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Differential regional responsiveness of astroglia in mild hepatic encephalopathy: An Immunohistochemical approach in bile duct ligated rat

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ABSTRACT

Hepatic encephalopathy (HE) is a neuropsychiatric disorder that occurs in both acute and chronic liver failure. However, the pathomechanisms of the disease remains obscure. Neuropathological studies have demonstrated a primary gliopathy in humans as well as in animal models of chronic and acute liver failure. Here, we have investigated in an animal model of mild HE: the bile duct ligated rat (BDL) at the cirrhotic stage (4 weeks after surgery), the expression of the key marker of mature astrocytes; the glial fibrillary acidic protein (GFAP) in different brain areas such as: Substantia nigra pars compacta (SNc), Ventral tegmental area (VTA), hippocampus, dorsal striatum and brain cortex by means of immunohistochemistry. The immunohistochemical study showed, in BDL compared to the operated controls (shams), a diminished astrocyte reactivity corresponding to a loss of GFAP expression within SNc, VTA, hippocampus and dorsal striatum ($p < 0.05$),whereas in the brain cortex astrocytes appeared strongly immunoreactive with increased GFAP expression ($p < 0.05$) as compared to shams. Our finding demonstrated differential astroglial responses which depend to the specificity of the area investigated and its particular neuronal neighboring environment, and could have possible outcomes on the diverse neuronal functions especially those observed during the different episodes of hepatic encephalopathy.

1. Introduction

Hepatic encephalopathy (HE) is a serious neuropsychiatric complication of chronic and acute liver failure featured partially by a neuronal impairments involving numerous neurotransmitter systems imbalances including the glutamatergic (Record et al., 1976; Lavoie et al., 1990), serotonergic (Young et al., 1975; Bergeron et al., 1995; El Hiba et al., 2013), GABAergic (Giguère et al., 1992; Rao et al., 1994) and catecholaminergic (Bergeron et al., 1989; El Hiba et al., 2012) systems. In neuropathology, several attempts report that HE in both acute and chronic liver failure is primarily a disorder of glial cells and HE is currently considered to be a classical example of a primary gliopathy involving astrocyte swelling (Kato et al., 1992; Willard-Mack et al., 1996), microglial activation (Rodrigo et al., 2010) and Alzheimer Type II astrocytosis (Adams and Foley, 1953; Norenberg et al., 1990; Butterworth et al., 1987) as well as other glial cells changes such as increased secretory activity of the ependymal and hypendymal cells of the subcommissural organ (SCO: a circumventricular organ located in the roof of the third ventricle) (El Hiba et al., 2013). Such impairments may impact on some neuropsychiatric features of HE, indeed Shawcross et al. (2004) proposed that systemic inflammation exacerbates the neuropsychological alterations induced by hyperammonemia (characteristic of liver failure). The authors showed that hyperammonemia deteriorates neuropsychological test scores during inflammatory state (Shawcross et al., 2004). Otherwise, our previous works have shown a general loss of tyrosine hydroxylase (TH) expression in substantia nigra pars compacta (SNc) and ventral tegmental area (VTA) as well as the striatal projections leading to a loss of dopamine synthesis in these brain areas (El Hiba et al., 2012). Several hypothesis were suggested to explain these finding including a possible implication of neurotoxins accumulated during liver failure (Butterworth et al., 1987; Weissenborn, 1992; Hawkins and Mans, 1994; Jones and Basile, 1997). Besides, we postulated a possible dysfunction

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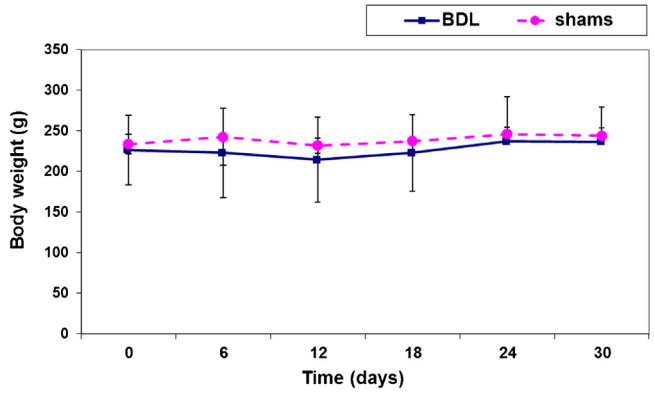

Fig. 1. Body weight evolution of BDL rats and their corresponding shams during 30 days of observation. Note a slight and insignificant reduction of body weight in BDL rats between 6 and 18 days post-surgery. Data are reported as mean ± SEM and were subjected to the Student *T*-test. A value of $P < 0.05$ was considered to indicate statistical significance between sham and BDL groups.

of the neighboring astroglia close to the dopaminergic neurons in those brain areas since several investigations have shown that co-cultured striatal astrocytes and mesencephalic dopaminergic neurons increased in the number and outgrowth as compared to cultures of mesencephalic neurons alone (Drukarch et al., 1997). Furthermore, some finding have demonstrated an astroglial release of some growth factors such as the Transforming Growth Factor (TGF-β) (Buchanan et al., 2000; Dhandapani et al., 2003) which could play a role in mediating astrocyte-induced neuroprotection, implicated in diverse processes as regulation of growth, differentiation, extracellular matrix formation, and immune regulation as well as induction of neuronal survival and repair following injury (Massague and Blain, 2000; Zhu et al., 2001). In spite of the numerous studies of the glial responses in HE episodes, many of them are controverted depending on the animal model, the brain region, the stage of HE studied. Consequently, information about the clear astroglia responses in each animal model of HE is still not fully understood. The present investigation aims to assess, in some brain areas such as the midbrain SNc, VTA, dorsal striatum, hippocampus and brain cortex, the astroglial responses, via GFAP immunostaining, in an animal model of mild HE using the bile duct ligated rat at the cirrhotic stage (4 weeks after surgery).

2. Material and methods

2.1. Animals

Animals were housed at a constant room temperature (25 °C), with a 12-h dark–light cycle and free access to food to all studied groups. Rats were treated in compliance according to the guidelines of the Cadi Ayyad University, Marrakesh (Morocco). All animals were treated according to the European decree, related to the ethical evaluation and authorization of projects using animals for experimental procedures, 1st February 2013, NOR: AGRG1238767A. Thus, all efforts were made to minimize the number and suffering of the animals used

2.2. Liver injury

Male Sprague Dawley rats were used for all the experiments and were divided into two groups: Group 1: with 6 rats weighted 237.83 ± 17.87 g were anesthetized with inhalational isoflurane then subjected to a midline abdominal incision. Liver injury was induced by obstruction of the common bile duct as described previously (El Hiba et al., 2012). Group 2: sham rats (n = 5, 233.6 ± 22.67) were subjected to a sham operation with only an abdominal incision and gentile manipulation without bile duct ligation. The cirrhotic and their sham-operated control groups were studied 4 weeks after surgery and pair fed to avoid confounds owing to diminished food intake (Jover et al., 2006).

2.3. Liver failure and cirrhosis assessment

Liver and renal failures in BDL rats were assessed via morphometric, histological and biochemical tools. Thus, after sacrifice of animals from the two groups, liver and kidneys were dissected photographed and then weighted. Samples of the organs were post fixed in 4% paraformaldehyde solution, then, dehydration in graded ethanol solution (50%, 70%, 90%, 100%). The tissues are then embedded in Polyethylene glycol (PEG), and sliced in a 10 μm thickness, stained with hematoxylin and eosin (H&E) for identifying histological features of liver cirrhosis and renal glomerular alterations indicating respectively liver and kidney failures. Besides, a biochemical urinalysis was performed to evaluate the levels of urinary urea, bilirubin and urobilinogens which constitute a biomarkers of liver dysfunction (26) (Binder et al., 1989) of the two groups using a Multistix 10SG Bayer reagent strips.

2.4. HE assessment

As previously described, our cirrhotic rats showed features of encephalopathy demonstrated using the open field test which shows a significant loss of locomotor performance following 4 weeks of BDL (Giguère et al., 1992; El Hiba et al., 2012).

2.5. Immunohistochemistry

Four weeks after surgery, the cirrhotic rats and their shams were anesthetised and perfused transcardially with chilled physiological saline and paraformaldehyde (4%) in phosphate buffer (PBS, 0.1 M, pH 7.4). Brains were post-fixed in the same fixative oven night at 4 °C, dehydrated in graded ethanol solutions (50–100%), passed through serial polyethylene glycol solutions (PEG) and embedded in pure PEG. Frontal sections (20 μm) were cut with a microtome, collected and rinsed in PBS to wash out the fixative. Immunohistochemistry with anti-glial fibrillary acidic protein (GFAP: (Dako,Copenhagen, Denmark)) (1/1000) containing PBS (0.1 M, pH 7.4), Triton (0.3%), and serum albumin bovin (BSA; 1%) was performed in slices through midbrain (substantia nigra pars compacta SNc, ventral tegmental area VTA), dorsal stratum, hippocampus and cerebral cortex. The slides are then washed three times with PBS (0.1 M, pH 7.4) containing BSA (1%) for 5 min. The slices are then incubated with the secondary antibody in goat anti-rabbit biotinylated immunoglobulins (1/1000, Dako, Copenhagen, Denmark)) for 2 h at room temperature. After three washes, the slides were incubated for 90 min in PBS buffer containing Triton (0.3%) and the Avidin-biotin peroxidase complex (Kit ABC, Vector; 1/500). diluted 1/5000 and 1/1000 in PBS containing 0.3% Triton X-100 and 1% bovine serum albumin. After three washes in the same buffer, peroxidase activity was revealed by incubating sections in 0.03% DAB (3-3-diaminobenzidine, Sigma, Oakville, Canada) in 0.05 M Tris buffer, pH 7.5, containing 0.01% H2O2. The sections were then collected, dehydrated and mounted in Eukit for optic microscopy observation. The specificity of the immunoreactive materials was tested following the subjection of the slides to same immunohistochemical protocol described above by either using the preimmune serum or omitting of the primary antibodies. These tests showed that both primary antibodies used against GFAP display specific labelling as has been previously published by our group (Sansar et al., 2011).