

8-4-2017

Effects of Hepatic Ischemia-Reperfusion Injury on the Blood-Brain Barrier Permeability to [14C] and [13C]Sucrose

Mohammad K. Miah

Texas Tech University Health Sciences Center

Ulrich Bickel

Texas Tech University Health Sciences Center

Reza Mehvar

Chapman University, mehvar@chapman.edu

Follow this and additional works at: https://digitalcommons.chapman.edu/pharmacy_articles

Recommended Citation

Miah MK, Bickel U, Mehvar R. Effects of hepatic ischemia-reperfusion injury on the blood-brain barrier permeability to [14C] and [13C]sucrose. *Metab Brain Dis*. 2017. doi: 10.1007/s11011-017-0069-2

This Article is brought to you for free and open access by the School of Pharmacy at Chapman University Digital Commons. It has been accepted for inclusion in Pharmacy Faculty Articles and Research by an authorized administrator of Chapman University Digital Commons. For more information, please contact laughtin@chapman.edu.

Effects of Hepatic Ischemia-Reperfusion Injury on the Blood-Brain Barrier Permeability to [14C] and [13C]Sucrose

Comments

This article was originally published in *Metabolic Brain Diseases* in 2017. DOI:10.1007/s11011-017-0069-2

[This link](#) will allow users without a subscription to view, but not download, the article.

Copyright

Springer

Effects of hepatic ischemia-reperfusion injury on the blood-brain barrier permeability to [¹⁴C] and [¹³C]sucrose

Mohammad K. Miah^{1,a} . Ulrich Bickel^{1,2} . Reza Mehvar^{1,2,3}

¹ Department of Pharmaceutical Sciences, Texas Tech University Health Sciences Center, Amarillo, Texas, USA

² Center for Blood-Brain Barrier Research, Texas Tech University Health Sciences Center, Amarillo, Texas, USA

³ Department of Biomedical and Pharmaceutical Sciences, School of Pharmacy, Chapman University, Irvine, California, USA

Corresponding authors: Reza Mehvar, Department of Biomedical and Pharmaceutical Sciences, School of Pharmacy, Chapman University, 9401 Jeronimo Road, Irvine, California, USA; phone: 714.516.5490; E-mail: mehvar@chapman.edu. Ulrich Bickel, Department of Pharmaceutical Sciences, School of Pharmacy, Texas Tech University Health Sciences Center, Amarillo, Texas, USA; phone: 806.414.9236; E-mail: ulrich.bickel@ttuhsc.edu.

^a Present address: School of Pharmacy, University of Pittsburgh, Pennsylvania, USA

Abstract Hepatic encephalopathy that is associated with severe liver failure may compromise the blood-brain barrier (BBB) integrity. However, the effects of less severe liver diseases, in the absence of overt encephalopathy, on the BBB are not well understood. The goal of the current study was to investigate the effects of hepatic ischemia-reperfusion (IR) injury on the BBB tight junction permeability to small, hydrophilic molecules using the widely used [^{14}C]sucrose and recently-proposed alternative [^{13}C]sucrose as markers. Rats were subjected to 20 min of hepatic ischemia or sham surgery, followed by 8 h of reperfusion before administration of a single bolus dose of [^{14}C] or [^{13}C]sucrose and collection of serial (0-30 min) blood and plasma and terminal brain samples. The concentrations of [^{14}C] and [^{13}C]sucrose in the samples were determined by measurement of total radioactivity (nonspecific) and LC-MS/MS (specific), respectively. IR injury significantly increased the blood, plasma, and brain concentrations of both [^{14}C] and [^{13}C]sucrose. However, when the brain concentrations were corrected for their respective area under the blood concentration-time curve, only [^{14}C]sucrose showed significantly higher (30%) BBB permeability values in the IR animals. Because [^{13}C]sucrose is a more specific BBB permeability marker, these data indicate that our animal model of hepatic IR injury does not affect the BBB tight junction permeability to small, hydrophilic molecules. Methodological differences among studies of the effects of liver diseases on the BBB permeability may confound the conclusions of such studies.

Keywords Blood-brain barrier permeability . [^{14}C]Sucrose . [^{13}C]Sucrose . Apparent brain uptake clearance . Hepatic ischemia-reperfusion injury

Introduction

Acute liver failure is a serious, life-threatening condition that may result in hepatic encephalopathy, coma, and death (Bernal and Wendon 2013). Although somewhat debatable (Butterworth 2013), several investigators have reported an increased BBB permeability in hepatic encephalopathy and coma (Butterworth 2016; Cauli et al. 2011; Chastre et al. 2013; Chen et al. 2009; Chen et al. 2013; McMillin et al. 2015). However, the effects of less severe hepatic diseases in the absence of overt encephalopathy or coma, such as hepatic ischemia-reperfusion (IR) injury, on the BBB permeability have not been well understood.

Our recent studies (Miah et al. 2014) demonstrated that hepatic IR injury increased the expression of P-glycoprotein (P-gp) at the BBB, resulting in a decrease in the apparent in vivo brain uptake clearance of the P-gp marker rhodamine 123. Additionally, an in vivo study using sodium fluorescein (FL) (Miah et al. 2015), as a BBB tight junction permeability marker to small hydrophilic molecules, indicated that IR significantly increased the brain concentrations of the marker. However, the brain concentrations corrected for the free drug concentration in plasma was similar in both groups, suggesting no change in the permeability through the BBB tight junctions as a result of IR. Because FL might also pass the BBB through some transporters (Hawkins et al. 2007; Huai-Yun et al. 1998; Loscher and Potschka 2005), the true effects of hepatic IR on the integrity of BBB tight junctions remain to be determined by using a marker that is not subject to BBB transporters and is devoid of confounding factors such as binding to plasma proteins and hepatic metabolism.

Radiolabeled [^{14}C]sucrose is a small (342 Da), hydrophilic molecule with a very low permeability across the intact BBB, which has no known binding to blood or plasma proteins and no significant systemic metabolism. Further, no significant facilitative or active transport of

sucrose in vivo has been demonstrated in mammals to date. Therefore, it has been one of the most widely used small molecules for quantitative determination of blood-brain barrier (BBB) integrity in many in vitro (Behrens et al. 2015; Kochi et al. 1999; Oppenheim et al. 2013; Paulson et al. 2006), in situ brain perfusion (Bickel et al. 1996; Cannon et al. 2012; Hawkins et al. 2010; Huber et al. 2002; Lochhead et al. 2012; Ronaldson et al. 2009), and in vivo (Bickel et al. 1998; Chavarria et al. 2010; Ferguson and Woodbury 1969; Jin et al. 2013; Lo et al. 1987; Ohno et al. 1978; Preston et al. 1984; Preston and Haas 1986; Preston and Webster 2002; Smith et al. 1988; Stolp et al. 2005; Yin et al. 2008; Ziylan et al. 1983; Ziylan et al. 1984; Ziylan et al. 1988) studies. Very recently (Miah et al. 2017), we demonstrated that the apparent brain uptake clearance (K_{in}) of [^{14}C]sucrose in normal rats, estimated by quantitation of total radioactivity in the brain, was around 6-7 fold higher than that of stable isotope labeled [^{13}C]sucrose, which was quantitated using a specific LC-MS/MS method. Upon chromatographic fractionation of the brain samples of rats injected in vivo with [^{14}C]sucrose, several peaks, in addition to the intact sucrose, were identified. Although the possibility of a minor systemic metabolism could not be ruled out, the higher K_{in} values for [^{14}C]sucrose were attributed, at least in part, to presence of small lipophilic impurities in the stock solution of [^{14}C]sucrose, which could easily penetrate BBB. Therefore, it was suggested that specific quantitation of the stable isotope (^{13}C) of sucrose is a more accurate alternative to the current widespread use of the radioactive sucrose as a BBB permeability marker.

The main objective of the current study was to investigate the effects of hepatic IR on the BBB permeability to sucrose using the specific [^{13}C]sucrose marker. Although [^{14}C]sucrose substantially overestimates the absolute permeability of BBB to sucrose, it may still be a valid marker in studies of the effects of a disease state on the BBB permeability when its brain uptake

clearance is compared in healthy and diseased conditions. Therefore, a secondary objective of the study was to test whether the use of [^{14}C]sucrose would affect the conclusion of such studies.

Materials and methods

Chemicals and reagents

We purchased [UL- $^{13}\text{C}_{12}$]sucrose (all the carbons in both glucose and fructose molecules are labeled with ^{13}C isotope; denoted [^{13}C]sucrose) and the internal standard (IS), which was [UL- $^{13}\text{C}_6^{\text{fru}}$]sucrose (all the carbons in the fructose molecule are labeled with ^{13}C isotope), from Omicron Biochemicals (South Bend, IN, USA). [^{14}C]Sucrose, Solvable, and Hionic-Fluor solution were purchased from PerkinElmer (Boston, Massachusetts, USA). Specific activity of the [^{14}C]sucrose stock solution was 400-700 mCi/mmol. ^3H -Labelled rat serum albumin (RSA) was prepared and chromatographically purified as described before (Bickel et al. 1998). The precipitability by trichloroacetic acid of the purified ^3H -RSA peak was greater than 98%. LC-MS grade water (J.T. Baker) was purchased from Avantor Performance Materials, Inc. (Center Valley, PA, USA). Analytical grade ammonium hydroxide and LC-MS grade acetonitrile (ACN) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). For anesthesia, ketamine and xylazine solutions were purchased from Lloyd Laboratories (Shenandoah, IA, USA). Heparin solution was purchased from APP Pharmaceuticals (Schaumburg, IL, USA). All other chemicals were analytical grade and obtained from commercial sources.

Animals

Adult, male Sprague-Dawley rats were purchased from Charles River laboratory (Wilmington, MA) and acclimated in single, ventilated cages with 12-h dark-light cycles in a temperature- and humidity-controlled room with free access to the food and water. All the animal procedures used in this study were approved by Texas Tech University Health Sciences Center's Institutional

Animal Care and Use Committee and were consistent with the guidelines set by the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011). Animals were randomly assigned to two experimental groups of [^{14}C] and [^{13}C]sucrose, which were further divided into two groups of sham surgery (Sham) and IR, resulting in a total of 4 groups ($n = 5-7/\text{group}$). The average \pm SD of body weights (g) of animals were 306 ± 12 (Sham) and 298 ± 14 (IR) for the [^{13}C]sucrose groups and 287 ± 3 (Sham) and 276 ± 13 (IR) for the [^{14}C]sucrose groups.

Hepatic ischemia-reperfusion (IR) injury model

To determine whether the use of [^{13}C]sucrose or [^{14}C]sucrose would affect the outcome of BBB permeability studies in a disease model, the BBB permeability to [^{13}C] and [^{14}C]sucrose was determined in different groups of rats subjected to hepatic IR or sham surgery (Sham), as described before (Miah et al. 2015). Briefly, after an overnight fast, rats were anesthetized with ketamine: xylazine (80:8 mg/kg) via intramuscular injection. For the IR group, after opening the abdomen, the blood supply to the entire liver was occluded by placing a microvascular clamp on the portal triad. Complete obstruction of blood flow to the liver was confirmed by visual inspection of the liver color. After 20 min of total hepatic ischemia (Pringle maneuver), the liver was reperfused with blood by removing the clamp. Finally, 5 ml of normal saline (37°C) was added to the abdominal cavity to compensate for the loss of fluids during the surgery, and the muscle and skin layers were closed. During the entire procedure, the rat body temperature was maintained at 37°C by using a heating lamp and a heating plate attached to a rectal temperature control probe. Sham animals underwent only laparotomy under identical experimental conditions but without any interruption of the blood supply to the liver. Rats were allowed to recover for 8 h before dosing with the markers.

Dosing and sampling

After 8 h of reperfusion, rats were anesthetized with ketamine: xylazine at a dose of 80:4 mg/kg. Subsequently, a catheter was placed into the femoral artery for the collection of blood samples. For the [¹³C]sucrose study, a single 10-mg/kg dose of the marker was administered as a bolus dose (via penile vein). For the [¹⁴C]sucrose study, the bolus dosing solution contained 3 μCi (~6.5 μg/kg) of the marker plus 10 μCi of ³H-RSA, as the vascular marker. Our recent report (Miah et al. 2017) indicated that the dose-corrected plasma/blood disposition of both markers were similar despite significant differences between the absolute doses of the two markers. Serial blood samples (~0.2 ml) were then collected for 30 min, a portion was centrifuged for collection of plasma and another portion was used for measurement of blood concentrations. Each time after collection of blood samples, the catheter was filled with heparin (10 U/ml) to prevent blood clotting. To remove the residual blood from the brains of animals injected with [¹³C]sucrose, at the end of sampling a catheter was placed in the left ventricle, and the whole body was perfused with ice-cold saline at a rate of 25 ml/min for 5 min. Finally, brain was collected from both groups and snap frozen in cold iso-pentane. All samples were kept at -80°C until analysis.

LC-MS/MS analysis of [¹³C]sucrose

Before analysis of [¹³C]sucrose by LC-MS/MS, plasma and blood samples were diluted 100- and 50-fold, respectively, and brain samples were homogenized in water (1:9). The LC-MS/MS method is described in a recent publication (Miah et al. 2016). In brief, brain homogenates and diluted plasma and blood samples (20 μl) were subjected to protein precipitation by the addition of 180 μl of acetonitrile: water (80:20), which also contained IS. Chromatographic separation was then performed using an Acquity BEH amide column (50 mm x 3 mm, 1.7 μm) and an isocratic mobile phase of acetonitrile: water: ammonium hydroxide (72:28:0.1, v/v), run a flow

rate 0.2 ml/min. Column temperature was maintained at 45°C, and autosampler was at room temperature. The retention time of sucrose was ~2.5 min, and the total run time was 6 min. The MRM was monitored in negative mode, and the transitions for [¹³C]sucrose and IS were 353→92 m/z and 347→89 m/z, respectively.

Liquid scintillation analysis of [¹⁴C]sucrose and [³H]-RSA

For [¹⁴C] and [³H] analysis, one hemisphere of the brain was cut into small pieces, and 0.2-0.3 g of the brain tissue was added to 2 ml of Solvable to digest the tissue. Additionally, 20 µl of blood and plasma samples were added to 2 ml of solvable. After 24 h at room temperature, 100 µl of hydrogen peroxide was added to each sample to remove any possible color quenching, 12 ml of scintillation fluid was added, and the samples were subjected to the scintillation counter.

Analysis of AST and ALT

Plasma AST and ALT levels were measured to confirm the IR-induced hepatic damage by a kit from TECO diagnostics (Anaheim, CA), according to the manufacturer's protocol.

Pharmacokinetic analysis

Apparent brain uptake clearance (K_{in}) values were estimated after a 30-min sampling period for both plasma ($K_{in-plasma}$) and blood ($K_{in-blood}$) AUC data (Gjedde 1981):

$$K_{in-blood} = \frac{C_{30}^{br}}{AUC_{0-30}^{blood}} \quad (1)$$

$$K_{in-plasma} = \frac{C_{30}^{br}}{AUC_{0-30}^{plasma}} \quad (2)$$

where C_{30}^{br} , AUC_{0-30}^{blood} , and AUC_{0-30}^{plasma} are the amount of the marker in the terminal (30 min) brain sample (in units of mass/g of brain) and the areas under the blood or plasma concentration-time curve from time zero to the last sampling time (30 min), respectively. The AUC_{0-30}^{blood} and AUC_{0-30}^{plasma} were estimated by non-compartmental analysis of the concentration-time data during

the sampling period (5-30 min) using the log-linear trapezoidal method in Phoenix® WinNonlin® software (Certara, Princeton, NJ). The method back extrapolates the concentration at time zero for AUC calculations (AUC_{0-30}). For [^{13}C]sucrose, C_{30}^{br} was the brain concentration of the marker after the in situ removal of the residual blood in the brain. For [^{14}C]sucrose, C_{30}^{br} was the brain concentration of the marker after correction for the residual contents of the radioactivity in the brain vasculature estimated using the following equations:

$$C_{30}^{br} = C_{30}^{br+vasc} - V_{blood} \times C_{30}^{blood} \quad (3)$$

$$C_{30}^{br} = C_{30}^{br+vasc} - V_{plasma} \times C_{30}^{plasma} \quad (4)$$

where $C_{30}^{br+vasc}$, $V_{blood/plasma}$, and $C_{30}^{blood/plasma}$ are the total (brain tissue plus vasculature) brain amount of [^{14}C]sucrose per g of tissue, volume of blood or plasma within the brain vasculature, and blood or plasma concentrations of [^{14}C]sucrose at 30 min, respectively. V_{blood} and V_{plasma} were estimated from the ^3H -RSA data:

$$V_{blood} = \frac{C_{30}^{br,RSA}}{C_{30}^{blood,RSA}} \quad (7)$$

$$V_{plasma} = \frac{C_{30}^{br,RSA}}{C_{30}^{plasma,RSA}} \quad (8)$$

where $C_{30}^{br,RSA}$, $C_{30}^{blood,RSA}$, and $C_{30}^{plasma,RSA}$ are the terminal brain, blood, and plasma concentrations of ^3H -RSA. Similar to the sucrose data, the AUC_{0-30}^{blood} and AUC_{0-30}^{plasma} values for RSA were also estimated using the log-linear trapezoidal method in Phoenix® WinNonlin® software.

To allow comparison of data between the [^{13}C] and [^{14}C]sucrose, the mass of the markers in the blood and brain were corrected for the injected dose and expressed as percentage of injected dose (%ID).

Statistical analysis

Comparison of two means for the IR and Sham groups was carried out by an unpaired, two-tailed Student's t-test. For ALT and AST measurements, two-way ANOVA, followed by Bonferroni post-hoc analysis was used. A p value of < 0.05 was considered significant. Data are presented as mean \pm SD or individual values.

Results

Plasma concentrations of the hepatic IR injury markers are presented in Fig. 1. As demonstrated in this figure, hepatic IR injury significantly increased the plasma levels of ALT (Fig. 1a) and AST (Fig. 1b) in the IR animals, as compared with their respective Sham groups. Comparison of ALT or AST values across the two sucrose marker groups showed no significant differences between the two marker groups for AST (Fig. 1b). However, the ALT levels in the [^{14}C]sucrose-IR group were significantly ($p < 0.01$) lower than those for the [^{13}C]sucrose-IR group (Fig. 1a).

The plasma and blood concentration-time courses of the vascular marker ^3H -labeled rat serum albumin (^3H -RSA) in the Sham and IR groups are presented in Fig. 2, and the corresponding zero to 30 min AUC (AUC_{0-30}) values and vascular volumes are presented in Table 1. IR caused a significant increase in the blood and plasma concentrations (Fig. 2) and AUC_{0-30} values (Table 1) of ^3H -RSA. However, there were no changes in the blood or plasma vasculature volume of the brain because of the IR injury (Table 1). On average, the brain plasma: blood volume ratios were 0.54 and 0.51 (Table 1) for the Sham and IR groups, suggesting hematocrit values of 0.46 and 0.49, respectively.

The plasma and blood concentrations-time profiles of [^{14}C] and [^{13}C]sucrose in the IR and Sham animals are depicted in Figure 3, and their corresponding AUC_{0-30} values are presented in Figure 4. The plasma and blood concentrations of [^{13}C]sucrose (Figs. 3a and 3b) and

[¹⁴C]sucrose (Figs. 3c and 3d) followed similar patterns, suggestive of multicompartment disposition. Additionally, for both markers, the plasma concentrations (Figs. 3a and 3c) were higher than the corresponding concentrations in blood (Figs. 3b and 3d), which is in agreement with the lack of penetration of sucrose to the red blood cells. Further, the blood and plasma concentrations (Fig. 3) and AUC₀₋₃₀ values (Fig. 4) of both markers in the IR animals were significantly higher than those in the Sham animals.

The terminal brain concentrations of [¹³C] and [¹⁴C]sucrose in the IR and Sham animals are presented in Fig. 5. Although the brain concentrations of both markers were higher in the IR animals, only for [¹⁴C]sucrose did the difference between the Sham and IR groups reach statistical significance ($p = 0.0026$, Fig. 5b); the p value for the difference between the Sham and IR groups for the brain concentrations of [¹³C]sucrose was 0.0502. Apparent brain uptake clearance (K_{in}) of [¹³C] (Figs. 6a and 6b) and [¹⁴C]sucrose (Figs. 6c and 6d), based on the plasma (Figs. 6a and 6c) or blood (Figs. 6b and 6d) AUC₀₋₃₀ values, are presented in Fig. 6. There were no significant differences in the plasma or blood K_{in} values between the Sham and IR groups for [¹³C]sucrose (Figs. 6a and 6b). However, blood K_{in} values for [¹⁴C]sucrose were significantly ($p = 0.0163$) higher in the IR animals, compared with the Sham group (Fig. 6d).

Discussion

In this study, we examined the effects of hepatic IR injury on the permeability of BBB tight junctions to sucrose using an animal model of Pringle maneuver (total hepatic ischemia) that is used clinically during liver surgery for trauma, resection of cancerous tissue, or liver transplantation. A significant increase in the plasma concentrations of the liver injury markers, ALT and AST, in both [¹³C] and [¹⁴C]sucrose groups (Fig. 1) confirmed the IR injury.

IR injury increased the plasma and blood concentrations and AUC_{0-30} values of the vascular marker RSA (Fig. 2 and Table 1) and those of both $[^{13}\text{C}]$ and $[^{14}\text{C}]$ sucrose (Figs. 3 and 4). The higher blood or plasma concentrations of $[^{13}\text{C}]$ and $[^{14}\text{C}]$ sucrose in the IR animals were also reflected in higher brain concentrations for both markers (Fig. 5), although the IR-induced increase in the brain concentrations of $[^{13}\text{C}]$ sucrose barely missed the significance level ($p = 0.0502$). When the terminal brain concentrations of the markers were corrected for their corresponding blood or plasma AUC_{0-30} values, the $K_{in-blood}$ values were significantly increased by 30% only in the case of $[^{14}\text{C}]$ sucrose (Fig. 6). Therefore, whereas the $[^{13}\text{C}]$ sucrose data do not suggest any changes in the BBB permeability to sucrose as a result of hepatic IR, the opposite is true based on the $K_{in-blood}$ values of $[^{14}\text{C}]$ sucrose (Fig. 6).

Accurate measurement of brain concentrations of BBB markers with low permeability requires removal of the blood content of brain by perfusion or the use of a vascular marker to eliminate the contribution of brain blood to the total brain concentrations of the marker. Consequently, $[^{14}\text{C}]$ sucrose is frequently used along with radioactive albumin as a vascular marker, a method that was also used in our studies. For the $[^{13}\text{C}]$ sucrose, we chose to remove the brain blood by perfusion before collection of brain, in order to avoid the use of radioactive vascular markers. It may be argued that the differences between the two methods might be due to residual blood after the brain perfusion in the $[^{13}\text{C}]$ sucrose group. However, any residual blood in the brain of rats injected with $[^{13}\text{C}]$ sucrose is expected to cause an increase in the K_{in} value of $[^{13}\text{C}]$ sucrose, compared with that in the $[^{14}\text{C}]$ sucrose group, which is opposite of our observations (Fig. 6).

The injection of RSA in the $[^{14}\text{C}]$ sucrose group allowed us to estimate the volumes of brain plasma and blood, which were 8.99 and 16.6 $\mu\text{l/g}$, respectively, in the Sham animals (Table

1), predicting a cerebral hematocrit of 0.46 in this group. These values are in agreement with the cerebral blood volume of 25.1 $\mu\text{l/g}$, measured by the summation of red blood cell volume ($^{99\text{m}}\text{Tc}$ -labelled red blood cells) and plasma volume ($[^{14}\text{C}]\text{dextran}$), and a hematocrit of 0.43 in control rats (Todd et al. 1992). The estimated hematocrit in the brain blood (0.46) of our Sham animals is also close to the estimated hematocrit in the systemic blood (0.47) in the same group, with the latter obtained from the RSA blood and plasma AUC_{0-30} values (Table 1). The V_{blood} and V_{plasma} values for the IR animals were not significantly different from those in the Sham animals (Table 1), suggesting that our IR model does not affect the cerebral blood or plasma volumes.

The lack of a significant effect of a 20-min Pringle maneuver plus 8 h of reperfusion on the $[^{13}\text{C}]\text{sucrose } K_{in}$ (Figs. 6a and 6b) is in agreement with our recent study using sodium fluorescein (FL) as a BBB permeability marker in the same disease model (Miah et al. 2015). Similar to sucrose, FL is a small, hydrophilic, low MW (374 Da) marker, which minimally crosses the intact BBB. However, the use of FL is subject to some inherent confounding problems, including disease state-induced potential changes in the protein binding of the marker. For example, when the K_{in} value of FL was estimated based on Eq. 2 using the total (free plus unbound) AUC_{0-30} of FL, hepatic IR significantly increased the marker's K_{in} . However, correcting for the free fraction of the marker in the plasma, the K_{in} values in the Sham and IR rats were similar (Miah et al. 2015). In addition to the problem of binding to plasma proteins, some investigators have suggested that FL might pass the BBB through some transporters (Hawkins et al. 2007; Huai-Yun et al. 1998; Loscher and Potschka 2005). Therefore, $[^{14}\text{C}]\text{sucrose}$, because of its lack of binding to plasma proteins and lack of any known transporters for its passage through the mammalian BBB, has so far been considered a more advantageous low MW marker for the BBB permeability. However, our current study indicates that the use of $[^{14}\text{C}]\text{sucrose}$ might also

result in inaccurate conclusions regarding the changes in the BBB permeability in pathological conditions.

Previous studies and our own recent investigation (Miah et al. 2017) suggest presence of minor (a few percentages) lipophilic impurities in the stock solutions of [^{14}C]sucrose. Although these impurities do not significantly affect the plasma or blood AUC of the total radioactivity, they can account for a significant portion of the total radioactivity count in the brain. This is because these lipophilic impurities can penetrate BBB to a much greater magnitude, compared with the almost impenetrable intact [^{14}C]sucrose. Consequently, the K_{in} values of [^{14}C]sucrose in control animals were, on average, ~ 7 fold higher than that of [^{13}C]sucrose, which was quantitated by a specific LC-MS/MS method that only measures the intact marker (Miah et al. 2017). Our current observation that the K_{in} values were significantly increased in the IR animals only in the case of [^{14}C]sucrose suggests that the IR injury differentially affects the pharmacokinetics of the impurities in the [^{14}C]sucrose and the intact [^{14}C]sucrose itself. For example, if IR injury increases the AUC of the impurities more than the AUC of the intact [^{14}C]sucrose, the more than proportionate increase in the AUC of the impurities, relative to that of the intact [^{14}C]sucrose, significantly increases the brain concentration of the total radioactivity without a significant effect on the total AUC itself, resulting in a higher K_{in} value for [^{14}C]sucrose.

The increase in the plasma and blood concentrations (Fig. 3) and AUC_{0-30} values (Fig. 4) of both [^{13}C] and [^{14}C]sucrose in IR animals suggest a decrease in the clearance of sucrose as a result of IR injury. Sucrose is almost completely eliminated by excretion of the unchanged drug in the urine through glomerular filtration (Winkler and Parra 1937). Because hepatic IR injury is known to decrease the renal function (Behrends et al. 2008; Lee et al. 2009; Lee et al. 2011), the

apparent decrease in the clearance of sucrose in the IR animals is most likely due to a reduced renal function in these animals.

Conclusions

Hepatic IR injury increased the plasma, blood, and brain concentrations of BBB permeability markers [^{13}C] and [^{14}C]sucrose in rats. However, the apparent brain uptake clearance, which is estimated from the brain concentrations corrected for blood AUC, was increased significantly after IR only in the case of [^{14}C]sucrose. Therefore, although [^{14}C]sucrose data indicated that hepatic IR injury significantly increases the BBB permeability, this conclusion was not supported by the [^{13}C]sucrose data. Because [^{13}C]sucrose is a more specific BBB permeability marker (Miah et al. 2016; Miah et al. 2017), these data indicate that our model of hepatic IR injury does not affect the BBB tight junction permeability to small, hydrophilic molecules. Further, nonspecific measurement of [^{14}C]sucrose by total radioactivity may result in an erroneous conclusion regarding disease state-induced alterations in the BBB permeability to small molecules.

Acknowledgements The Authors would like to thank Dr. Raktima Bhattacharya from Texas Tech University School of Pharmacy for assistance with the radiolabeled studies.

Author contributions MKM, UB, and RM designed the experiments, analyzed the data and wrote the manuscript. MKM performed the experiments.

Compliance with ethical standards

Conflict of interest The authors declare that they do not have any conflict of interest.

Grant support Financial support for this study was provided by the Center for Blood–Brain Barrier Research at Texas Tech School of Pharmacy.

References

- Behrends M, Hirose R, Park YH, Tan V, Dang K, Xu F, Park SH, Niemann CU (2008). Remote renal injury following partial hepatic ischemia/reperfusion injury in rats. *J Gastrointest Surg* 12: 490-495.
- Behrens M, Huwel S, Galla HJ, Humpf HU (2015). Blood-brain barrier effects of the Fusarium mycotoxins deoxynivalenol, 3 acetyldeoxynivalenol, and moniliformin and their transfer to the brain. *PLoS One* 10: e0143640.
- Bernal W, Wendon J (2013). Acute liver failure. *N Engl J Med* 369: 2525-2534.
- Bickel U, Schumacher OP, Kang YS, Voigt K (1996). Poor permeability of morphine 3-glucuronide and morphine 6-glucuronide through the blood-brain barrier in the rat. *J Pharmacol Exp Ther* 278: 107-113.
- Bickel U, Grave B, Kang YS, del Rey A, Voigt K (1998). No increase in blood-brain barrier permeability after intraperitoneal injection of endotoxin in the rat. *J Neuroimmunol* 85: 131-136.
- Butterworth RF (2013). The liver-brain axis in liver failure: neuroinflammation and encephalopathy. *Nat Rev Gastroenterol Hepatol* 10: 522-528.
- Butterworth RF (2016). The concept of "the inflamed brain" in acute liver failure: mechanisms and new therapeutic opportunities. *Metab Brain Dis* 31: 1283-1287.
- Cannon RE, Peart JC, Hawkins BT, Campos CR, Miller DS (2012). Targeting blood-brain barrier sphingolipid signaling reduces basal P-glycoprotein activity and improves drug delivery to the brain. *Proc Natl Acad Sci U S A* 109: 15930-15935.

- Cauli O, Lopez-Larrubia P, Rodrigo R, Agusti A, Boix J, Nieto-Charques L, Cerdan S, Felipo V (2011). Brain region-selective mechanisms contribute to the progression of cerebral alterations in acute liver failure in rats. *Gastroenterology* 140: 638-645.
- Chastre A, Belanger M, Nguyen BN, Butterworth RF (2013). Lipopolysaccharide precipitates hepatic encephalopathy and increases blood-brain barrier permeability in mice with acute liver failure. *Liver Int* 34: 353-361.
- Chavarria L, Oria M, Romero-Gimenez J, Alonso J, Lope-Piedrafita S, Cordoba J (2010). Diffusion tensor imaging supports the cytotoxic origin of brain edema in a rat model of acute liver failure. *Gastroenterology* 138: 1566-1573.
- Chen F, Ohashi N, Li W, Eckman C, Nguyen JH (2009). Disruptions of occludin and claudin-5 in brain endothelial cells in vitro and in brains of mice with acute liver failure. *Hepatology* 50: 1914-1923.
- Chen F, Radisky ES, Das P, Batra J, Hata T, Hori T, Baine AM, Gardner L, Yue MY, Bu G, Del Zoppo G, Patel TC, Nguyen JH (2013). TIMP-1 attenuates blood-brain barrier permeability in mice with acute liver failure. *J Cereb Blood Flow Metab* 33: 1041-1049.
- Ferguson RK, Woodbury DM (1969). Penetration of ¹⁴C-inulin and ¹⁴C-sucrose into brain, cerebrospinal fluid, and skeletal muscle of developing rats. *Exp Brain Res* 7: 181-194.
- Gjedde A (1981). High- and low-affinity transport of D-glucose from blood to brain. *J Neurochem* 36: 1463-1471.
- Hawkins BT, Ocheltree SM, Norwood KM, Egleton RD (2007). Decreased blood-brain barrier permeability to fluorescein in streptozotocin-treated rats. *Neurosci Lett* 411: 1-5.

Hawkins BT, Sykes DB, Miller DS (2010). Rapid, reversible modulation of blood-brain barrier P-glycoprotein transport activity by vascular endothelial growth factor. *J Neurosci* 30: 1417-1425.

Huai-Yun H, Secrest DT, Mark KS, Carney D, Brandquist C, Elmquist WF, Miller DW (1998). Expression of multidrug resistance-associated protein (MRP) in brain microvessel endothelial cells. *Biochem Biophys Res Commun* 243: 816-820.

Huber JD, Hau VS, Borg L, Campos CR, Egleton RD, Davis TP (2002). Blood-brain barrier tight junctions are altered during a 72-h exposure to lambda-carrageenan-induced inflammatory pain. *Am J Physiol Heart Circ Physiol* 283: H1531-1537.

Jin L, Nation RL, Li J, Nicolazzo JA (2013). Species-dependent blood-brain barrier disruption of lipopolysaccharide: amelioration by colistin in vitro and in vivo. *Antimicrob Agents Chemother* 57: 4336-4342.

Kochi S, Takanaga H, Matsuo H, Naito M, Tsuruo T, Sawada Y (1999). Effect of cyclosporin A or tacrolimus on the function of blood-brain barrier cells. *Eur J Pharmacol* 372: 287-295.

Lee HT, Park SW, Kim M, D'Agati VD (2009). Acute kidney injury after hepatic ischemia and reperfusion injury in mice. *Lab Invest* 89: 196-208.

Lee SY, Kim DH, Sung SA, Kim MG, Cho WY, Kim HK, Jo SK (2011). Sphingosine-1-phosphate reduces hepatic ischaemia/reperfusion-induced acute kidney injury through attenuation of endothelial injury in mice. *Nephrology (Carlton)* 16: 163-173.

Lo WD, Ennis SR, Goldstein GW, McNeely DL, Betz AL (1987). The effects of galactosamine-induced hepatic failure upon blood-brain barrier permeability. *Hepatology* 7: 452-456.

Lochhead JJ, McCaffrey G, Sanchez-Covarrubias L, Finch JD, Demarco KM, Quigley CE, Davis TP, Ronaldson PT (2012). Tempol modulates changes in xenobiotic permeability and

- occludin oligomeric assemblies at the blood-brain barrier during inflammatory pain. *Am J Physiol Heart Circ Physiol* 302: H582-593.
- Loscher W, Potschka H (2005). Blood-brain barrier active efflux transporters: ATP-binding cassette gene family. *NeuroRx* 2: 86-98.
- McMillin MA, Frampton GA, Seiwel AP, Patel NS, Jacobs AN, DeMorrow S (2015). TGFbeta1 exacerbates blood-brain barrier permeability in a mouse model of hepatic encephalopathy via upregulation of MMP9 and downregulation of claudin-5. *Lab Invest* 95: 903-913.
- Miah MK, Shaik IH, Bickel U, Mehvar R (2014). Effects of hepatic ischemia-reperfusion injury on the P-glycoprotein activity at the liver canalicular membrane and blood-brain barrier determined by in vivo administration of rhodamine 123 in rats. *Pharm Res* 31: 861-873.
- Miah MK, Shaik IH, Bickel U, Mehvar R (2015). Effects of Pringle maneuver and partial hepatectomy on the pharmacokinetics and blood-brain barrier permeability of sodium fluorescein in rats. *Brain Res* 1618: 249-260.
- Miah MK, Bickel U, Mehvar R (2016). Development and validation of a sensitive UPLC-MS/MS method for the quantitation of [¹³C]sucrose in rat plasma, blood, and brain: Its application to the measurement of blood-brain barrier permeability. *J Chromatogr B* 1015: 105-110.
- Miah MK, Chowdhury EA, Bickel U, Mehvar R (2017). Evaluation of [¹⁴C] and [¹³C]sucrose as blood-brain barrier permeability markers. *J Pharm Sci* doi: 10.1016/j.xphs.2017.1002.1011.
- Ohno K, Pettigrew KD, Rapoport SI (1978). Lower limits of cerebrovascular permeability to nonelectrolytes in the conscious rat. *Am J Physiol* 235: H299-H307.
- Oppenheim HA, Lucero J, Guyot AC, Herbert LM, McDonald JD, Mabondzo A, Lund AK (2013). Exposure to vehicle emissions results in altered blood brain barrier permeability and

expression of matrix metalloproteinases and tight junction proteins in mice. *Part Fibre Toxicol* 10: 62.

Paulson JR, Roder KE, McAfee G, Allen DD, Van der Schyf CJ, Abbruscato TJ (2006). Tobacco smoke chemicals attenuate brain-to-blood potassium transport mediated by the Na,K,2Cl-cotransporter during hypoxia-reoxygenation. *J Pharmacol Exp Ther* 316: 248-254.

Preston E, Haas N, Allen M (1984). Reduced permeation of ¹⁴C-sucrose, ³H-mannitol and ³H-inulin across blood-brain barrier in nephrectomized rats. *Brain Res Bull* 12: 133-136.

Preston E, Haas N (1986). Defining the lower limits of blood-brain barrier permeability: factors affecting the magnitude and interpretation of permeability-area products. *J Neurosci Res* 16: 709-719.

Preston E, Webster J (2002). Differential passage of [¹⁴C]sucrose and [³H]inulin across rat blood-brain barrier after cerebral ischemia. *Acta Neuropathol* 103: 237-242.

Ronaldson PT, Demarco KM, Sanchez-Covarrubias L, Solinsky CM, Davis TP (2009). Transforming growth factor-beta signaling alters substrate permeability and tight junction protein expression at the blood-brain barrier during inflammatory pain. *J Cereb Blood Flow Metab* 29: 1084-1098.

Smith QR, Ziylan YZ, Robinson PJ, Rapoport SI (1988). Kinetics and distribution volumes for tracers of different sizes in the brain plasma space. *Brain Res* 462: 1-9.

Stolp HB, Dziegielewska KM, Ek CJ, Potter AM, Saunders NR (2005). Long-term changes in blood-brain barrier permeability and white matter following prolonged systemic inflammation in early development in the rat. *Eur J Neurosci* 22: 2805-2816.

- Todd MM, Weeks JB, Warner DS (1992). Cerebral blood flow, blood volume, and brain tissue hematocrit during isovolemic hemodilution with hetastarch in rats. *Am J Physiol* 263: H75-82.
- Winkler AW, Parra J (1937). The Measurement of Glomerular Filtration. Creatinine, Sucrose and Urea Clearances in Subjects without Renal Disease. *J Clin Invest* 16: 859-867.
- Yin D, Wang X, Konda BM, Ong JM, Hu J, Sacapano MR, Ko MK, Espinoza AJ, Irvin DK, Shu Y, Black KL (2008). Increase in brain tumor permeability in glioma-bearing rats with nitric oxide donors. *Clin Cancer Res* 14: 4002-4009.
- Ziylan YZ, Robinson PJ, Rapoport SI (1983). Differential blood-brain barrier permeabilities to [¹⁴C]sucrose and [³H]inulin after osmotic opening in the rat. *Exp Neurol* 79: 845-857.
- Ziylan YZ, Robinson PJ, Rapoport SI (1984). Blood-brain barrier permeability to sucrose and dextran after osmotic opening. *Am J Physiol* 247: R634-638.
- Ziylan YZ, LeFauconnier JM, Bernard G, Bourre JM (1988). Effect of dexamethasone on transport of alpha-aminoisobutyric acid and sucrose across the blood-brain barrier. *J Neurochem* 51: 1338-1342.

Figure Legends

Fig. 1 Plasma concentrations of ALT (a) and AST (b) in [^{13}C] and [^{14}C]sucrose groups. Different groups of animals ($n = 5-7/\text{group}$) were subjected to 20 min of Pringle maneuver (total hepatic IR) or sham surgery, and plasma concentrations of ALT and AST were measured 8 h after reperfusion. Columns and bars represent mean and SD values, respectively. Statistical significance (p value) is based on two-way ANOVA, followed by Bonferroni post-hoc analysis.

Fig. 2 Plasma and blood concentration-time courses of [^3H]-RSA in the IR and Sham animals. Different groups of animals ($n = 5/\text{group}$) were subjected to 20 min of Pringle maneuver (total hepatic IR) or sham surgery, followed by 8 h of reperfusion. A single intravenous dose of [^3H]-RSA (10 μCi) was administered, and serial blood and plasma samples were obtained. Symbols and bars represent mean and SD values, respectively.

Fig. 3 Plasma (a and c) and blood (b and d) concentration-time courses of [^{13}C] (a and b) and [^{14}C] (c and d) sucrose in the IR and Sham animals. Different groups of animals ($n = 5-7/\text{group}$) were subjected to 20 min of Pringle maneuver (total hepatic IR) or sham surgery, followed by 8 h of reperfusion. A single intravenous dose of [^{13}C]sucrose (10 mg/kg) or [^{14}C]sucrose (3 μCi) was administered, and serial blood and plasma samples were obtained. Symbols and bars represent mean and SD values, respectively.

Fig. 4 Plasma (a and c) and blood (b and d) AUC_{0-30} of [^{13}C] (a and b) and [^{14}C] (c and d) sucrose in the IR and Sham animals. Different groups of animals ($n = 5-7/\text{group}$) were subjected to 20 min of Pringle maneuver (total hepatic IR) or sham surgery, followed by 8 h of reperfusion. A single intravenous dose of [^{13}C]sucrose (10 mg/kg) or [^{14}C]sucrose (3 μCi) was administered, and serial blood and plasma samples were obtained. Symbols and vertical lines represent

individual and mean values, respectively. Statistical significance (p value) is based on unpaired, two-tailed Student's t -test.

Fig. 5 Terminal brain concentrations of [^{13}C] (a) and [^{14}C] (b) sucrose in the IR and Sham animals. Different groups of animals ($n = 5-7/\text{group}$) were subjected to 20 min of Pringle maneuver (total hepatic IR) or sham surgery, followed by 8 h of reperfusion. A single intravenous dose of [^{13}C]sucrose (10 mg/kg) or [^{14}C]sucrose (3 μCi) was administered, and brain was collected at 30 min. Symbols and vertical lines represent individual and mean values, respectively. Statistical significance (p value) is based on unpaired, two-tailed Student's t -test.

Fig. 6 Plasma (a and c) and blood (b and d) K_{in} values of [^{13}C] (a and b) and [^{14}C] (c and d) sucrose in the IR and Sham animals. Different groups of animals ($n = 5- 7/\text{group}$) were subjected to 20 min of Pringle maneuver (total hepatic IR) or sham surgery, followed by 8 h of reperfusion. A single intravenous dose of [^{13}C]sucrose (10 mg/kg) or [^{14}C]sucrose (3 μCi) was administered, and serial blood and plasma samples and terminal brain samples were obtained. Symbols and vertical lines represent individual and mean values, respectively. Statistical significance (p value) is based on unpaired, two-tailed Student's t -test.

Table 1 Plasma and blood AUC₀₋₃₀ values and brain volume of distribution (V) values (mean ± SD) of ³H-RSA in Sham and IR rats. Animals in each group (*n* = 5/group) received a single intravenous dose of ³H-RSA (10 μCi), and serial blood samples were taken over 30 min.

Parameter	Plasma		Blood	
	Sham	IR	Sham	IR
AUC ₀₋₃₀ , %ID.min/ml	307 ± 32	410 ± 57**	164 ± 19	198 ± 16*
V, μl/g	8.99 ± 1.03	8.40 ± 1.10	16.6 ± 1.1	16.6 ± 3.1

* *p* < 0.05, ** *p* < 0.01: Significantly different from the Sham group based on unpaired, two-tailed Student's t-test.

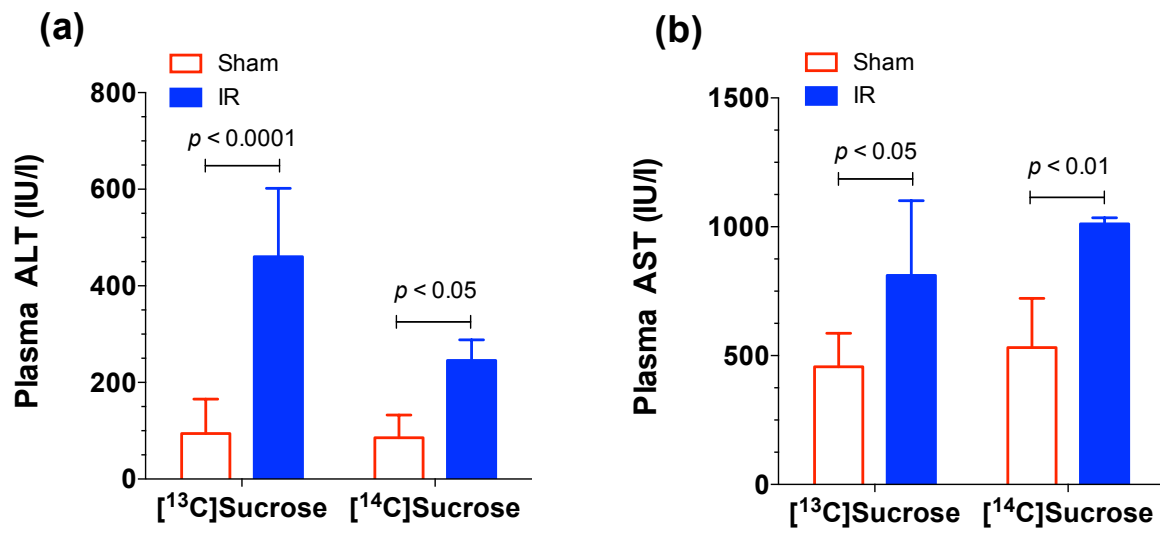


Figure 1

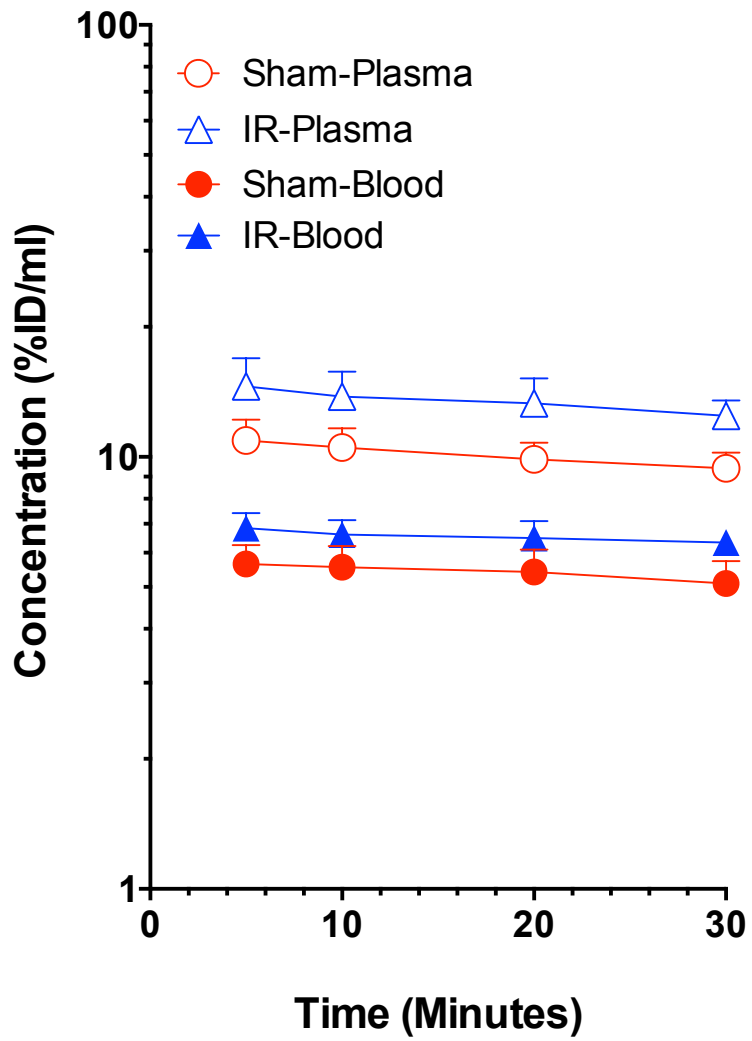


Figure 2

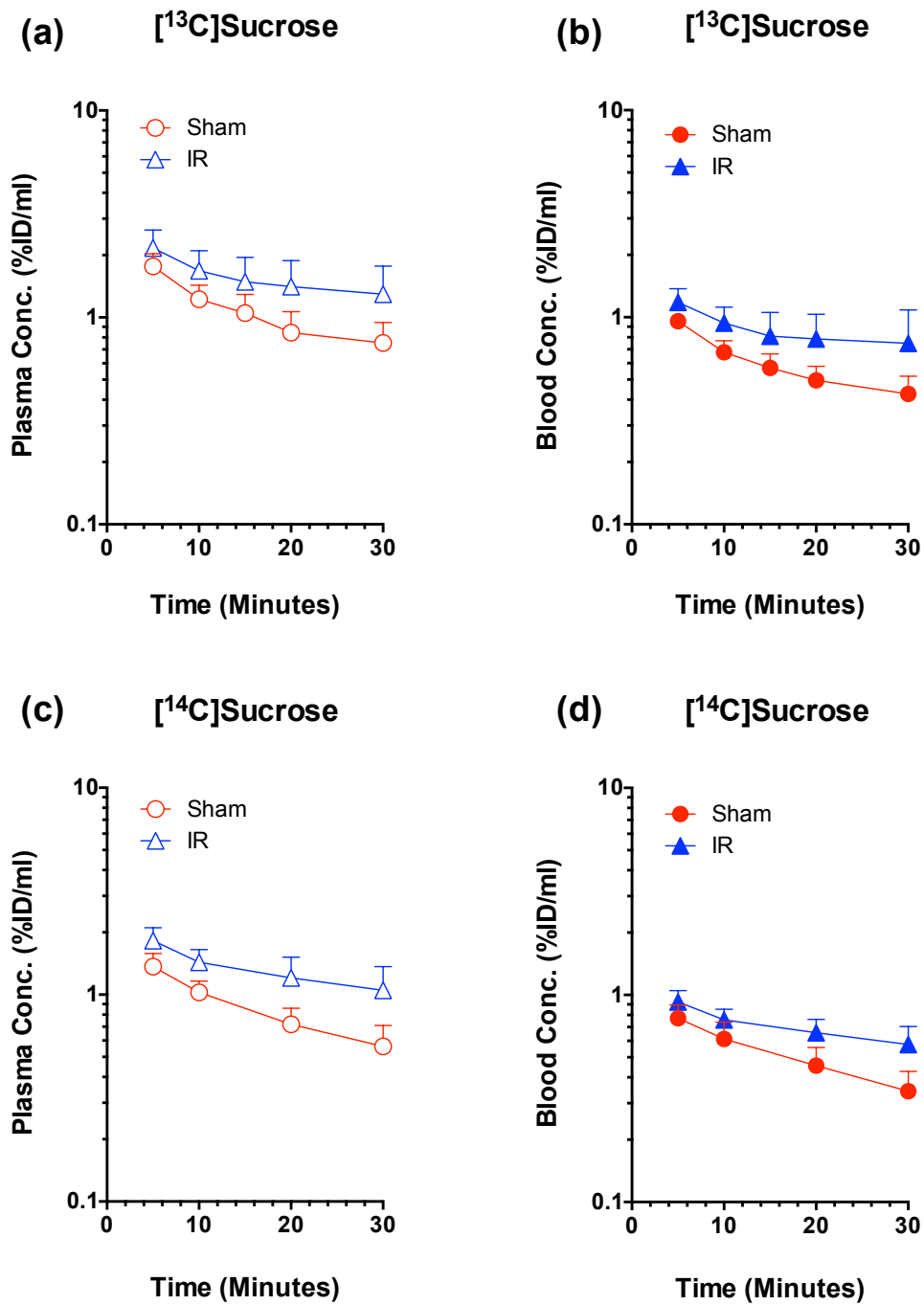


Figure 3

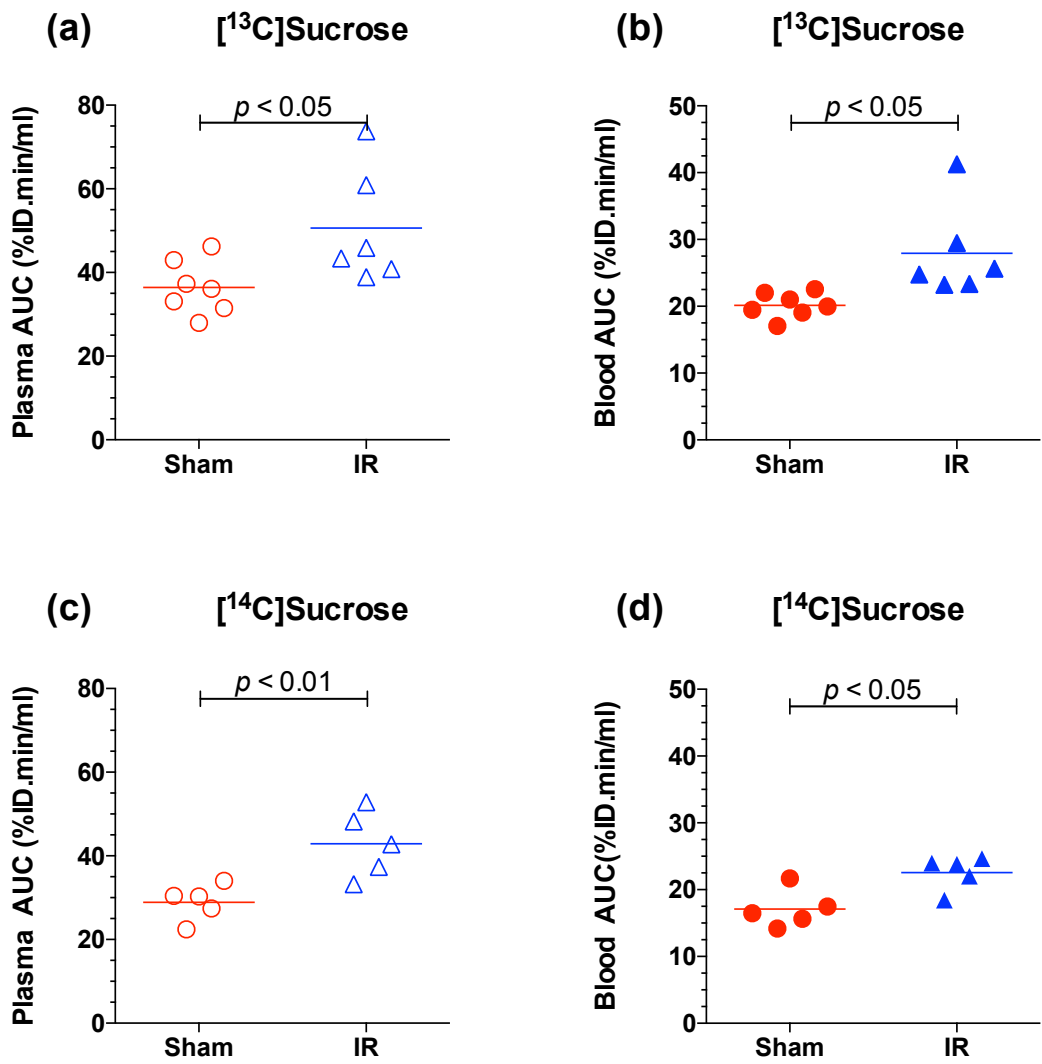


Figure 4

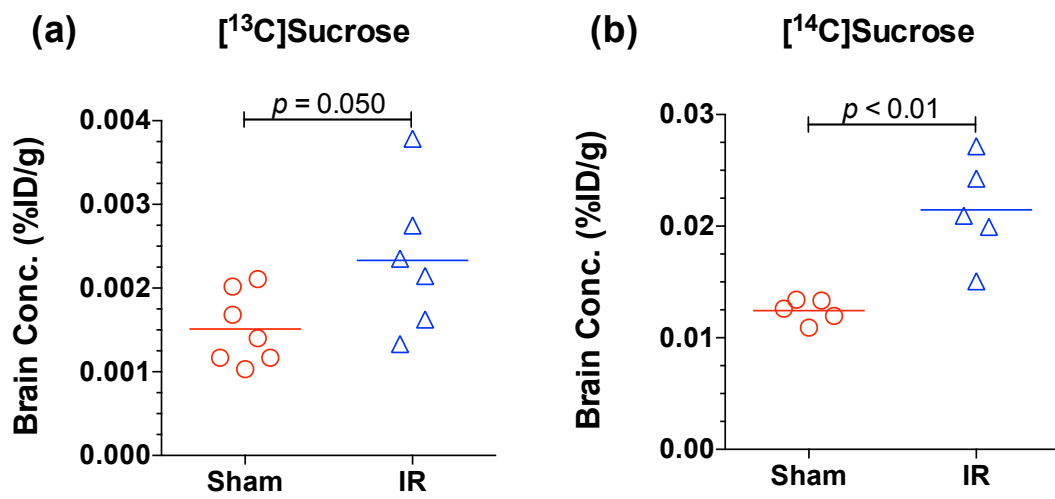


Figure 5

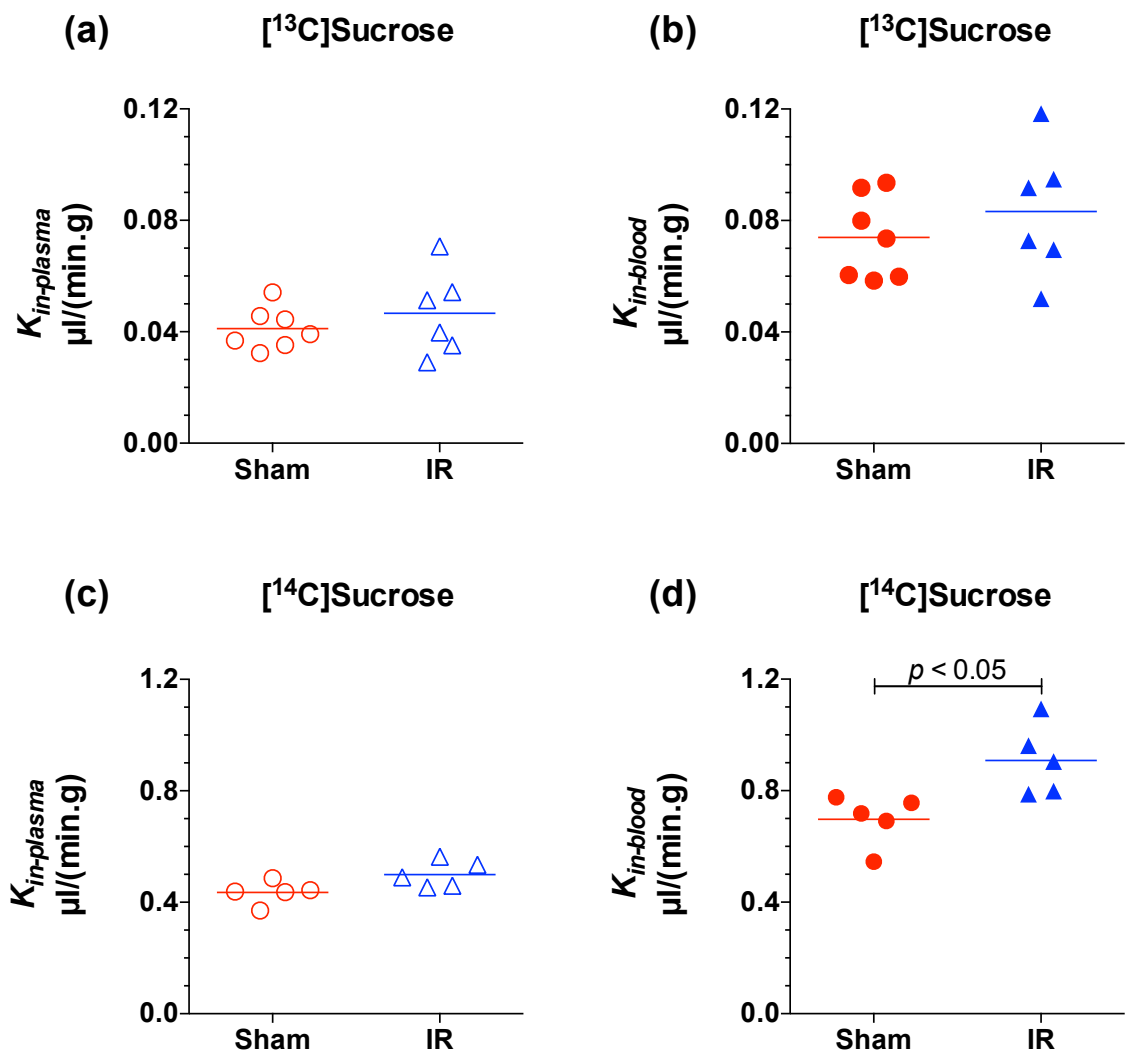


Figure 6