brain lactate increased at similar rates shortly before brain death. Also in support of this hypothesis is the absence of intracranial hypertension and the absence of increased brain lactate in the hypoglycemic dog (Fig. 1D) that died 44 hours after infusion of D-galactosamine. Production of lactic acid is not possible during hypoglycemia because of glucose (substrate) deficiency.[8] Conversely, in children with central nervous system disorders, hyperglycemia was associated with increased brain lactate when compared with similar patients with normoglycemia.[2]

Lactic acidosis resulting from cerebral ischemia and increased anaerobic glycolysis may also cause lysosomal instability and cerebral damage by the activation of lysosomal enzymes.[41] Alternatively, it has been proposed that lactate accumulation during liver failure is the result of ammonia-induced inhibition of the malate-aspartate shuttle and/or inhibition of tricarboxylic acid cycle flux in the brain.[42] Of note, in vivo ^{13}C MRS studies suggest that the rate of the tricarboxylic acid cycle is not significantly altered during ammonia infusion.[43]

The anesthetized model of FHF, first described by Sielaff et al,[14] was used in this study. This dog model provided a large brain for intraventricular catheter placement and MRS data acquisition. FHF, including significant intracranial hypertension and brain death within 48 hours, was reproducible after the administration of 1.7 and 2.0 g/kg D-galactosamine, which was greater than the dose (1.0 g/kg) used to produce liver failure by Sielaff et al. We found that brain death did not occur within 48 hours at doses of D-galactosamine of <1.7 g/kg. These findings are in agreement with the study of Diaz-Buxo et al.[44] No signs of endotoxic shock were observed in our study because SBP remained >100 mm Hg before brain death. Intracranial hypertension was an important finding in our study, although no evidence of edema was observed on gross or microscopic examination of the brain in the study of Diaz-Buxo et al.[44] A higher dose of 1.7–2.0 g/kg of D-galactosamine may be needed in the dog for the formation of cerebral edema. The development of cerebral edema and intracranial hypertension after infusion of D-galactosamine is supported by past reports using rats[36,45,46] and rabbits.[47,48]

Although the current study was conducted in an experimental facility at 9.4 T, observations made in this study regarding brain lactate are transferable to the clinical setting. Namely, identification of the lactate peak at 1.33 ppm is currently possible on clinical MRS scanners (1.5 T) provided care is taken to eliminate contributions from extraneous lipid resonances. Proton spectra of brain lactate at 1.5 T have been reported in a case of Reye's syndrome[35] and in a variety of other diseases with neurological manifestations.[1-7] Unfortunately, unambiguous separation of the glutamine/flutamate complex (2.2–2.5 ppm) is not possible at 1.5 T. It was for this reason that the current study was performed at 9.4 T.

We concluded from this feasibility study that the measurement of brain lactate by MRS is a noninvasive test of advanced encephalopathy during acute hepatic failure. In particular, our data suggest that brain death is likely in the setting of an increasing brain lactate during FHF. It cannot be determined from this feasibility study whether brain lactate is also a marker of reversible brain injury because both animals died after development of elevated brain lactate and because therapies such as osmotic diuretics, hyperventilation, or a bioartificial liver were not attempted to lower ICP. This study supports the role of MRS in the evaluation of encephalopathy during FHF.

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