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Lipid-lowering drugs prevent neurovascular and cognitive consequences of cardiopulmonary bypass

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ABSTRACT

Inflammatory injury and hypoperfusion following cardiopulmonary bypass (CPB) are associated with potential brain injury in relationship between CPB, memory impairment, changes in cerebral vascular reactivity and both systemic and cerebral inflammatory reaction. The objective of this study was to assess the preventive effect of a pretreatment with simvastatin or fenofibrate on neurovascular and cognitive consequences of CPB. Male Sprague–Dawley rats were treated by control diet, simvastatin 10 mg/kg/day or fenofibrate 200 mg/kg/day for 14 days before CPB surgery and were sacrificed immediately after surgery or 24 h later. Cognitive function, vascular reactivity, neuronal counts in CA1 and CA3 hippocampal regions, and inflammatory markers were assessed. CPB induced memory impairment and endothelial dysfunction 24 h after surgery associated with neuronal loss. Neuronal loss was reduced by simvastatin or fenofibrate treatment in parallel to memory alteration prevention. Pretreatment by simvastatin and fenofibrate prevented CPB-induced endothelial dysfunction. CPB led to early and marked release of TNFα and overexpression of ICAM-1. Both inflammatory marker expression was decreased in the pretreated groups by lipid-lowering drugs. In a rat model of CPB, we demonstrated that simvastatin and fenofibrate protected against CPB-induced endothelial dysfunction, cerebral and systemic inflammation in parallel to memory impairment prevention.

1. Introduction

Cardiopulmonary bypass (CPB) is widely used in cardiovascular surgery. Nevertheless it is associated with neurological complications. Associated ischemic cerebral vascular events are rare (3%) and caused by gas or fibrin emboli [1,2]. A number of publications reported cognitive deficits that appear without any macroscopic cerebral lesions and seem to be more worrying. First cognitive deficit concerns more than half of the patients at discharge and persists in 1/3rd of them six months following surgery [3]. Secondly they are responsible for quality of life impairments as well as socio-economic over-cost [4]. In term of pathophysiology surgical stress and anesthesia seem to be partially involved, as cognitive impairments concern 25% of non-cardiovascular surgical patients [5,6,7]. Other factors have to be taken into consideration, including brain micro-embolisms, cerebral perfusion disorders and the activation of inflammatory process [8]. The latest has been well described in clinical practice [9,10,11,12] and in experimental models [13,14].

Cerebral perfusion plays an important role in cognitive impairment induced by CPB as demonstrated clinically [15,16,17]. Previously we have shown that CPB was responsible for a middle cerebral artery endothelial dysfunction as well as early systemic and cerebral inflammatory activation [18]. Endothelial dysfunction appears early after the onset of CPB. Since the nervous system is dependent upon a consistent blood supply, any changes in the microvasculature could affect neuronal function, resulting in a significant loss of neuronal plasticity and a reduction in the ongoing replacement of neurons and synapses. One possible mechanism is that inflammatory events induce endothelial impairment, which may lead to disruption of the brain micro-vessel vasomotor tonus with, as a consequence, a diffuse cerebral hypoperfusion. It is suggested that inflammation is initiated by ischemia/reperfusion phenomena caused by aortic clamping on the one hand, and by blood contact with the synthetic CPB circuit, resulting in “alternative pathway” complement activation on the other hand [8,19,20,21,22]. Under activated complement component action, monocytes secrete TNFα that recruits other cells implicated in the inflammation process by inducing NF-κB transcriptional activity for synthesis of pro-inflammatory cytokines (TNFα, IL6, IL8, IL12), adhesion molecules (ICAM-1, VCAM-1) and vasoactive substances capable of injuring the endothelium and impairing its vasomotor characteristics [20,21,23,24].

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A number of works focusing on cerebral ischemia/reperfusion experimental models have shown that statins and fenofibrate have preventive effects on inflammatory response intensity and endothelial dysfunction explaining their neuroprotective effects [25,26]. Fenofibrate activates Peroxisome Proliferator-Activated Receptor-α (PPAR-α) resulting in inflammatory transcriptional repression on factors like NF-κB and AP-1 [27]. The anti-inflammatory effect of simvastatin has been shown to be mediated by indirect PPAR-α activation, via the inhibition of the protein kinase C [28]. These two molecules have also been shown to have beneficial effects on endothelial dysfunction [29,30,31,32]. Indeed statins enhance the endothelial nitric oxide synthase (eNOS) activity by forwardly activating the phosphatidylinositol 3-kinase/protein kinase Akt and by inhibiting system Rho proteins geranyl-geranylation, involved in enzyme stability [32,33,34,35]. Statins enhance nitric oxide (NO) production, which has an important role on endothelial function preservation and on inhibition of the endothelial adherence of polymorphonuclear cells. As for fenofibrate, its endothelial beneficial effect seems to be only due to oxidative stress inhibition enhancing NO bioavailability, since it has no effect on NO production [29,36].

The purpose of this work was to assess in a model of CPB the pharmacological prevention by simvastatin or fenofibrate on (i) cognitive impairment, (ii) vascular compartment, (iii) neuronal loss and iv) involvement of inflammatory pathway.

2. Materials and methods

2.1. Animals

All experiments were performed in strict accordance with the European community legislation (2010/63/UE). A local review board approved the study. Male Sprague–Dawley rats (Charles River, Saint-Aubin les Elbeuf, France), weighing 420 ± 30 g were used. Animals were housed in a light- and temperature-controlled environment with unlimited access to food and water. Animals were randomly assigned to the different groups. Experimental data were monitored by a blinded investigator for group allocation.

2.2. Experimental protocols

Three types of diet were administered: a control diet (standard chow from Harlan), a simvastatin-enriched diet (the standard chow containing 0.01% of simvastatin, corresponding to a dose of about 10 mg/kg) and a fenofibrate-enriched diet (the standard chow containing 0.2% of fenofibrate, corresponding to a dose of about 200 mg/kg). Twelve groups of 12 rats were randomly constituted and compared early (T0) or 24 h (T24) after the sham or CPB procedure: "Sham" group (animals received the control diet for 14 days). They were anesthetized, cannulated and anti-coagulated similarly to the animal of the CPB group, but not connected to the circuit; "Sham Feno" group (animals received the fenofibrate-enriched diet for all 14 days); "Sham Simva" group (animals received the simvastatin-enriched diet for all 14 days); "CPB" group (animals received the control diet for 14 days before induction of CPB procedure); "CPB Feno" group (animals received a fenofibrate-enriched diet for all 14 days before induction of CPB procedure) and "CPB Simva" group (animals received a simvastatin-enriched diet for all 14 days before induction of CPB procedure). Animals were sacrificed early (T0) or 24 h (T24) after the sham or surgical procedure for in vitro cerebral vascular study, plasma assay and immunohistochemistry studies (for each group, 6 animals were used for vascular study and 6 animals for immunohistochemistry studies). Neurobehavioral testing was achieved only at 24 h because the animals were not yet awakened from the anesthesia at T0 (6 animals for Y maze test and 6 animals for passive avoidance test for each group).

2.3. Cardiopulmonary bypass procedure

Model of cardiopulmonary bypass in rat was validated and described previously [18,37]. All circuits were single use and set up under sterile conditions. Rats were anesthetized with a single intra-peritoneal injection of chloral hydrate (300 mg/kg). Palpebral and pedal withdrawal reflexes are used to assess the depth of anesthesia. Arterial pressure was continuously monitored using a femoral artery catheter. Central temperature was maintained at normal levels with a warming mattress and a heat lamp, and measured using a rectal temperature probe. Femoral arterial blood gases were taken every 15 min throughout the CPB period to ensure adequacy of oxygenation. Polyvinyl chloride (PVC) circuit (3 mm of internal diameter and 30 cm length) was connected to a non-pulsatile roller pump (Watson Marlow 101 U/R, France) equipped with a 15 cm long silicone tube with a 5 mm internal diameter. The cardiotomy reservoir was made of a 20 ml syringe. The membrane oxygenator used was specifically conceived for rodent experimentation (Pheresis research, Scotland). The entire circuit was primed with a 10 ml total volume composed of 5 ml of physiologic serum and 4 ml of Gelofusine 4% and 1 ml of bicarbonates 1.4% (B Braun Medical Ltd., France). The oxygenator was ventilated initially with pure oxygen at a flow of 75 ml/min that was subsequently adjusted according to the blood gases during the CPB period. Partial right–left CPB was carried out after right atrium cannulation with a 6 Fr drain placed with a right trans-jugular approach, via a surgical cut-down, and caudal cannulation with a 20 G catheter. Heparin (500 UI/kg) was administrated via the arterial cannulae. CPB duration was 30 min selected in accordance with our earlier studies in which we demonstrated that endothelial dysfunction appears within 30 min of initiating CPB. CPB was established and maintained at a flow rate of 100 $ml \cdot kg^{-1} \cdot min^{-1}$, corresponding to normal cardiac output of 3.5 $l \cdot m^{-2} \cdot min^{-1}$. This flow rate is similar to that employed in routine clinical practice.

2.4. Behavioral assessment

2.4.1. Y-maze test

Evaluation of spatial and learning memory using a Y maze was performed [38]. A Y maze consists of a wooden 3 arm black box, containing a central stem of 50 cm length and two side-arms of 43 cm in length and 16 cm in width, surrounded by 33 cm high walls. In the initial trial, the rats were placed in the stem with the right arm blocked by a plastic barrier (forced choice). After entering the left arm (four-paw criterion), the rats were kept inside for 1 min, retrieved, and placed back in the stem for a free-choice trial. One arm of the Y maze is blocked off and the rat is allowed to explore the other two arms for about 15 min. Then several hours after the familiarization time, the rat is positioned in the start arm and the blocked arm is uncovered to test the memory and the earning abilities of rats. The time spent in the new arm is measured in 1-min trials. Data were recorded using EthoVision® XT Noldus software.

2.4.2. Passive avoidance test

To evaluate short-term memory we use the passive avoidance test, a test based on fear memory consolidation. The apparatus was a 2 compartment box, consisting of one dark small compartment, and one large, bright chamber connected by a guillotine door. In the conditioning trial each rat was first placed into the lighted chamber and the guillotine door was opened. After rat entered the dark chamber, a 1 s footshock at 0.5 mA was delivered to rat. Animal that did not enter the dark chamber within 300 s were excluded from the analysis. Animals were tested for retention by placing each animal into the lighted chamber and the latency of the rat entering the dark chamber was recorded using ShutAvoid (Panlab®, Bioseb, France).