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Phospholipid and cholesterol alterations accompany structural disarray in myelin membrane of rats with hepatic encephalopathy induced by thioacetamide

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Abstract

Fulminant hepatic failure is often associated with a wide range of neurological symptoms which are collectively referred to as hepatic encephalopathy. Fulminant hepatic failure with associated hepatic encephalopathy has a poor prognosis with the currently available sure treatment being only liver transplantation. This is largely owing to the lack of understanding of critical factors involved in the etiology of the condition. Lipid changes have been implicated in cerebral derangements characteristic of hepatic encephalopathy. About 79% of the brain lipid is concentrated in the myelin fraction where they play an important role in ion balance and conduction of nerve impulses. Hence, in the present study we aimed to investigate changes in myelin lipid composition and structure.

Myelin was isolated by sucrose density gradient centrifugation from cerebral cortex of male Wistar rats (250–300 g body weight) treated with 300 mg/kg body weight thioacetamide administered twice at 24 h interval to induce hepatic encephalopathy. Significant decrease was observed in the cholesterol and phospholipids content of myelin from treated rats. Sphingomyelin, phosphatidylserine and phosphatidylethanolamine content also decreased significantly following 18 h of thioacetamide administration. However, phosphatidylcholine levels remained unaltered. Transmission electron microscopic observation of myelin membrane from cerebral cortex sections showed considerable disorganization in myelin structure.

Increase in malondialdehyde levels precede lipid changes leading to the speculation that oxidative damage may be the critical factor leading to decrease in the anionic phospholipids. Changes in myelin were evident only in later stages of hepatic encephalopathy indicating that myelin alteration may not play a role in early stages of hepatic encephalopathy. Nevertheless, myelin alteration may have a crucial role to play in various psycho-motor alterations during later stages of hepatic encephalopathy.

Keywords: Hepatic encephalopathy; Thioacetamide; Myelin lipid changes; Oxidative stress

1. Introduction

Hepatic encephalopathy (HE) is a common neurological complication of severe liver disease and occurs in two forms: acute and chronic (Hazell and Butterworth, 1999). The acute form of HE – a common complication of fulminant hepatic failure (FHF) or acute hepatic failure – develops rapidly progressing from altered mental status and delirium to seizures and coma within a short period generally hours or few days. The mortality rate of HE associated FHF approaches 80% despite intensive care therapy and can be currently reduced only by timely orthotopic liver transplantation. The most frequent cause of FHF is viral hepatitis (Saunders et al., 1972) followed closely by overdose of drugs like paracetamol, halothane (Barnard et al., 1981) and toxicity due to drugs such as tetracycline or valproic acid and in patients with surgically constructed jejunoileal bypass constructed for morbid obesity (Burroughs et al., 1982; Delong and Glick, 1982). Rarer causes include congenital or acquired biochemical disorders, vascular anomalies and acute hepatic failure associated with pregnancy and trauma.

Though several factors like elevated ammonia levels, imbalance in amino acid levels, false neurotransmitters like octapamine, accumulating in systemic circulation following hepatic dysfunction have been implicated in the etiology of HE

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(Zieve, 1981; Cooper and Plum, 1987; Hazell and Butterworth, 1999), the exact mechanisms behind the cerebral changes encountered in HE still remains an enigma. Changes in lipid composition of whole brain homogenates from cerebral cortex have been implicated in HE (Osada et al., 1990) along with changes in CSF fatty acid composition. These studies though providing initial evidence in the direction of lipid changes being involved in pathogenesis of HE, reflect on average changes occurring in whole brain. They do not however, elaborate on changes in specific cerebral components, which could vary significantly from the total brain picture.

Lipids are essential functional and structural constituents of the nervous system. Changes in lipid composition could have far reaching implications on the functionality of brain, thus making this an important focus of study in many neurodegenerative conditions. Seventy percent of the cerebral lipids are concentrated in the myelin fraction where they act to insulate ionic currents and have an important functional role in saltatory conduction of action potentials. Changes in myelin lipid content and integrity have been shown to occur in several neurodegenerative conditions like Alzheimer's disease (Miu et al., 2004) and multiple sclerosis (Kieseier et al., 2005). Myelin changes would effect conduction of action potentials and thus many neural functions. The high lipid content in myelin makes it particularly susceptible to oxidative changes like lipid peroxidation. Oxidative stress has been earlier reported in mitochondria and cortex (Reddy et al., 2004) during HE. It however remains unclear, how these intracellular changes in astrocytes and neurons would affect myelin structure and composition. Hence, in the present study we aimed to investigate changes if any, in myelin during the pathogenesis of HE. The current study also proposes oxidative stress as a possible causative factor for effects on myelin.

In the present study HE was induced in rats by the administration of thioacetamide (TAA), a known hepatotoxin. TAA causes marked hepatocellular necrosis in a dose dependent manner after biotransformation to a active metabolite via the flavin adenine dinucleotide monoxygenase pathway resulting in formation of TAA-S oxide (Chieli and Malvaldi, 1984; Hunter et al., 1977; Porter and Neal, 1978). In rats the clinical picture following intraperitoneal administration of TAA includes metabolic acidosis, high serum transaminases, abnormal coagulopathy and histologically centrolobular necrosis in liver and encephalopathy that are at a maximum by 12–24 h (Peeling et al., 1993; Bruck et al., 1998). The dosage and reproducibility of this model have been reported earlier from our lab (Reddy et al., 2004).

2. Experimental procedure

2.1. Materials

All standards (phospholipids, cholesterol and malondialdehyde) were purchased from Sigma–Aldrich Chemicals Private Ltd., St. Louis (USA). Silica gel 60 sheets for thin layer chromatography (TLC) were purchased from Merck, Damstadt, Germany. All other chemicals and solvents were obtained from Qualigens (Glaxo India Pvt. Ltd., India) and Sisco Research Laboratories (SRL, India). Cholesterol estimation was done using commercial kit supplied by Qualigens diagnostic kits (Glaxo, India).

2.2. Animals

Male Wistar rats (250–300 g body weight) were used for the study. All the animals were housed four per cage at 25 ± 2 °C with 12 h day–12 h night cycles in the animal house facility available at the University of Hyderabad. Food and water were provided to the animals ad libitum. Rats were fed balanced pellet diet supplied by Hindustan Lever Ltd. All the protocols followed for the use in animal experimentation were approved by the Institutional (IAEC) as well as the national animal ethical committee (CPCSEA) guidelines. Special approval has been obtained to sacrifice the rats used in the present study without anesthesia.

2.3. Drug treatment

Thioacetamide (300 mg/kg body weight) was dissolved in physiological saline and administered intraperitoneally for two days at 24 h interval (Reddy et al., 2004). Animals were killed at different time periods (12 h and 18 h) after the administration of the second dose. Control rats received normal saline to serve as vehicle controls. All the rats were given a 25 ml/kg body weight of supportive therapy which consisted of 5% dextrose and 0.45% saline with 20 mEql of potassium chloride (Norton et al., 1997). Brains were dissected, weighed and immediately processed for further studies. No anesthesia was used prior to sacrificing. This was done to avoid any changes that may occur in response to administered anesthetics (Sirois et al., 1998).

2.4. Isolation of myelin membranes

Myelin membranes were isolated from the cerebral cortex of adult Wistar rats by a modification of the method described by Locher et al. (1985). Cerebral cortex collected from control and thioacetamide treated rats were homogenized in 0.32 M sucrose and centrifuged at 1500 × *g*. The supernatant was centrifuged at 10,000 × *g*. The supernatant is discarded and the pellet (p2) containing myelin, synaptosomes and mitochondria is resuspended in 0.32 M sucrose and loaded on a discontinuous density gradient (1 M and 0.8 M sucrose) which is centrifuged at 60,000 × *g*. The fraction separating at the interface between 0.32 M and 0.8 M sucrose represents the myelin fraction (Locher et al., 1985). This fraction is collected, resuspended in 0.32 M sucrose and centrifuged at 12,000 × *g*. The pellet is collected and used for all further assays. Purity of isolated myelin fraction was assessed by assaying activity of marker enzymes. Succinate dehydrogenase, Glutamate decarboxylase and 2′3′-cyclic nucleotide 3′ phosphohydrolase were used as marker for mitochondria, synaptosomes and myelin respectively.

2.5. Ammonia, prothrombin time and enzyme assays

Ammonia levels in liver, brain and serum were determined according to the method of Ratnakumari and Murthy (1989). Prothrombin time was determined by using standard commercial kit (obtained from Tulips, India). Serum and Liver aminotransferases activities were measured as per the method of Bergmayer and Brent (1974).

Activities of succinate dehydrogenase and glutamate decarboxylase were determined as per the method described by Nandakumar et al. (1973) and Sadasivudu and Murthy (1978), respectively. Activity of 2′3′-cyclic nucleotide 3′ phosphohydrolase was estimated by the method described by Fujimoto et al. (1976). Recovery of enzyme activity was calculated by taking total activity in homogenates as 100% and was expressed as percent recovery. For purpose of better clarity in assessing extent of purity of isolated fractions the folds increase in specific activity is also presented.

2.6. Lipid analysis

2.6.1. Lipid extraction

Myelin lipids were extracted with chloroform:methanol (2:1) according to the method of Folch et al. (1951). The combined lower phase obtained by Folch extraction were dried under nitrogen gas and used for further estimations of phospholipids.