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Modifying the vessel walls in porcine kidneys during machine perfusion

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ABSTRACT

Background: Endothelial glycocalyx regulates the endothelial function and plays an active role in maintaining vascular homeostasis. During ischema and reperfusion, the glycocalyx is rapidly shed into the blood stream. A Corline heparin conjugate (CHC; Corline systems AB, Uppsala, Sweden) consists of 70 heparin molecules that have the capacity to adhere strongly to biological tissues expressing heparin affinity. We hypothesized that CHC could be used to restore disrupted glycocalyx *in vivo* in kidneys from brain-dead pigs.

Materials and methods: Brain death was induced in male landrace pigs ($n = 6$) by inflating a balloon catheter in the epidural space until obtaining negative cerebral perfusion. The recovered kidneys ($n = 5 + 5$) were perfused by hypothermic machine perfusion using two Lifeport kidney transporters (Organ Recovery Systems, Chicago, IL). CHC (50 mg) (including 25 mg biotinylated CHC) or 50 mg unfractionated heparin (control) was added to the perfusion fluid in the respective machines. In one case, the kidneys were used only for dose escalation of CHC with the same procedure.

Results: CHC was detected by immunofluorescence and confocal microscopy in the inner surface of the vessel walls. The binding of CHC in the kidney was confirmed indirectly by consumption of CHC from the perfusion fluid.

Conclusions: In this first attempt, we show that CHC maybe used to coat the vessel walls of perfused kidneys during hypothermic machine perfusion, an approach that could become useful in restoring endothelial glycocalyx of kidneys recovered from deceased donors to protect vascular endothelium and possibly ameliorate ischemia and reperfusion injuries.

The primary results of this study were presented as an oral presentation in the first international meeting on ischemia reperfusion injury in transplantation on March 5, 2012 in Poitiers France.

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1. Introduction

The endothelial glycocalyx consists of a mesh of membrane-bound proteoglycans and glycoproteins, which cover the entire endothelial surface and interact with both endothelium and soluble molecules in plasma [1]. Together with the endothelium, it constitutes a competent dual barrier function against cellular edema, inflammation, and leukocyte and platelet adhesion [1]. It becomes increasingly evident that damage to the glycocalyx is directly linked to the earliest stage of the ischemia and reperfusion injury (IRI), and initiation of reperfusion appears to be essential for the shedding process [2–6].

A macromolecular heparin conjugate, the Corline heparin conjugate (CHC; Corline Systems AB) consisting of about 70 heparin molecules covalently attached to an inert aliphatic polymer [7], has been used to improve blood compatibility of biomaterials. CHC inhibits coagulation and complement activity, reduces platelet adhesion, and stimulates endothelial infiltration [8–13]. Based on the finding that CHC binds strongly to collagen, which heparin does not (Table), we hypothesized that CHC could be used to restore disrupted glycocalyx of organs from deceased donors and potentially help to inhibit IRI.

A growing number of transplant centers worldwide are using hypothermic machine perfusion (HMP) as the preservation modality of choice because of its multifaceted benefits in comparison with conventional cold storage. HMP improves immediate posttransplant graft function, gives better graft survival rates, and enhances microcirculatory integrity [14–16]. In particular, the pulsatile flow is associated with the expression of flow-dependent, vasoprotective endothelial genes [16]. The modulation of the organ during its transfer from the donor to the recipient represents a major area of advancement. The modulation could potentially be provided during HMP of kidneys recovered from deceased donors to protect vascular endothelium and possibly ameliorate IRI after transplantation.

In this first attempt to restore disrupted glycocalyx, we investigated the ability of CHC to bind to the vessel walls of recovered organs in kidneys from brain-dead pigs during HMP.

2. Materials and methods

2.1. Animals, anesthesia, and induction of brain death

This study is consistent with the guidelines for the use of laboratory animals published by the Swedish National Board for Laboratory Animals and the European Convention on Animal Care and has been approved by the regional ethics committee for animal experiments in Uppsala, Sweden.

A total of six Swedish male landrace pigs aged 10–12 wk with a mean weight of 24.6 ± 1.7 kg were included in the study. Five pigs were used for the perfusion experiments and one for the dose escalation test of CHC. All experiments were performed under general anesthesia and the animals were subjected to standardized brain death induction as previously described, using stepwise intracranial hypertension by volume expansion of the epidurally placed catheter until obtaining negative cerebral perfusion [17].

Table – Surface concentration of heparin expressed on collagen after incubation with CHC or heparin.

Surface concentration of heparin (μg/cm^2)	CHC ($n = 5$)	UFH ($n = 5$)	P^*
Without pH 9	0.45 ± 0.08	0.03 ± 0.01	<0.05
Without pH 9	0.44 ± 0.06	0.01 ± 0.01	<0.05

Data are presented as mean ± standard deviation.
*Mann–Whitney U-test.

2.2. Kidney recovery and storage

Recovery of the kidneys was started 120 min after confirmation of brain death. In total, 2500 U heparin (LEO Pharma, Malmö, Sweden) was given intravenously as a single dose. The renal arteries were ligated at the level of the abdominal aorta, and the renal veins were divided at the junction of the inferior vena cava. Both ureters were isolated and divided close to the bladder. The kidneys were removed to the back table and placed on ice, and the blood was flushed out with 100 mL ice-cold (4°C) saline.

2.3. Hypothermic machine perfusion

The kidneys ($n = 5 + 5$) were placed on two separate perfusion systems (LifePort; Organ Recovery Systems) and the chambers prefilled with 1 L of kidney perfusion solution (KPS-1; Organ Recovery Systems). All kidneys were preserved by a pulsatile flow of recirculating KPS-1, perfused at a mean arterial pressure of 15 mm Hg at a temperature of 5.0 ± 0.47°C for 20 h.

2.4. Biotinylation of CHC

To enable visualization of histologic sections using fluorescent probes and to measure consumption of CHC (Corline System AB) in the perfusate, CHC was labeled with biotin. The CHC was labeled with biotin using residual free amine groups on the carrier chain. CHC was dissolved in saline (0.15 M NaCl) and mixed with Sulfo-NHS-LC-Biotin (Soltec Ventures, Beverly, MA). The reaction was then allowed to proceed for 30 min after which the solution was passed through a PD10 column (Amersham Biosciences, Uppsala, Sweden) and the void fraction collected. The presence of biotin linked to CHC was confirmed by allowing CHC or CHC-biotin to bind to a cationic matrix, 96-well plates precoated with polyamine compound, (PAV; Lab Site Heparin Coating Kit, Corline Systems AB), and measuring the uptake of streptavidin conjugated with horseradish peroxidase. Although binding of streptavidin-horseradish peroxidase to the test surface with CHC generated 0.02 absorbance units, the test surface with CHC-biotin showed a value of 3.50 absorbance units, thus confirming that biotin had bound to the CHC.

This assay was also used to measure the consumption of CHC after perfusion. A linear standard curve was obtained by diluting the starting solution 1.0–0.8, 0.5, and 0.25, respectively. The readouts obtained with samples from the perfusate