

## Cardiopulmonary bypass and neurologic dysfunction – Results from animal models

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Neurologic deficits following cardiac surgery persist as common complications with a significant impact to the patient's quality of life [1,2]. Clinical manifestations of these deficits are variable, ranging from frank stroke as worst case scenario to the more subtle neurocognitive deficits [2,3]. Despite extensive research in the field, the mechanisms leading to neurologic dysfunctions are not fully understood and neuroprotective drugs or strategies have limited effects. To further elucidate the etiology of cerebral injury following cardiac surgery an appropriate non-clinical disease model is needed. Such a disease model is also required to screen potential neuroprotective drugs in a preclinical environment with regard to safety concerns and effectiveness.

### The ideal animal model

Ideally, any potential neuroprotective drug or strategy should be tested to be effective and well-tolerated in an appropriate animal model before it is used in humans. But what is an appropriate animal model and which demands does it need to meet? Embolization and hypoperfusion are discussed as contributing factors to cerebral injury following cardiac surgery [4-7] and several models are established to study the impact of these factors on the brain [8-11]. However, how cardiopulmonary bypass (CPB) modifies the influence of these factors on the neurologic outcome is not fully understood. On account of this, an animal model of CPB is required which allows to incorporate different insults like cerebral emboli or hypoperfusion and various comorbidities like diabetes, age or atherosclerosis.

To study neurologic outcome following CPB an ideal animal model first needs to allow long-term survival, second should be simple to be performed ideal-

ly by one person, third should mirror clinical standards as close as possible and last but not least established and validated learning tasks should be available to assess gross neurologic as well as neurocognitive outcome.

Large animal models of CPB in dogs, lambs, pigs and rabbits have been used but their use is limited by the costs of operating in a full-scale environment and the personnel required [12-14]. Further, tests to assess cognitive performance are not validated yet. In contrast, the rat is a well studied species in neuroscience with validated learning tasks available and the entire experiment can be performed by a single investigator. Therefore, a rat model of CPB would allow to study a larger sample size and to assess long-term neurologic outcome at a reasonable price. However, the rat models of CPB used so far were confronted with two problems: first the rat does not easily survive sternotomy and second venous drainage via peripheral inserted catheters (to avoid sternotomy) was not optimal with the consequence of only partial CPB to be performed [15-18]. An important step forward is presented by the introduction of a rat model avoiding sternotomy but allowing the conduct of complete CPB by optimizing venous drainage [19,20]. Using this model Mackensen and colleagues demonstrated neurocognitive deficits during the first 12 postoperative days utilizing the Morris water maze [19]. Dieleman and colleagues studied the impact of CPB on long-term cognitive outcome in young and healthy rats and did not detect any difference between the animals exposed to CPB and the sham-operated animals [21]. Consequently, they exposed old and diabetic rats to CPB and assessed the short-term cognitive outcome with the Morris water maze with again no deficit in the CPB-groups [22]. Taken these results together it seems that CPB alone without any cardiac surgery, embolization, aortic manipulation, etc. does not lead to reproducible neurolog-

ic deficits which is in accordance to clinical trials [23,24]. However, this model of CPB in rats presents an important starting point as it allows to incorporate further insults like emboli and hypoperfusion. By simulating these factors it can be distinguished between virtual etiologic factors contributing to neurologic deficits following cardiac surgery and pure epiphenomena.

### **Disease model of cardiopulmonary bypass in rats**

Emboli, gaseous or solid, are discussed as one important contributing factor to adverse cerebral outcome following cardiac surgery [4,6]. Therefore, we incorporated cerebral air embolization into the existing model of CPB in rats. Based on established models of cerebral air embolism in rodents [11], the emboli were directly injected into the cerebral circulation to ensure standardized and controlled embolization even though that does not entirely mirror the clinical scenario where the air is entrained during open-chamber procedures or generated in the CPB circuit. A dose-escalating study showed that significantly larger volumes of cerebral air emboli are tolerated in the absence of CPB suggesting an additive negative effect of CPB and emboli on the brain [25].

Cerebral hypoperfusion is discussed as another contributing factor leading to neurologic dysfunction following cardiac surgery [7]. The extreme variant of cerebral hypoperfusion is represented by circulatory arrest, which is performed as deep hypothermic circulatory arrest (DHCA) for the repair of congenital heart disease and for complex aortic surgery. Using the model of CPB in rats we performed DHCA for different durations and assessed survival, motor function and histological outcome. The survival rate decreased dramatically after DHCA durations exceeding 60 min while the percentage of animals without any motor deficits after 14 days decreased to 50% following 45 min of DHCA [26].

With the incorporation of emboli and DHCA in the existing model of CPB we were able to establish a reproducible disease model leading not only to motor and cognitive dysfunction but also to histological alterations.

### **The need for a disease model**

In a recent editorial Hindman et al. stated that there is a lack of an appropriate non-clinical disease model, which is required A) to study mechanisms and etiologies leading to cerebral injury and B) to screen potential neuroprotective drugs and strategies before use in humans [27]. Using a rabbit model of CPB combined with cerebral air emboli he showed that in the presence of CPB the recovery of somatosensory evoked potentials was decelerated [10]. These findings are in accordance with own results demonstrating a worse cerebral outcome in rats exposed to cerebral air emboli during CPB compared to rats subjected to air emboli during normal circulation [25]. Systemic and cerebral inflammatory response to CPB seems to play an important role in the development of neurologic dysfunction. However, human studies examining the impact of systemic inflammatory reaction on cerebral outcome following CPB have revealed conflicting results [28; 29; 30]. In accordance, preclinical studies using a long-term recovery model of CPB in rats showed varying effects of CPB on systemic inflammatory response and on neurocognitive outcome [21,22,31]. Recently, the link between systemic IL-6 and cerebral COX-2 mRNA expression was demonstrated by Hindman et al. using the rat model of CPB without studying neurologic function [32]. In this context we demonstrated an increased cerebral TNF $\alpha$  expression following both CPB and sham-operation accompanied by a transient cognitive impairment with no difference between the CPB and the sham-group [33].

### **Animal model as preclinical screening tool**

Ideally, each potential neuroprotective drug should be administered safely in animals before use in humans. With regard to safety concerns, an animal model can be adjusted and tailored to focus on the question which needs to be answered. In this context, we performed a study to investigate the impact of the noble gas xenon on neurocognitive function when administered during CPB in the presence of cerebral air emboli. Xenon has been shown to be neuroprotective in several models of cerebral injury including CPB [34-36]. However, it also has been demonstrated that according to its physical characteristics xenon has the disposition to expand air bubbles (present as cerebral air emboli during cardiac surgery) which may abolish any neuroprotective effect or even amplify neurologic injury following

CPB with cerebral air emboli [37,38]. Our study using the combined model of CPB and cerebral air emboli confirmed our safety concerns and demonstrated a worse fine-motor, cognitive and histological outcome in animals treated with xenon and exposed to CPB in the presence of cerebral air emboli [39].

De Lange et al. studied another safety concern regarding the artificial oxygen carrier perfluorocarbon. They showed that administration of perfluorocarbon during CPB resulted in a remarkable inflammatory response, loss of vasomotor tone and significantly increased mortality rate [40]. These experimental results confirmed the concerns raised during the conduct of a phase 3 trial which was abandoned prematurely due to a higher incidence of neurologic complications in patients treated with perfluorocarbon [41].

## Conclusion

In summary, neurologic dysfunction following cardiac surgery remains a common and significant complication. An appropriate animal model is needed to further elucidate the mechanisms leading to these deficits and to screen potential neuroprotective drugs and strategies before their use in humans. However, history shows that drugs tested safe and effective in the lab are not always effective in human trials and raises the question about translational research. One needs to keep in mind that any preclinical study deals with animals and that the results of these animal studies require cautious interpretation. Nevertheless, these studies are important not only to improve our knowledge of the underlying mechanism but also to study safety concerns about potential neuroprotectants before advancing them into the clinical sphere.

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