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Blood Brain Barrier is Impermeable to Solutes and Permeable to Water after Experimental Pediatric Cardiac Arrest

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Abstract

Pediatric asphyxial cardiac arrest (CA) results in unfavorable neurological outcome in most survivors. Development of neuroprotective therapies is contingent upon understanding the permeability of intravenously delivered medications through the blood brain barrier (BBB). In a model of pediatric CA we sought to characterize BBB permeability to small and large molecular weight substances. Additionally, we measured the percent brain water after CA.

Asphyxia of 9 min was induced in 16-18 day-old rats. The rats were resuscitated and the BBB permeability to small (sodium fluorescein and gadoteridol) and large (immunoglobulin G, IgG) molecules was assessed at 1, 4, and 24 h after asphyxial CA or sham surgery. Percent brain water was measured post-CA and in shams using wet-to-dry brain weight.

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Conflict of Interest: none

Fluorescence, gadoteridol uptake, or IgG staining at 1, 4 h and over the entire 24 h post-CA did not differ from shams, suggesting absence of BBB permeability to these solutes. Cerebral water content was increased at 3 h post-CA vs. sham.

In conclusion, after 9 min of asphyxial CA there is no BBB permeability over 24 h to conventional small or large molecule tracers despite the fact that cerebral water content is increased early post-CA indicating the development of brain edema. Evaluation of novel therapies targeting neuronal death after pediatric CA should include their capacity to cross the BBB.

Keywords

cardiac arrest; asphyxia; rat; blood brain barrier; permeability

Introduction

Cardiac arrest (CA) is secondary to asphyxia in most pediatric patients [1]. Despite the significant morbidity and mortality associated with pediatric CA, there are few therapeutic options to improve neurologic outcomes. Development of successful neuroprotective therapies for pediatric CA is likely to be contingent upon understanding the permeability of intravenously delivered medications through the blood brain barrier (BBB). Successful treatment of pediatric CA victims may also be contingent upon assessment and treatment of cerebral edema [2], which can occur in the presence or absence of BBB permeability to solutes and medications [3].

The BBB is a complex anatomic and physiological barrier between the vasculature and central nervous system. Increased BBB permeability to solutes and water secondary to BBB disruption, although a pathologic process, has the theoretical advantage of allowing intravenously delivered neuroprotective agents to reach their target in the nervous system. BBB permeability has been shown to be critical to the efficacy of pharmacological therapies after focal cerebral ischemia [4, 5]. Increased BBB permeability to solutes after global cerebral ischemic insults such as CA is more variable compared with focal ischemia and may depend on myriad factors such as insult type, insult duration, temperature, species, model, and age [6-9]. BBB permeability to solutes after traumatic brain injury (TBI) and stroke is accompanied by permeability to water, which leads to development of cerebral edema and its deleterious consequences [10, 11]. Pathologic passage of water through the BBB can also occur in the absence of permeability to solutes and can lead to cerebral edema and worse neurological outcomes [3]. BBB permeability to small or large molecular weight substances or water after pediatric asphyxial CA is thus an important aspect of post-resuscitation therapy and warrants characterization.

Our pediatric asphyxial CA model in postnatal day 17 rats produces selective cortical, thalamic, hippocampal and cerebellar neuronal death along with neurological impairment. This model is being used to study potential neuroprotective strategies for possible clinical translation. Our previous work suggested that the BBB was not permeable to gadoteridol at 3 h after CA [12]. BBB permeability is dependent on many factors including molecular weight, polarity of the tracer used, protein binding, and timing of the insult [13]. Given the

importance of knowing the state of the BBB in our model, we sought to investigate the permeability of BBB to small and large molecular weight tracers at three time points during the initial 24 h after CA. We also sought to assess the change in percent brain water after CA in our model.

Materials and Methods

We used postnatal day 16-18 mixed gender Sprague-Dawley rats (30-45 g). We assessed the permeability of the BBB to conventional small and large molecular tracers at three time points during the initial 24 h, namely immediate (1 h), early (4 h), and delayed (24 h) times after asphyxial CA, and the percent brain water at 3 h after CA. Sham operated rats served as negative controls and rats subjected to traumatic brain injury (TBI) served as positive controls.

To assess the BBB permeability to conventional tracers during the first 24 h after CA we performed the following experiments (Fig. 1):

1. Assessment of BBB permeability to a small molecule (sodium fluorescein, MW=367) at immediate (1 h) and early (4 h) time points after CA via spectrofluorophotometry (n=12, 3/group).
2. Assessment of BBB permeability to a small molecule (gadoteridol, MW=550) at a delayed (24 h) time point after CA via magnetic resonance imaging (MRI) (n=11, 5-6/group).
3. Assessment of BBB permeability to a large molecule (IgG, MW=150,000) during the first 24 h after CA via immunohistochemistry (assessing immediate, early and delayed time points) (n=3/group).

To assess the change in percent brain water at 3 h and 24 h after CA we determined brain wet-to-dry weight ratio in shams and after CA (n=17, 5-6/group).

Asphyxial Insult

We used an established model of asphyxial CA developed at our center [14]. Rats were anesthetized with 3% isoflurane/50% N₂O/balance oxygen until unconscious and then the trachea was intubated and mechanical ventilation was initiated. Central femoral arterial and venous catheters were inserted via inguinal cut-down. Anesthesia was maintained with 1% isoflurane/50% N₂O/O₂. A bolus of vecuronium (1 mg/kg, iv) was administered two minutes prior to asphyxia to prevent respirations. Asphyxia was produced by disconnecting the tracheal tube from the ventilator for 9 min. Resuscitation was started by reconnecting the ventilator. Epinephrine 0.005 mg/kg and sodium bicarbonate 1 mEq/kg were administered iv, followed by manual chest compressions until return of spontaneous circulation. Sham rats underwent identical procedures, without CA or medications. The rats were sacrificed at 1, 3-4, or 24 h as detailed below.

Traumatic Brain Injury

Rats subjected to TBI served as positive controls for BBB disruption. An established TBI protocol using the controlled cortical impact model was used [15, 16]. The rats were

subjected to a pneumatically driven 3-mm metal impactor tip at a velocity of 6 m/s and a depth of penetration of 1.2 mm.

Assessment of BBB Permeability to Sodium Fluorescein

Permeability of BBB to sodium fluorescein was evaluated at 1 and 4 h after CA, sham (negative control) or TBI (positive control) (n=18, n=3/group at each time point). Sodium fluorescein (5 ml/kg, 2%) was injected at resuscitation or 3 h post injury or sham surgery and was allowed to circulate for 60 min. At 60 min after the infusion of sodium fluorescein, the rats were perfused with 60 ml of heparinized saline. Brains were removed and frozen in liquid nitrogen. Cerebral hemispheres were then thawed on ice, weighed, and homogenized in 2.5 ml of phosphate-buffered saline. Trichloroacetic acid (2.5 ml, 60%) was added and the specimens were ultrasonicated. Fluorescence was measured by spectrophotofluorometry at 440 nm emission and 516 nm excitation wavelengths [17].

Assessment of BBB Permeability to Gadoteridol

Permeability of BBB to gadoteridol was evaluated at 24 h following CA (n=6) and sham surgery (n=5) using magnetic resonance imaging (MRI). T1-weighted images were obtained immediately before and 30 min after infusion of gadoteridol (0.2 mg/kg, iv), using a 7 Tesla Bruker Biospec AVIII (MSME sequence with the following parameters: TR/TE=600/12 ms, 256x256 matrix, FOV=3x3 cm). BBB permeability was determined by subtracting pre- and post-infusion image intensities for the hemispheric, hippocampal, thalamic and cortical regions [18].

Assessment of BBB Permeability to IgG

Permeability of BBB to the large molecular weight endogenous IgG was evaluated during the first 24 h after CA (n=3/group). IgG is a large molecular weight protein found in plasma, unable to penetrate through the intact BBB. If any time after an insult the BBB becomes permeable, IgG accumulates in the brain parenchyma and can be detected by immunofluorescence. Positive immunofluorescent staining for IgG in brain sections of rats sacrificed at 24 h would indicate BBB permeability at any time during the first 24 h after injury. At 24 h after CA, sham surgery, or TBI, rats were perfused with normal saline and the brains were isolated. For IgG staining, coronal brain sections were obtained, washed with PBS, and then blocked in 3% donkey serum. Goat-Anti-Rat-IgGFITC was added to the tissue in a final dilution of 1:250 in 0.5% BSA. Nuclei were stained with Vectashield with 4', 6-diamidino-2-phenylindole (DAPI).

Assessment of Percent Brain Water

We assessed the change in percent brain water after CA using the brain wet-to-dry weight ratio at 3 and 24 h after CA or sham surgery (n=5-6/group). Cerebral wet weight was measured immediately with a precision scale. Cerebral dry weight was determined after the brain was dried to a constant weight in a 100°C oven. Global hemispheric tissue water content was calculated according to the formula: Percent water = (1-dry weight/wet weight) x 100 [19].

Statistical Analysis

Data were analyzed with the SPSS version 17 software and expressed as mean $\pm$ SEM. We used one-way ANOVA testing with Bonferroni post-hoc test for comparing BBB permeability to sodium fluorescein between CA, sham and TBI rats. We used the Student's *t* test or the Mann-Whitney U test for comparing BBB permeability to gadoteridol and the percent brain water in CA and sham rats. *P*<0.05 was considered significant.

Results

Assessment of BBB Permeability to Sodium Fluorescein

We first measured the global hemispheric BBB permeability to the small molecular weight tracer sodium fluorescein at 1 and 4 h after CA. At 1 h after CA sodium fluorescein fluorescence was similar to shams (575 ± 71 IU/g and 542 ± 16 IU/g CA and shams, respectively, *p*=1.0) and markedly lower than positive control rats subjected to TBI (2130 ± 74 , *p*=0.001 vs. CA). At 4 h after CA, sodium fluorescein fluorescence was similar to shams (405 ± 44 IU/g and 525 ± 82 IU/g, CA and shams, respectively, *p*=1.0) and lower compared with TBI (980 ± 38 IU/g, *p*=0.001 vs. CA). These results indicate that the BBB is not permeable to the small molecular weight tracer sodium fluorescein at 1 or 4 h after CA.

Assessment of BBB Permeability to Gadoteridol

Next, we measured global hemispheric and regional BBB permeability to gadoteridol, another tracer of small molecular weight and established MRI contrast agent for assessment of BBB. In a previous study we showed that BBB was not permeable to gadoteridol at 3 h after CA. In this study we assessed BBB permeability to gadoteridol at 24 h after CA. Mean values of T1 weighted signal intensities were similar in sham and post-CA in the hemispheric areas (3.7 ± 0.9 vs. 2.5 ± 0.7 , respectively, *p*=0.36) and regionally in the hippocampus, cortex and thalamus (4.8 ± 1.7 vs. 4.4 ± 2.2 , 4.4 ± 1.1 vs. 2.2 ± 0.9 , 3.9 ± 1.2 vs. 4.4 ± 2.8 , *p*=0.88, 0.14, and 0.91, respectively). These results indicate that BBB is not permeable to gadoteridol at 24 h after CA.

Assessment of BBB Permeability to IgG

We next measured BBB permeability to a large molecular weight endogenous tracer, IgG, during the first 24 h after CA via immunohistochemistry. IgG was not detected in brain tissues obtained at 24 h after CA, indicating that the BBB remains impermeable to this large molecule for the first 24 h following resuscitation (data not shown).

Assessment of Percent Brain Water

The percent of brain tissue water at 3 h after CA was greater than sham (83.667 ± 0.108 vs. 83.100 ± 0.136 %, *p*=0.02, Fig. 2). At 24 h after CA the percent of brain tissue water, although showing a trend of increase, was not different than shams (83.9 ± 0.417 vs. 83.1 ± 0.136 %, *p*=0.09, Fig. 2).

Discussion

To our knowledge, this is the first study to assess the permeability of the BBB after experimental pediatric asphyxial CA in rats, which models CA in a young child rather than adult or newborn. We demonstrated that the BBB is not permeable to conventional small or large molecular weight tracers during the first 24 h after asphyxial CA in immature rats. Despite BBB impermeability to these tracers, cerebral water content was increased early after CA.

Relevant to improving neurological outcome after pediatric CA is intravenous administration of neuroprotective agents with the goal of delivering these medications across the BBB to neurons, microglia and astrocytes. For example, minocycline, a microglial activation modulator with excellent penetration through the intact BBB was found to be beneficial in attenuating neuronal death after pediatric CA [20]. Our study suggests that neuroprotective medications given in the first 24 h after CA in our model are effective if they have the ability to either freely cross the intact BBB or ought to be coupled with compounds that are transported through the BBB or possibly that act via modulating endothelial signaling [21].

BBB permeability to small or large molecular tracers was previously assessed after ventricular fibrillation (VF) CA, as well as in models of hypoxia and ischemia. Permeability of BBB after VF CA appears to be insult duration-, age- and species-specific. Similar to our study, the BBB was not permeable to small or large tracers either immediately or at 4 h after VF CA in adult dogs [22, 23]. After prolonged periods of hypoxemia without CA, the BBB was not permeable to mannitol, sodium, or antipyrine in newborn piglets [24]. The BBB also lacked permeability to albumin after prolonged CA in adult rats resuscitated with cardiopulmonary bypass [25]. In contrast to our study, BBB permeability to the small molecule α -aminoisobutyric acid (AIB, MW=103) was biphasic after VF CA in immature piglets; the BBB was impermeable immediately after CA and then permeable at 4 h [26, 27]. Of note, AIB is slightly smaller than the tracers used in our current study (MW 103 for AIB vs. 367 and 550 for sodium fluorescein or gadoteridol, respectively). Focal or global ischemia in models of carotid ligation [5] or vascular compression [6-9], in contrast to our model, often demonstrate permeability of BBB. However, developmental differences in the structure and function of the BBB could contribute to age-related differences in permeability in response to ischemia. For example, in a neonatal model, BBB was largely intact even after focal ischemia, and post-ischemic endothelial gene expression differed compared to the adult brain [28].

There is evidence that post resuscitative hypertension contributes to BBB permeability. After VF CA, Caceres et al. demonstrated the presence of endothelial cell defects such as vacuolation, plasma membrane blebs and discontinuities as well as perivascular astrocyte swelling. These defects were also seen in non-ischemic hypertensive controls suggesting that hypertension might play a role in endothelial cell damage [29]. After focal ischemia in cats, while in most subjects BBB showed the characteristic biphasic permeability, the BBB was impermeable in subjects lacking post-reperfusion reactive hyperemia [30]. Dogs subjected to VF CA lacked severe and prolonged hypertensive episodes, which was

postulated by the authors as being the reason for the absence of BBB permeability [22]. In our model, post-resuscitation MAP reaches a maximum of 25% above baseline [12], whereas after VF CA in piglets the maximum MAP reported was 180% above baseline [27]. The absence of detectible global or regional BBB permeability in our asphyxial CA model may thus be due to either lack of intense post resuscitative hypertension vs. VF CA or to using a relatively shorter insult duration. Nevertheless, this insult duration is sufficient to produce considerable neuronal death [31].

Brain tissue water content was increased in our model at 3 h after CA. In light of normal or slightly decreased hemispheric CBF at 3 h after CA in our model [12], the increased cerebral water content is likely not the result of an increase in cerebral blood volume. This finding suggests that water crosses through the intact BBB. Permeability of water through the BBB in the absence of BBB disruption was previously demonstrated [3] and could be accomplished via aquaporins (AQP), water-selective transport proteins and key players for water transport through the BBB. AQP 4 was found to be elevated in adult rats with post-CA cerebral edema [32]. As AQP have increased expression during brain development [33, 34], our findings may suggest that AQP are implicated in BBB selective permeability to water in the absence of permeability to the tracers studied. At 24 h after CA, although brain water content had a trend toward increase, it may not have reached statistical significance due to higher variability—although a larger sample size is needed to specifically test that hypothesis.

While the BBB was not permeable to various tracers in the first 24 h after CA, it is important to remark we did not demonstrate that BBB components remain morphologically normal after CA. Selective damage to the endothelial cell, pericytes or astrocytes may be present, however, not to an extent that permits extravasation of small and large molecular tracers. In light of absence of BBB permeability to the tracers tested, the increase in cerebral water after CA likely represents intracellular or interstitial edema and could also reflect impaired ion homeostasis secondary to energy failure [35]. It was previously shown that hypoxemia alone did not result in permeability of BBB [36], while endothelial cell cultures exposed to hypoxia had a decrease in antioxidant enzymes, rearrangement of F-actin filaments, and decrease in ATP [37]. Likewise, we cannot exclude that an electron microscopic evaluation of BBB would have shown changes at the level of the endothelial cells or astrocytes.

In conclusion, the BBB is not permeable to small and large molecular weight substances in an pediatric asphyxial CA model with considerable selective neuronal death in the hippocampus, cerebellum, striatum and layer V cortex, CBF disturbances (cortical hypoperfusion and thalamic hyperemia), and post-resuscitation increase in brain water content. These results suggest that the development of neuroprotective therapies for asphyxial CA may need to focus on pharmacologic agents that are permeable, can be transported across the BBB, or act via endothelial signaling. Additionally, clinical assessment of BBB permeability in pediatric CA is needed to determine if this important aspect of pathobiology in our model is clinically translatable.

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Abbreviations

CA	cardiac arrest
TBI	traumatic brain injury
BBB	blood brain barrier
MRI	magnetic resonance imaging
MAP	mean arterial pressure
AQP	aquaporin

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- We assessed blood brain barrier permeability in a pediatric model of cardiac arrest
- We evaluated the percent brain water in a pediatric model of cardiac arrest
- Blood brain barrier was impermeable to small or large molecular weight substances
- Cerebral water content was increased at 3 hours post-resuscitation
- Evaluation of neuroprotective therapies in pediatric CA should include their ability to cross BBB

Fig. 1.

Schematic representation of our methods. The shaded areas represent the time window for BBB assessment for each tracer. Permeability to sodium fluorescein was assessed at 1 and 4 h after CA. Permeability to gadoteridol was assessed at 24 h after CA. Permeability to IgG was assessed during the first 24 h after CA.

Fig. 2.

Assessment of percent brain water after CA. * $p < 0.05$ vs. *sham*.