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Research Report

Effects of short-term portacaval anastomosis on the peripheral and brain disposition of the blood–brain barrier permeability marker sodium fluorescein in rats

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ABSTRACT

Contradictory results have been reported with regard to the effects of various models of hepatic encephalopathy on the blood–brain barrier (BBB) permeability, which may be due partly to the use of brain concentrations of BBB markers without attention to their peripheral pharmacokinetics. The purpose of the current study was to investigate the effects of short-term portacaval anastomosis (PCA), a type B model of hepatic encephalopathy, on the peripheral pharmacokinetics and brain distribution of sodium fluorescein (FL), which is a small molecule marker of BBB passive permeability. A single 25 mg/kg dose of FL was administered intravenously to 10-day PCA and sham-operated rats, and serial blood and bile (0–30 min) and terminal (30 min) brain samples were collected, and the concentrations of FL and its glucuronidated metabolite (FL-Glu) were measured by HPLC. Additionally, the free fractions of FL (f_u) in all the plasma samples were determined, and the effects of bile salts on f_u were investigated *in vitro*. Passive permeability of BBB to FL was estimated by brain uptake clearance (K_{in}) based on both the brain concentrations of FL and plasma concentrations of free (unbound) FL. PCA caused a 26% increase in the f_u of FL in plasma, which was due to competition of bile acids with FL for binding to plasma proteins. Additionally, PCA reduced the biliary excretion of FL-Glu by 55%. However, free K_{in} values (μl/min/g brain) for the sham (0.265±0.034) and PCA (0.228±0.038) rats were not significantly different. It is concluded that whereas 10-day PCA alters the peripheral pharmacokinetics of FL, it does not significantly affect the BBB permeability to the marker.

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1. Introduction

Hepatic encephalopathy (HE) is a neuropsychiatric disorder associated with hepatocellular failure or portal–systemic venous shunting, which results in exposure of the brain to high concentrations of toxins that are otherwise removed by the liver. In addition to significant effects on the brain function, it has been suggested that HE may also increase the blood–brain barrier (BBB) permeability in different models of HE (Cauli et al., 2011; Chen et al., 2013; Dixit and Chang, 1990; Horowitz et al., 1983; Laursen and Westergaard, 1977; Livingstone et al., 1977; Nguyen, 2012; Shimojima et al., 2008; Zaki et al., 1984). However, other studies have shown that HE does not affect the BBB passive permeability (Alexander et al., 2000; Bémeur et al., 2010; Bosoi et al., 2012; Jin et al., 2013). One obvious explanation for these apparently contradictory results is the heterogeneity of the animal models of HE used in these studies. According to the latest definitions (Ferenci et al., 2002), HE is divided into three types based on the extent and type of liver injury that are associated with acute liver failure (type A), portal–systemic bypass (type B), and cirrhosis (type C). Therefore, it is likely that different types of HE have different effects on the BBB permeability.

A second complicating factor in the reported contradictory results may be the methodology to determine the BBB permeability. For example, in some reports (Bosoi et al., 2012; Chen et al., 2013; Shimojima et al., 2008) sodium fluorescein (FL) is used as a small molecule, non-permeable marker to study the passive permeability of the BBB. Generally, these studies use the brain concentration of FL as a measure of BBB integrity. However, HE may also alter the peripheral pharmacokinetics and area under the plasma concentration–time curve (AUC) of FL, thus potentially affecting the brain concentrations of the marker even in the absence of any changes in the BBB passive permeability. Additionally, FL is bound to proteins in the plasma (Li and Rockey, 1982; Manzini and Crescenzi, 1979), and only the free (unbound) fraction of the drug is available for distribution to other organs (Mehvar, 2005), including the brain. Therefore, studies using FL as an *in vivo* marker of BBB permeability in HE should consider the effects of the disease on both the extent of systemic exposure (AUC) and free fraction of the drug, in addition to its brain concentrations.

Rats with portacaval anastomosis (PCA) are considered one of the best models of latent encephalopathy (type B) in humans (Butterworth et al., 2009). Additionally, few studies have reported the effects of PCA in rats on the integrity of the BBB (Alexander et al., 2000; Bosoi et al., 2012; Laursen and Westergaard, 1977; Sumner, 1982). Whereas earlier studies (Laursen and Westergaard, 1977; Sumner, 1982) suggested that the BBB permeability to the macromolecule horseradish peroxidase (HRP) is increased at 10, 14, or 30 days after PCA, more recent studies (Alexander et al., 2000; Bosoi et al., 2012) using mannitol, Evans blue, or FL after 4 or 16 weeks of PCA reported no effects of PCA on the BBB passive permeability. The disagreement among these studies may be related, at least in part, to the time course of the PCA effects on the BBB permeability and/or the size and mechanism of the passage of the marker through the BBB. However, we are not aware of any quantitative studies on the short-term ($\leq$ 14 days) effects of PCA on the BBB permeability of small, non-permeable markers, such as FL. Therefore, the purpose of the current investigation was to study the effects of short-term (10 day) PCA on the BBB passive permeability using a quantitative analysis of the peripheral and brain disposition of FL, including consideration of its free fraction in plasma (f_u). Based on the reported increase in the BBB to HRP at 10–14 days after PCA (Laursen and Westergaard, 1977; Sumner, 1982), we hypothesized that short-term PCA increases the BBB passive permeability to FL.

2. Results

The liver weight (as a fraction of total body weight) and plasma biochemical parameters for the Sham and PCA rats are shown in Fig. 1. PCA caused a 16% decrease ($p<0.05$) in the liver: total body weight ratio (Fig. 1a). Additionally, PCA caused a 40% increase ($p<0.05$) in the plasma concentration of ammonia (Fig. 1b) and an almost 7-fold increase ($p<0.0001$) in the concentrations of total bile acids in plasma (Fig. 1c). Although PCA also modestly (11%) reduced ($p<0.01$) the total plasma protein concentrations (Fig. 1d), the plasma albumin concentration was not affected by the surgery (Fig. 1e). Furthermore, the plasma concentrations of aspartate aminotransferase (AST) in the Sham and PCA animals were not significantly different (Fig. 1f).

The total (free plus bound) and free plasma concentration-time courses of FL, along with f_u values of the marker, are presented in Fig. 2. Additionally, the zero to 30 min AUC values of total (AUC_{total}) and free (AUC_{free}) FL during the sampling time are presented in Table 1. Although the plasma concentrations or AUC values for the total or free FL were not significantly different in the PCA and Sham groups, the AUC_{free}/AUC_{total} ratio in the PCA animals was 26% higher ($p<0.01$) than that in the Sham group (Table 1). This was due to significantly higher f_u of FL in the PCA animals in most of the samples (Fig. 2c), which also resulted in reversal of PCA: Sham AUC ratios when total (ratio of 0.903) and free (ratio of 1.15) AUCs were considered (Table 1).

The biliary excretion data for FL and its glucuronidated metabolite (FL-Glu) in the Sham and PCA rats are depicted in Fig. 3. Although PCA did not significantly affect the biliary recovery (Fig. 3a) or biliary clearance of total (Fig. 3b) or free (Fig. 3c) FL, it significantly ($p<0.01$) reduced the biliary excretion of FL-Glu from 17.6% of the dose to 7.93% of the dose (Fig. 3d). Consequently, the overall (FL plus FL-Glu) biliary recovery in the PCA rats (17.9% of dose) was significantly ($p<0.05$) lower than that in the Sham animals (28.7% of dose) (Fig. 3e). Further, the bile flow rate (ml/h) in the PCA animals (1.09 ± 0.27) was significantly ($p<0.01$) lower than that in the Sham group (1.74 ± 0.27) (Fig. 3f).

The concentration and amount of FL in the liver of Sham and PCA rats are depicted in Fig. 4. Whereas the liver concentrations of FL in the Sham and PCA rats were not significantly different from each other (Fig. 4a), the amount of FL recovered in the liver of PCA rats (5.89 ± 0.93% of dose) was significantly ($p<0.05$) lower than that in the Sham animals (7.74 ± 1.33% of dose) (Fig. 4b), most likely due to the lower