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Laboratory Investigation

LABORATORY INVESTIGATION

Reduction of vascular leakage by imatinib is associated with preserved microcirculatory perfusion and reduced renal injury markers in a rat model of cardiopulmonary bypass

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Abstract

Background: Cardiopulmonary bypass (CPB) during cardiac surgery leads to impaired microcirculatory perfusion. It was hypothesized that vascular leakage is an important contributor to microcirculatory dysfunction. Imatinib, a tyrosine kinase inhibitor, has been shown to reduce vascular leakage in septic mice. We investigated whether the prevention of vascular leakage using imatinib preserves microcirculatory perfusion and reduces organ injury markers in a rat model of CPB.

Methods: Male Wistar rats underwent CPB after treatment with imatinib or vehicle (n=8 per group). Cremaster muscle microcirculatory perfusion and quadriceps microvascular oxygen saturation were measured using intravital microscopy and reflectance spectroscopy. Evans Blue extravasation was determined in separate experiments. Organ injury markers were determined in plasma, intestine, kidney, and lungs.

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Results: The onset of CPB decreased the number of perfused microvessels by 40% in the control group [9.4 (8.6–10.6) to 5.7 (4.8–6.2) per microscope field; $P<0.001$ *vs* baseline], whereas this reduction was not seen in the imatinib group. In the control group, the number of perfused capillaries remained low throughout the experiment, whilst perfusion remained normal after imatinib administration. Microvascular oxygen saturation was less impaired after imatinib treatment compared with controls. Imatinib reduced vascular leakage and decreased fluid resuscitation compared with control [3 (3–6) *vs* 12 ml (7–16); $P=0.024$]. Plasma neutrophil-gelatinase-associated-lipocalin concentrations were reduced by imatinib.

Conclusions: Prevention of endothelial barrier dysfunction using imatinib preserved microcirculatory perfusion and oxygenation during and after CPB. Moreover, imatinib-induced protection of endothelial barrier integrity reduced fluid-resuscitation requirements and attenuated renal and pulmonary injury markers.

Keywords: cardiopulmonary bypass; endothelium; microcirculation

Key points

- Cardiopulmonary bypass (CPB) results in impaired microcirculatory perfusion.
- Imatinib is a drug that reduces vascular leakage in animal sepsis models and may consequently improve microcirculatory perfusion.
- Intravital microscopy showed imatinib protected against loss of perfused capillaries and microvascular oxygen saturation caused by CPB in a rat model.
- Renal and pulmonary injury markers were reduced by imatinib, and fluid-resuscitation requirements were reduced.
- Imatinib may benefit postoperative organ function in high-risk cardiac-surgery patients.

Microcirculatory perfusion is disturbed during cardiac surgery with cardiopulmonary bypass (CPB),[1–3] but the underlying mechanisms remain to be elucidated. We recently showed in a rat CPB model that inflammation and endothelial activation contribute more to these microcirculatory-perfusion disturbances than haemodilution.[3] Moreover, microcirculatory dysfunction was paralleled by increases in markers of renal injury, and renal and pulmonary endothelial activation.[3]

Clinical studies and *in vitro* experiments using cultured endothelial cells show that endothelial activation and inflammation during CPB elicit endothelial barrier dysfunction with subsequent vascular leakage.[4–6] Moreover, vascular leakage is associated with postoperative pulmonary and renal dysfunction,[4,5] which may be mediated by a reduction in capillary perfusion due to interstitial fluid accumulation.[7] Whilst the association of vascular leakage with impaired microcirculatory perfusion seems obvious, there is only one study showing that endothelial barrier dysfunction as a result of inhibition of platelet endothelial cell adhesion molecule-1 was paralleled by preserved capillary perfusion in a rat cremaster muscle ischaemia–reperfusion model.[8]

Imatinib, an inhibitor of multiple tyrosine kinases, including c-KIT, platelet-derived growth factor receptor, c-Abl, and Abl-related gene (Arg),[9] prevents endothelial barrier dysfunction after inflammatory stimulation of an endothelial monolayer. It further reduces vascular leakage in animal models of sepsis and acute lung injury, attributed to blockade of the c-Abl and Arg pathways.[10,11] These findings led to our hypothesis that vascular leakage is an important contributor to impaired microcirculatory perfusion during CPB, and that prevention of vascular leakage by imatinib might preserve microvascular perfusion and oxygenation via protection of endothelial integrity and barrier function. We additionally investigated whether the preservation of endothelial barrier function reduces soluble and histological markers for organ injury, and diminishes the consequences of systemic inflammation induced by CPB.

Methods

Animals

All experiments were approved by the Institutional Animal Care and Use Committee of the VU University Medical Center. The experiments were conducted following the EU Directive on the protection of animals used for scientific purposes (EU2010/63), the Dutch Animal Experimentation Act, and are reported in accordance with relevant aspects of the Animal Research: Reporting of In Vivo Experiments guidelines. Animals were housed four per cage with unrestricted access to food and water in a temperature-controlled room (20–23°C; 40–60% humidity) with a 12/12 h light/dark cycle. Animal care was undertaken according to the national guidelines for care of laboratory animals. A total of 24 male Wistar rats of 375–425 g (Charles River Laboratories, Brussels, Belgium) underwent CPB procedures. Sixteen rats were randomized to treatment with imatinib (CPB imatinib; $n=8$) or saline (CPB control; $n=8$) before CPB. All measurements were performed in these groups, except for vascular-leakage quantification. Vascular leakage was determined in separate experiments, in which eight additional rats were randomized to undergo CPB with ($n=4$) or without imatinib ($n=4$).

Anaesthesia and surgical preparation

Anaesthesia was induced as described previously.[3] Briefly, anaesthesia was induced with isoflurane 5% in oxygen, and when the pedal withdrawal response to a nociceptive stimulus was absent, tracheal intubation with a 16G catheter was performed (Venflon Pro; Becton Dickinson, Helsingborg, Sweden). Mechanical ventilation (UMV-03; UNO Roestvaststaal BV, Zevenaar, The Netherlands; tidal volume 10 ml kg^{-1}, frequency 60–65 min^{-1}, PEEP 2–4 cm H_2O) was initiated and anaesthesia was maintained with isoflurane 2–3% with the