Downsizing: What have we learned from rodent models of cardiopulmonary bypass?

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Learning Objectives:
1. To review the progress that has been made in developing new small animal models of cardiopulmonary bypass.
2. To understand how these rodent models are offering unique abilities to examine bypass related organ dysfunction.

Major cardiovascular surgery employing cardiopulmonary bypass (CPB) and deep hypothermic circulatory arrest (DHCA) represents a unique biologic stimulus leading to profound perturbations in inflammatory, hemostatic, and oxidative stress pathways, all implicated in the pathogenesis of perioperative organ injury. Organ injury following cardiac surgery and associated morbidity concerns a number of organs including brain, heart, kidneys, liver, and lung.\(^1\)\(^-\)\(^6\) Despite significant advances in surgical, anesthetic, and organ protective strategies, the incidence of perioperative organ injury remains high. The clinical and financial implications of these problems can be profound, with prolonged hospitalization, reduced quality of life, and increased health care resource utilization having been associated with minor and major organ injuries. Preventing or treating these organ injuries remains difficult because the underlying mechanisms associated with ischemic reperfusion injury and the generalized inflammatory response introduced by CPB and DHCA are incompletely understood. The use of appropriate disease models of CPB and DHCA to study these injury mechanisms together with the testing of pharmaceutical treatments and other strategies should first occur in suitable animal models before applying them in costly multi-center trials.

The unphysiological state of CPB that occurs when the function of the heart and lungs are bypassed and taken over by mechanical devices has been extensively studied, both in laboratory and clinical settings. Although several animal models (including piglets, pigs, rabbits, dogs, and primates) exist in which to study the effects of CPB on various organ systems, they all have certain limitations. These limitations include the complexity of the preparations and models, significant cost, the availability of suitable species applicable to the organ system targeted for study, and the lack of feasible long-term recovery of these large animals. While partial CPB in rodents has been reported as early as 1969,\(^7\) it was during the last decade that a number of descriptions of complete CPB models in the rat were described.\(^8\)\(^-\)\(^10\) These newer models not only allow for complete CPB but also for more clinically relevant recovery times from CPB. Further, most of the earlier descriptions had incomplete methodology making the replication of these experimental settings difficult. In addition, some of these earlier model descriptions are more
than three decades old, and, while there has been marked improvement in associated technology and conduct of CPB, these older models appear now to be less applicable to the current clinical standards. Recent models of rodent CPB and DHCA not only include a miniaturized oxygenator but also have reduced prime volume requirements, thereby making the use of donor blood to fill the circuit unnecessary.\textsuperscript{11} Rat-specific oxygenators contain clinically used layers of polypropylene hollow-fiber mats. Overall reduction in prime volume to about 10 ml generally allows the conduct of CPB and DHCA without blood transfusion at hematocrit levels commonly seen in clinical practice. Today’s rodent models of CPB also allow for adequate CPB flows that are equivalent to normal cardiac outputs in the rat ranging from 160-180 ml/kg/min. Resulting CPB flows of 100-150 ml/kg/min result in sufficient oxygenation of the non-ventilated animals. Besides these small circuit components, it is the surgical preparation for CPB and DHCA that defines the uniqueness of these models. The surgical preparation used in most current rodent models consists of 10-14 week old rats that are anesthetized, intubated, and ventilated. Cannulation for CPB and DHCA consists of a multi-orificed venous return cannula that is positioned in the right atrium via the external jugular vein accessed from the neck, arterial inflow via the tail artery and descending aorta, with blood pressure measurement via a superficial branch of the femoral artery. With this model, others and we have been able to demonstrate neurologic and neurocognitive injury both after CPB alone and in combination with superimposed cerebral emboli or middle cerebral artery occlusion (MCAO) as well as following DHCA.\textsuperscript{8,9,12,13} We have also described myocardial and renal injury following CPB and DHCA in this model.\textsuperscript{14,15} Other organs of interest, such as the liver, the lungs, and the hematologic system, have also been addressed using similar model preparations.\textsuperscript{16,17} This lecture will review the progress that has been made in developing specific rodent models of CPB and DHCA. Individual organs of interest will be addressed separately and emphasis will be laid on organ-specific modification of the models.

1. Cerebral injury following CPB/DHCA

Following initial descriptions of both neurologic and neurocognitive injury after CPB alone or in conjunction with cerebral air emboli, MCAO or DHCA, these models have been also utilized to assess specific neuroprotective strategies. As part of that work, translational research using the rodent CPB model demonstrated neuroprotective benefits of the noble gas xenon.\textsuperscript{18} This successful pre-clinical experiment was used in support for subsequent phase I clinical trial as well as having been used as the basis for a planned phase II neuroprotective intervention trial in adult cardiac surgery in patients, demonstrating the enormous translational potential of established pre-clinical models.\textsuperscript{19} Of interest, xenon has been further examined in the context of CPB and cerebral air emboli in a model that combines the injection of pre-defined cerebral air emboli into the cerebral circulation with the conduct of CPB.\textsuperscript{13,20} In that setting, exposure to xenon during cerebral air emboli resulted in impairment of fine motor, cognitive, and histological outcomes. Potential neuroprotective properties of xenon may, at least in this study, been masked by the effect of xenon on cerebral air emboli dislodged into the cerebral circulation. Therefore, the use of xenon for its neuroprotective potential may have to be restricted to those cardiac surgery procedures that are not prone to cerebral air emboli. In other work, de Lange et al., provided evidence for the potential value of rodent models of CPB in discriminating the effects of CPB conduct. Rats exposed to normothermic CPB had deficits in Morris Water Maze testing 3 to 9 days following CPB. In rats where hyperthermia was induced, either only during
CPB with rapid rewarming, or where hyperthermia was induced only after CPB, Morris Water Maze performance was not improved. In contrast, when hypothermia was instituted during CPB and combined with slow rewarming to 35°C during CPB, post-CPB neurocognitive deficits were decreased. This laboratory work complements human trials within the time frame the outcome analysis was conducted and again demonstrates the potential utility of rodent CPB models.

As a number of human studies have identified age and diabetes as important risk factors for cerebral injury after cardiac surgery, several investigators have attempted to show similar correlations in rodent models of CPB. Of interest, one particular study was unable to demonstrate that exposure of old or diabetic rats to 90 minutes or normothermic CPB caused persistent changes in neurocognitive performance. This suggests that, at least in that model, CPB along was not sufficient to cause any cerebral injury, not even in animals with assumed increased susceptibility for cerebral injury. These results are in agreement with recent clinical studies suggesting that avoidance of CPB using off-pump coronary artery bypass grafting (CABG) techniques will not necessarily result in improved cognitive outcomes in humans when compared to regular CPB. The availability of certain diseased rodent strains and future availability of knock-out and other genetically altered rats makes these small CPB models perhaps even more applicable to the study of cerebral injury following CPB.

In other work, we have recently demonstrated that perfluorocarbons may be useful in producing the volume of gaseous bubbles present during CPB. However, in a secondary experiment designed to test the potential neuroprotective effects of perfluorocarbons by potentially improving oxygen delivery and attenuating ischemia-reperfusion and generalized inflammation, demonstrated deleterious effects of this compound. This again demonstrates the pre-clinical usefulness of suitable small animal models to assess certain compounds that appear to be promising in the pre-clinical environment.

2. Myocardial injury following CPB/DHCA

Since the advent of CPB, cardioplegic arrest has been an essential component of cardiac surgery but remains associated with significant ischemia reperfusion injury to the myocardium. An initial report on the use of microarray technology to elucidate cardiac transcriptional programs in response to CPB-specific injury in vivo was a first step towards elucidating specific injury mechanism underlying the ischemia-reperfusion injury. Recently, we have defined a novel survival model of cardioplegic arrest and CPB in rats. This model combines the administration of antegrade cardioplegia with endoaortic cross-clamping during CPB. This is achieved with the ultrasound-guided placement of a balloon angioplasty catheter via the right-common carotid artery with its tip proximal to the aortic valve. To initiate cardioplegic arrest, the intra-aortic balloon is inflated and cardioplegic solution injected. This novel model of cardioplegic arrest and CPB allows for both the study of myocardial ischemia-reperfusion injury as well as new cardioprotective strategies. Significant advantages of this model include its overall feasibility and cost-effectiveness. We have examined the long-term echocardiographic outcomes as well as enzymatic, genetic, and histologic characterization of myocardial injury with this model. Also, initial research towards myocardial protection using a number of different anti-inflammatory compounds has been initiated and is considered as an important avenue of research.
Acute kidney injury following CPB/DHCA

Acute kidney injury (AKI) is a common problem in the context of cardiac surgery. There are both similarities and differences with AKI occurring in other clinical scenarios. AKI following DHCA is also common with an incidence in patients of 25% to 50%. In addition to its association with serious morbidity, AKI is also associated with significant mortality. The development of renal protective strategies has been hampered by a lack of experimental models of CPB or DHCA-related renal injury. In order to further understand the etiology and to develop renal protective strategies, others and we have begun to use specific rodent models of CPB and DHCA to characterize AKI and to improve understanding of the underlying mechanism of injury. Following and early description of AKI after DHCA in rats, we have recently demonstrated significant up-regulation of neutrophil gelatinase-associated lipocalin (NGAL) gene and protein in renal medulla coincident with caspase-3 activation following experimental DHCA. In the human setting, NGAL was recently recognized as potentially useful early biomarker of AKI. In other work, Mazer and colleagues demonstrated that normothermic CPB was associated with higher endothelial nitric oxide synthase (eNOS) messenger mRNA in the kidney compared to sham animals. These data suggest a potential role for eNOS in the context of AKI following CPB.

In summary, it is important to remember that interpretation of data obtained from rodent models of CPB must always take into consideration the limitation of the model itself. Even the most sophisticated and clinically relevant animal models will likely fail to simulate the clinical situation completely. Models such as the ones described offer important insights into certain aspects of clinical problems and therefore, but one needs to use caution when making an interpretation or comparison.

References:


