

Bile Acid and Ammonia-Induced Brain Edema in Rats

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ABSTRACT

Infusion of bile acid such as chenodeoxycholic acid or deoxycholic acid through the carotid artery of rats produced reversible and unilateral opening of the blood-brain barrier without any tissue damage. Intravenous drip infusion of ammonium acetate during the opening resulted in severe edema of the brain. The results suggest the importance of bile acid and ammonia for the pathogenesis of brain edema frequently observed in acute hepatic failure.

Cerebral edema is a major cause of death in acute hepatic failure. This is a grievous problem in hepatology, since the pathogenesis is unclear, and thus no effective preventative or therapeutic method has been established yet. Biochemical metabolites such as ammonia, amino acid, mercaptan and short-chain fatty acid induce alterations in the blood-brain barrier¹⁾. We have observed that intravenous infusion of an ammonium acetate solution into dogs during mannitol-induced reversible opening of the blood-brain barrier results in a marked rise in intracranial pressure³⁾.

The present study describes the importance of bile acid in opening the blood-brain barrier and of ammonia in inducing cytotoxic edema of the brain in rats.

Male Sprague-Dawley rats, weighing 350-450 g each, were anesthetized with pentobarbital (35 mg/kg body weight, intraperitoneally). One-half ml of 1.0% chenodeoxycholic acid or 0.1% deoxycholic acid solution in physiological saline was infused directly into the right internal carotid artery via the external carotid for 60 sec at a constant rate⁶⁾. If bile acid infused for 60 sec is distributed in the blood passed through the right internal carotid artery for this period, the blood concentrations of these bile acids might be

within the physiological variations. Immediately after the bile acid infusion, 0.5 ml of 2% Evans blue (a visual tracer) solution in saline was administered through the cervical vein. The rats were killed by decapitation 60 min later. The degree of barrier opening to intravenous Evans blue was classified into 4 grades (0, no staining; 1+, faint; 2+, light but extensive, and 3+, deep and extensive staining)⁵⁾, and water contents were determined by our previous methods³⁾. A 4% ammonium acetate solution was drip-infused into the femoral vein for 90 min at a constant rate of 1.6 ml/hr. The bile acid solutions were infused as mentioned above 30 min after the initiation of ammonium acetate infusion, and the rats were sacrificed 60 min later. The blood ammonia concentration was determined enzymatically³⁾ and the brain ammonia content similarly measured²⁾.

Carotid infusion of chenodeoxycholic acid or deoxycholic acid induced staining by Evans blue in the right hemisphere of the brain and an increase in the brain water content (Table 1). The intensity of Evans blue staining depended on the concentrations infused, but regardless of the concentration, Evans blue staining was not observed more than 24 hr after the infusion, indicating the reversibility of opening. The bile

Table 1. Effect of bile acid and ammonia on brain water content and Evans blue staining in the right hemisphere of the brain

	Brain water content (%)	Grade of opening			
		0	1+	2+	3+
Saline	78.1 ± 0.3 ^{a)}	4 ^{b)}			
Ammonium acetate	78.3 ± 0.4	4			
Chenodeoxycholic acid	78.8 ± 0.3 ^{c)}	1	2		
Deoxycholic acid	79.1 ± 0.2 ^{c)}	1	4	1	
Ammonium acetate + chenodeoxycholic acid	80.6 ± 0.6 ^{d,e)}				3
Ammonium acetate + deoxycholic acid	81.2 ± 1.3 ^{d,e)}				5

a Mean ± SD.

b No. of rats.

c $p < 0.05$, saline (S) vs chenodeoxycholic acid (C) or deoxycholic acid (D).

d $p < 0.02$, ammonium acetate (A) vs (A + C) or (A) vs (A + D).

e $p < 0.05$, (C) vs (A + C) or (D) vs (A + D).

acid-treated rats were generally much less active than saline-treated rats. The brain water content correlated closely with the grade of Evans blue staining. Ammonium acetate alone, however, did not cause opening of the barrier. The blood ammonia levels under these conditions increased gradually after initiation of ammonium acetate infusion and finally reached 250-300 $\mu\text{g}/\text{dl}$.

Ammonium acetate infusion during chenodeoxycholic acid or deoxycholic acid-induced opening of the barrier resulted in severe staining by Evans blue and in a marked rise in brain water content. The brain ammonia content in the Evans blue-stained hemisphere was much higher ($1652 \pm 385 \mu\text{g}/\text{kg}$ brain, $p < 0.05$, No. of deoxycholic acid and ammonia-treated rats = 3) than in the unstained hemisphere (406 ± 59). All the brains were macroscopically normal and microscopically showed edematous changes.

Primary and secondary bile acids including unconjugated and conjugated forms induce Evans blue staining of the brain (data not shown), but deoxycholic acid (unconjugated form) is the most potent one (the minimum concentration to induce staining is 0.05%, compared with more than 0.1% for other bile acids). The present experiments on brain ammonia contents indicate that ammonium ion (NH_4^+) can be actively transported into the brain during bile acid-induced opening of the blood-brain barrier. We already reported that ammonia-induced cytotoxic edema was induced only during the mannitol-induced (osmotic) opening of the blood-brain barrier in dogs³⁾. The present observations also suggest

that ammonia-induced cytotoxic edema further accelerates opening of the blood-brain barrier (vasogenic edema) which in turn increases the edema. This vicious circle is important to the irreversibility of brain edema.

Intravenous drip infusion of conjugated bile acids dissolved in rat sera also induced brain edema more markedly with a prior and simultaneous infusion of ammonium acetate. However, this physiological approach did not induce Evans blue-staining of the brain but did the accelerated permeability, i.e., the increased brain uptake of [^{14}C]-inulin (Tominaga et al., unpublished observation). We are now investigating effects of severe liver dysfunction and ligation of common bile duct on transport of middle molecular substances into the brain.

In acute hepatic failure, permeability of the blood-brain barrier might be subclinically accelerated by severe and continuous elevations of blood bile acids, protease and kinin, and by stimulated entry of blood ammonia (even if at the normal levels) into the brain to induce cytotoxic edema. Fulminant hepatitis patients show a marked increase in serum total bile acid concentrations (50-fold increase) including chenodeoxycholic acid (40-fold increase). However, the most outstanding feature of bile acid pattern in fulminant hepatitis is a characteristic increase in deoxycholic acid (10-fold increase), since there is no significant increase of deoxycholic acid even in icteric patients with liver cirrhosis or biliary stone. We recently succeeded in producing brain edema in rats by infusing 2 ml of serum from two fulminant

hepatitis patients with severe brain edema (50-year-old female and 52-year-old male, serum total bile acid 6.1 and 3.3 mg/dl, chenodeoxycholic acids 3.7 and 2.3 mg/dl, deoxycholic acids 371 and 87 μ g/dl and blood ammonia 174 and 145 μ g/dl, respectively). The present study thus indicates that chenodeoxycholic acid, deoxycholic acid and other bile acids might additively induce the opening of the blood-brain barrier.

With early institution of charcoal hemoperfusion and plasma exchange (i.e., at Stage III encephalopathy), blood bile acid concentrations were found to be effectively prevented from rising to dangerously high levels, due to the bile acid pool in the body being relatively small⁷. This finding is consistent with the clinical observation that cerebral edema developed significantly less frequently in an early perfusion group than in patients in whom hemoperfusion was started later in the course of the disease⁴.

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