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Effect of normovolemic hematocrit changes on blood pressure and flow

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ABSTRACT

Aims: In patients with chronic kidney disease (CKD), severe anemia is associated with increased cardiovascular risk. Although elevating hemoglobin (Hb) and hematocrit (Hct) levels with erythropoiesis-stimulating agents (ESA) improves patients' quality of life, normalization of Hb does not reduce cardiovascular risk and the reason remains unclear.
Main methods: We measured the effect of acute isovolumic changes in Hct from 37 ± 5 to 50 ± 2% (mean ± SD) on arterial blood pressure (BP), cardiac output (CO), and carotid and renal blood flow (BF), (1) in control rats and (2) after acute blockade of the nitric oxide (NO) pathway by L-NAME.
Key findings: 1) In control conditions, BP, CO and carotid and renal BF remained stable for Hct values between 38 ± 2 and 46 ± 1%; 2) for higher Hct values, BP rose together with increasing blood viscosity whereas CO and renal BF decreased; 3) during acute NO blockade, CO, and carotid and renal BF were significantly reduced and remained low whereas BP increased with Hct thus increasing blood viscosity.
Our results suggest (1) the ceiling level of endothelium-mediated vasodilation for high values of blood viscosity under control conditions, and (2) the need for efficient endothelial function for vasomotor adaptation of hemodynamic resistances to blood viscosity.
Significance: (1) Clinical benefits of ESA in CKD patients with severe endothelial dysfunction are primarily due to increased oxygen transport and supply and, (2) normalization of Hct values in these patients may prove deleterious because of significant increases in BP and reductions in BF associated with high blood viscosity.

1. Introduction

Anemia is commonly observed in patients with chronic kidney disease (CKD) and is associated with an increased risk of cardiovascular events in these patients. Anemia is strongly predictive of complications and death from cardiovascular causes [1] and observational data suggest that correcting anemia in hemodialysis patients improves cardiovascular outcomes [2–8]. Extrapolation of these favorable observations to patients with anemia and earlier stages of CKD has led to wider use of erythropoiesis-stimulating agents (ESA). However, normalization of hemoglobin (Hb) levels in prospective trials including patients on hemodialysis did not result in further reductions in the risk of cardiovascular death [9,10]. Several large prospective clinical trials [11,12,13] have shown that Hb values within the normal ranges in patients with CKD were not associated with improved survival rates and may even be deleterious, with an increased risk of stroke, thus outweighing any potential benefit [13].

In 2007, the National Kidney Foundation conducted an extensive review of the results from six randomized controlled trials comparing the risks and benefits of a range of target Hb levels in CKD patients (on dialysis or not), who were receiving ESA therapy. The authors recommended target Hb levels should be between 11.0 and 12.0 g/dL and should not exceed 13.0 g/dL [14].

However, it is still unclear why higher Hb values do not improve cardiovascular outcomes of CKD patients. Ajay K. Singh pointed out that although much of the focus has centered on the Hb level targeted in these trials, ESA exposure itself may well account for the increased risk observed [15–17]. Indeed, 'targeting' higher Hb levels with ESA appeared to be the underlying problem rather than the Hb level itself. This interesting analysis of previous trials has highlighted the need for further investigation to clearly distinguish between the effect of the target Hb level and that of ESA treatment.

Our study therefore set out to quantify the isolated effect of acute isovolumic changes in hematocrit (Hct) on blood viscosity and on

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systemic and renal hemodynamics (arterial blood pressure [BP], cardiac output [CO] and carotid and renal blood flow [BF]), (i) under control conditions (normotensive healthy rats) and (ii) after acute blockade of the NO pathway with L-NAME to reflect the endothelial dysfunction that is a hallmark feature of CKD.

2. Material and methods

2.1. Animals

Experiments were conducted according to the French veterinary guidelines and those formulated by the European Community for experimental animal use. All rats were provided by Charles River (Lyon, France).

Normotensive male Wistar rats (14 weeks old) were randomized to either a control, untreated group (N = 10) or a treated group (N = 10) receiving N ω-nitro-L-arginine methyl ester (L-NAME) in the drinking water (10 mg/kg/day) for one day before hemodynamic measurements. Rats were anesthetized (1.5% isoflurane) and maintained in the dorsal decubitus position on a heating blanket (39 °C). They were shaved on the neck, thorax and the left side of the abdomen to facilitate catheterization of the left common carotid artery and ultrasound examination of the pulmonary and right carotid and left renal arteries.

2.2. Protocol

Packed red cell and plasma aliquots were prepared from freshly drawn, inbred rat blood that had been centrifuged for 15 min at 7000 rpm. After anesthesia and stabilization of hemodynamic parameters, one milliliter (mL) of blood was withdrawn from the jugular vein and immediately replaced by 1 mL of plasma or 1 mL of packed red cells. Ten minutes later, ultrasound measurements were performed; arterial BP was recorded continuously (Fig. 1). Hct and blood viscosity were then measured in venous blood samples. In all rats, successive plasma or packed red-cell infusions were repeated step by step: two 1-mL intravenous (IV) injections of plasma were administered to induce successive hemodilution, followed by five to seven 1-mL IV injections of packed red cells, to produce successive increases in Hct. Total duration of the experiments did not exceed 150 min for each rat.

2.3. Pressure measurements

A Teflon catheter (0.9 mm id), filled with saline and coupled to a Statham P23ID pressure transducer (Gould Statham®, Newcastle under Lyme, UK), was inserted through the left carotid artery and the tip placed into the ascending aorta. The pressure transducer was connected to an A/D converter (Bioseb®, Vitrolles, France) connected to a PC (Dell). Arterial BP was monitored continuously throughout the experiment. Systolic BP (SBP), diastolic BP (DBP), mean arterial BP (MBP) and heart rate (HR) were calculated on a beat-to-beat basis for 30 s and then averaged.

2.4. Blood flow measurements

CO, and carotid and renal artery BF velocity were recorded according to previously described methods [18–20] using an ultrasound device (ACUSON S3000 ultrasound system, Siemens®, Erlangen, Germany) equipped with a 14-MHz linear transducer (14L5-SP). Cardiac output (mL/min) was calculated as follows:

$$CO = 60 \times \left\{ [mBFV] \times \left[\pi \times (Dpa/2)^2 \right] \right\}.$$

CO, cardiac output; mBFV, mean blood flow velocity (cm/s); Dpa, pulmonary artery internal diameter (cm). Cardiac index (COI) was calculated as CO normalized to body weight (kg).

The ultrasound probe was placed on the left side of the abdomen then on the neck, to examine the left renal and right common carotid arteries. Peak SBP, end-diastolic BP and mBFV were recorded on a beat-to-beat basis for 30 s and then averaged.

2.5. Hematocrit and blood viscosity measurements

Hct was measured after blood centrifugation (Jouan-Hema-C, Saint Herblain, France) immediately after BF recordings.

Blood viscosity (η) was determined immediately after sampling in EDTA tubes, according to the guidelines for hemorheological laboratory techniques [21], using a cone-plate viscometer (Brookfield DVII +, with CPE40 spindle, Middleboro, Massachusetts, USA) at 37 °C. For each level of Hct, blood viscosity was estimated for different given levels of shear rate ranging from 10/s to 500/s (Fig. 2).

2.6. Statistical analysis

Data are expressed as means ± standard deviation (SD). Normality of distribution was assessed by the Kolmogorov-Smirnov test. The effects of Hct were tested by analysis of variance for independent groups (ANOVA). The Bonferroni test, and post hoc paired or unpaired Student's *t*-tests were used when the ANOVA was statistically significant. A P value < 0.05 was considered statistically significant. Statistical analysis, and linear or polynomial regression between MBP, CO, mBFV measured in the renal and common carotid arteries and Hct were carried out using MedCalc Software (Mariakerke, Belgium).

3. Results

Successive plasma or packed red-cell infusions modified Hct in both control and L-NAME-treated rats from 37 ± 5 to 50 ± 2%, corresponding to blood viscosity increases from 4.52 ± 1.21 to 5.95 ± 1.91 cP.

In control conditions, for Hct levels ranging from 38 ± 2 to 46 ± 1%, MBP remained stable around basal values (85 ± 6 mm Hg) and then showed a linear increase up to 106 ± 13 mm Hg when Hct rose to 50 ± 1% (P < 0.01 vs. baseline, Fig. 3A). The CO index did not change significantly from basal values (306 ± 17 mL · min^{-1} · kg^{-1}) for Hct levels

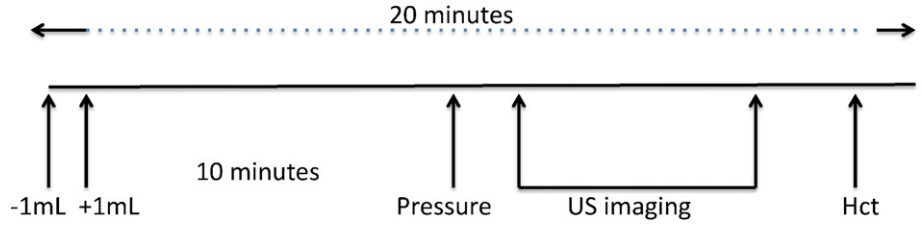

Fig. 1. Plotted mean arterial blood pressure (mBP, mean ± SEM), cardiac output index (COI = cardiac output/weight, mean ± SEM) and mean blood flow velocity (mBFV, mean ± SEM) measured in the right renal artery (RA) with hematocrit (Hct, mean ± SEM) in normotensive (•) and L-NAME treated (■) rats.