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Effects of Deep Hypothermic Circulatory Arrest on the Blood Brain Barrier in a Cardiopulmonary Bypass Model – A Pilot Study

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Abstract

Background—Neurologic injury is common after cardiac surgery and disruption of the blood brain barrier (BBB) has been proposed as a contributing factor. We sought to study BBB characteristics in a rodent model of cardiopulmonary bypass (CPB) and deep hypothermic circulatory arrest (DHCA).

Methods—Adult rats were subjected to CPB/DHCA or to sham surgery. Analysis included Western blotting of relevant BBB proteins in addition to in vivo brain magnetic resonance imaging (MRI) using a clinically used low-molecular contrast agent.

Results—While quantitative analysis of BBB proteins revealed similar expression levels, MRI showed evidence of BBB disruption after CPB/DHCA compared to sham surgery.

Conclusions—Combining molecular BBB analysis and MRI technology in a rodent model is a highly translatable approach to study adverse neurologic outcomes following CPB/DHCA.

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Keywords

Blood brain barrier; Deep hypothermic circulatory arrest; Cardiopulmonary bypass; Cardiac surgery; Neurologic injury

Introduction

Neurologic injury after cardiac surgery is common and the mechanisms leading to injury are often poorly understood. Disruption of the BBB has been proposed as a key-contributing factor.[1-3]

The BBB forms a complex interface that regulates transfer of ions, neurotransmitters, macromolecules, nutrients, and neurotoxins into and out of the central nervous system.[4-6] BBB function can be altered during pathologic states, specifically in the setting of inflammation and hypoxia, such as occurs during cardiac surgery. Previous work characterising BBB permeability after CPB/DHCA has mostly relied on detection of extravasation of large macromolecular proteins, such as albumin. Using this approach some studies have found no evidence of BBB disruption,[7] while others have detected it after only 30 minutes of CPB.[8]

Here, we performed a pilot study to investigate our hypothesis that DHCA will cause alterations in BBB function through: First, altered expression of BBB tight junction proteins Claudin-5 and Occludin, and the BBB efflux transporter P-glycoprotein. Second, mechanical disruption of BBB integrity as assessed by brain MRI using the low-molecular weight marker gadobutrol that is available also for use in humans. Moreover, its smaller molecular mass (gadobutrol 605; albumin 67,000) is likely to permit detection of much smaller disruptions in barrier integrity than commonly used albumin-based techniques.

Materials and Methods

The Institutional Animal Care & Use Committee approved all animal experiments, which conformed to the National Institutes of Health guide for the care and use of laboratory animals.[9] Fasting adult male Sprague-Dawley rats (10-12 weeks old) were randomly assigned to undergo CPB/DHCA or sham surgery as previously described.[10] Briefly, animals were anaesthetised with inhaled isoflurane 2-2.5%, intubated and mechanically ventilated. Cannulas were placed in the tail artery and the right external jugular vein. Animals were then cooled on CPB for 30 minutes, and DHCA was instituted at a pericranial temperature of 16-18°C. Following 60 minutes of DHCA, CPB was reinitiated, animals were rewarmed for 30 minutes, and separated from CPB at a temperature 35.5°C. Sham operated animals were anaesthetised, cannulated and heparinised, but did not undergo CPB/DHCA.

In-vivo rat brain MRI: On day 1 after the CPB/DHCA experiment animals were re-anaesthetised with 1-2% isoflurane, and MRI was performed using a 7.0 T Bruker Biospec horizontal bore scanner. Images were collected using a send/receive volume coil and

processed using Paravision 4.0 software. Intravenous gadobutrol (0.1mg/kg; Gadovist®, Bayer, Inc., Leverkusen, Germany) was used as contrast agent.

Rat brain capillary preparation: Animals were sacrificed and brain tissue collected for isolation of brain capillaries as described previously.[11] Purified rat brain capillary proteins were quantitatively analysed by Western blot using the following antibodies: #C219 (Covance, Inc., Princeton, New Jersey) against P-glycoprotein, #ab31721 (Abcam, Cambridge, Massachusetts) against Occludin, and #ABT45 (Millipore, Billerica, Massachusetts) against Claudin-5. Protein expression levels were compared using Student's t-test using Graph Pad Prism 5 software.

Results

Although Western blot analysis revealed a possible trend toward a lower expression of the tight junction proteins Occludin and Claudin-5, no significant differences between DHCA (n=5) and sham animals (n=6) were detected in protein expression (Figure 1). Comparison of MRI signals from DHCA and sham animals following intravenous administration of low-molecular weight contrast agent revealed increased local presence of tracer in the brain parenchyma following DHCA (n=2) relative to sham controls (n=2) (Figure 2).

Conclusion

In this pilot study of experimental CPB/DHCA in a rat model, MRI was successfully utilised to detect increased brain capillary permeability to commercially available low molecular weight contrast agent. While no significant quantitative changes in select proteins relevant for BBB structure and function were detected one day after reperfusion, differential protein expression could have been more pronounced at other time points. Therefore, future studies should include a time course analysis of protein expression as well as a characterisation of post-translational modifications, protein-protein interactions, and associated second-messenger pathways of relevant BBB proteins.

Most previously performed experimental studies of perioperative BBB function have assessed barrier integrity through the extravascular presence of large serum proteins such as albumin.[3, 7, 8] Our study used a contrast agent (gadobutrol) that is not only detectable in processed brain tissue, but permits in vivo administration allowing survival analysis. This approach thus renders animal-based studies with serial neurological exams possible, and furthermore, since gadobutrol is an intravenous contrast agent approved for patient use, is directly applicable to clinical studies.

The strength of this pilot study is that we have demonstrated the feasibility of using molecular analysis in combination with clinically relevant imaging technology to describe BBB characteristics in a rodent model of CPB/DHCA. The study is limited by its small group size. However, our goal was to lay the groundwork for future studies that will also include measurement of neurologic function. The isolation of brain capillaries offers the opportunity to analyse capillary BBB proteins in a highly purified state. This technique allows for measurement of transport rates of BBB substrates (for example for P-

glycoprotein) across the purified capillaries, thereby permitting analysis of transport protein function.[11]

In summary, our innovative approach of molecular BBB analyses combined with MRI technology in a rodent model is highly translatable and may improve our understanding of BBB function after CPB/DHCA. Further studies to assess the feasibility of strategies to maintain BBB integrity and their effects on neurologic function are needed.

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Figure 1.

Western blot analysis of protein extracted from purified rat grey matter brain capillaries showed no significant differences in expression of the blood brain barrier proteins P-glycoprotein, Occludin, or Claudin-5 between sham animals (n=6) and animals after deep hypothermic circulatory arrest (DHCA; n=5). Densitometry ratios relative to Actin depicted as fold-difference after DHCA compared to mean expression after sham surgery. Error bars depict SEM, statistical analysis performed using t-test (A-C). Western blot image of two experiments (D).

Figure 2.

Magnetic resonance imaging using low-molecular weight contrast agent showed increased local presence of gadobutrol (Gadovist®) following deep hypothermic circulatory arrest (DHCA; n=2) compared to sham procedure (n=2). On routine T1-weighted images, the local presence of gadobutrol is observed as a qualitative increase in signal through shortening of longitudinal relaxation. Quantitation of the amount of T1 shortening (represented as a mathematical map), however, is necessarily depicted as a decrease in pixel signal intensity, as above.