Archives of Biochemistry and Biophysics 536 (2013) 171–175

Contents lists available at SciVerse ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

Brain edema in acute liver failure: Role of neurosteroids

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ARTICLE INFO

Article history:
Available online 6 April 2013

Keywords:
Acute liver failure
Ammonia
Astrocyte swelling
Brain edema
Blood–brain barrier
Hepatotoxins
Neurosteroids

ABSTRACT

Brain edema is a major neurological complication of acute liver failure (ALF) and swelling of astrocytes (cytotoxic brain edema) is the most prominent neuropathological abnormality in this condition. Elevated brain ammonia level has been strongly implicated as an important factor in the mechanism of astrocyte swelling/brain edema in ALF. Recent studies, however, have suggested the possibility of a vasogenic component in the mechanism in ALF. We therefore examined the effect of ammonia on blood–brain barrier (BBB) integrity in an *in vitro* co-culture model of the BBB (consisting of primary cultures of rat brain endothelial cells and astrocytes). We found a minor degree of endothelial permeability to dextran fluorescein (16.2%) when the co-culture BBB model was exposed to a pathophysiological concentration of ammonia (5 mM). By contrast, lipopolysaccharide (LPS), a molecule well-known to disrupt the BBB, resulted in an 87% increase in permeability. Since increased neurosteroid biosynthesis has been reported to occur in brain in ALF, and since neurosteroids are known to protect against BBB breakdown, we examined whether neurosteroids exerted any protective effect on the slight permeability of the BBB after exposure to ammonia. We found that a nanomolar concentration (10 nM) of the neurosteroids allopregnanolone (THP) and tetrahydrodeoxycorticosterone (THDOC) significantly reduced the ammonia-induced increase in BBB permeability (69.13 and 58.64%, respectively). On the other hand, we found a marked disruption of the BBB when the co-culture model was exposed to the hepatotoxin azoxymethane (218.4%), but not with other liver toxins commonly used as models of ALF (thioacetamide and galactosamine, showed a 29.3 and 30.67% increase in permeability, respectively). Additionally, THP and THDOC reduced the effect of TAA and galactosamine on BBB permeability, while no BBB protective effect was observed following treatment with azoxymethane. These findings suggest that ammonia does not cause a significant BBB disruption, and that the BBB is intact in the TAA or galactosamine-induced animal models of ALF, likely due to the protective effect of neurosteroids that are synthesized in brain in the setting of ALF. However, caution should be exercised when using azoxymethane as an experimental model of ALF as it caused a severe breakdown of the BBB, and neurosteriods failed to protect against this breakdown.

Introduction

Hepatic encephalopathy (HE) is a major clinical complication in patients with severe liver disease. HE in the setting of chronic liver disease (HE Type C or portal-systemic encephalopathy) usually occurs after alcoholic liver cirrhosis (for review, see [1]). HE Type C is characterized by alterations in cognition, consciousness and motor function. The encephalopathy associated with acute liver failure (ALF) occurs following massive liver necrosis, generally due to viral hepatitis (predominantly hepatitis B), hepatic neoplasms, vascular causes or exposure to various hepatotoxins. It presents with the abrupt onset of delirium, seizures, and coma and has an extremely poor prognosis (70% mortality) (for reviews, see [2,3]) While cerebral edema and associated increased intracranial pressure and brain herniation occur in up to 80% of patients with ALF [2,4], and represents the most frequent cause of death in these individuals [2,5,6], the actual percentage of deaths remains unclear [2,7], as recent reports indicate that mortality has decreased in recent years (for review, see [3]).

Swelling of astrocytes (cytotoxic edema) represents the major component of the edema in ALF (for review, see [8]). Swollen astrocytic processes were identified in patients dying with ALF [9,10]. Magnetic resonance and diffusion-weighted imaging studies in patients with ALF revealed a reduction in the size of the extracellular space, consistent with an intracellular accumulation of water [11,12]. Animal studies also support the cytotoxic aspect of ALF-related brain edema (for review, see [13]). Further, exposure of

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0003-9861/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.abb.2013.03.007

rat cerebral cortical slices or organotypic slice cultures from mouse forebrain to 1–10 mM ammonia was shown to induce astrocyte swelling [14,15]. Additionally, treatment of cultured astrocytes with a pathophysiologically relevant concentration of ammonia (5 mM) was shown to induce cell swelling [8,16–19]. In aggregate, these findings strongly support that the brain edema in ALF is cytotoxic, principally as a result of astrocyte swelling.

While a generalized breakdown of the BBB (as occurs in vasogenic edema) is not a feature in humans with ALF, a recent study [20] suggested that in patients dying with ALF, the brain edema is vasogenic in origin since there was evidence of tight junctional abnormalities, brain microvessel endothelial cell shrinkage, and a reduction in occludin protein expression. While the reason for the BBB breakdown in animal models is not clear, it is possible that liver toxins employed in these studies may have direct toxic effect on components of the BBB that are independent of ALF. We therefore examined to what extent various liver toxins commonly used to induce ALF, contribute to a breakdown of the BBB using an *in vitro* co-culture model of the BBB. Since increased neurosteroid biosynthesis is known to protect against BBB breakdown [21–24], and increased neurosteroid production has been reported in brains of rats with ALF (for review, see [25]), we also examined whether neurosteroids exert a protective effect on the slight BBB permeability observed after exposure to ammonia and liver toxins.

Materials and methods

All chemicals used in the study were purchased from Sigma–Aldrich (St. Louis, MO, USA) unless noted otherwise: thioacetamide (TAA, cat# 163678), ammonium chloride (Cat# A0171), D-(+)-Galactosamine hydrochloride (Cat# G0500), and azoxymethane (Cat# A5486).

Co-culture of brain microvessel endothelial cells and astrocytes

A co-culture model of BBB (containing brain microvessel endothelial cells and astrocytes) was employed to examine the effect of toxins on BBB permeability using a model previously described [26]. Briefly, primary endothelial cells from brain microvessels were seeded on transwell inserts (3.0 mm pore size) (Fig. 1). Primary cultures of astrocytes were seeded at the bottom of the transwell inserts. Ammonia, liver toxins, as well as lipopolysaccharide (LPS), a well-known inducer of the BBB breakdown [26], were added to the lower compartment (A) (Fig. 1). Endothelial permeability was measured by adding fluorescein isothiocyanate-labeled dextran (40 kDa) in the lower compartment (A) to a final concentration of 1 mg/ml at 37 °C for 20 min. Fluorescence was measured in the culture media from the upper compartment (B) at an excitation wavelength of 485 nm and emission wavelength of 520 nm.

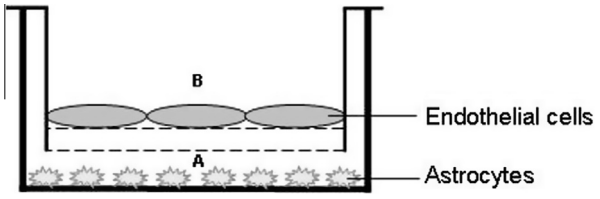

Fig. 1. Co-culture model of BBB (brain microvessel endothelial cells and astrocytes). Primary cultures of astrocytes were seeded at the bottom of the transwell inserts. Ammonia, liver toxins, LPS, as well as the fluorescein isothiocyanate-labeled dextran (40 kDa), were added to the lower compartment (A). Fluorescence was measured in the culture media from the upper compartment (B).

Results

Effect of ammonia and LPS on BBB permeability

Co-culture of microvessel endothelial cells and astrocytes were treated for 24 h with 5 mM ammonia (NH_4Cl), the concentration detected in brains of experimental animals with ALF [27–29], and the extent of endothelial permeability was measured by adding fluorescein isothiocyanate-labeled dextran as noted above in Materials and Methods. Ammonia caused only a slight increase in dextran fluorescein (16.2%) (Fig. 2). However, we found a BBB breakdown when co-cultures were exposed to LPS (5 μg/ml) for 24 h (87.1% permeability) (Fig. 2). A recent report also describes a slight increase in the paracellular permeability (27%) using cultured endothelial cell line (RBE-4) after exposure to ammonia; however, this culture system did not include astrocytes ([30]).

Effect of neurosteroids on the ammonia-induced BBB permeability

Since increased neurosteroid biosynthesis has been reported in brains of rats with ALF (for review, see [25]) and neurosteriods are known to protect the BBB from breakdown in other conditions [31], we examined whether neurosteroids also exert a protective effect on the slight BBB permeability observed after exposure to ammonia. Co-cultures were exposed to the neurosteroids allopregnanolone (THP) and tetrahydrodeoxycorticosterone (THDOC) (10 nM, a level found in brains in experimental ALF) (for review, see [25]), along with ammonia (5 mM) for 24 h, and BBB permeability was determined as described above. Both THP and THDOC significantly reduced the slight endothelial permeability observed following ammonia treatment (69.13 and 58.64%, respectively) (Fig. 2). Similar BBB protection was observed when these neurosteroids were added in the co-culture system along with LPS (55.67% with THP and 50.74% with THDOC) (Fig. 2).

Effect of liver toxins on the BBB breakdown

As noted above, controversies exist surrounding the integrity of the BBB in animal models of ALF induced by the hepatotoxins galactosamine and azoxymethane, although there is no report of a BBB breakdown in the TAA-induced ALF in rats. This variable effect of hepatotoxins suggested the possibility that some hepatotoxins may additionally injure the BBB, independent of their effect on

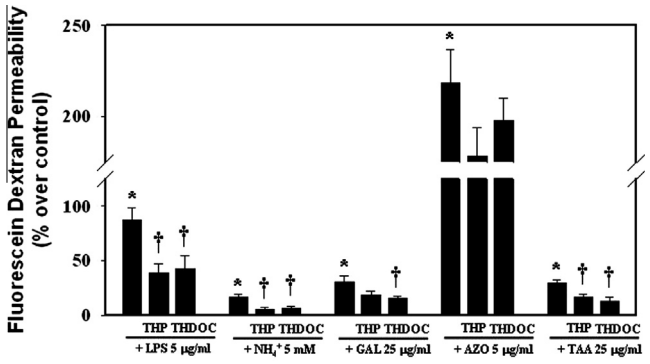

Fig. 2. Effect of neurosteroids on BBB disruption. Co-culture model of BBB were exposed to LPS, ammonia, galactosamine, azoxymethane and thioacetamide (TAA) for 24 h, with and without the neurosteroids THP and THDOC (10 nM), and the extent of permeability to fluorescein isothiocyanate–dextran was determined. Data of all experiments were subjected to analysis of variance followed by Tukey's post hoc comparisons. $^{*}p < 0.05$ vs. control; $^{\dagger}p < 0.05$ vs. toxins. Error bars, mean ± S.E. THP, allopregnanolone (3α-hydroxy-5α-pregnan-20-one); THDOC, 3α, 21-dihydroxy-5α-pregnan-20-one (tetrahydrodeoxycorticosterone/3α, 5α).