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Profiling biochemical and hemodynamic markers using chronically instrumented, conscious and unrestrained rats undergoing severe, acute controlled hemorrhagic hypovolemic shock as an integrated in-vivo model system to assess new blood substitutes

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Abstract

The aim of the present study was to assess several biochemical and physiological endpoint parameters alongside controlled hemorrhagic and recovery phases of chronically instrumented, conscious and unrestrained healthy rats. Male Sprague-Dawley rats (12–14 weeks; 430±20 g; $n=22$–18) were instrumented with a saline-perfused femoral arterial catheter and placed individually in a metabolic cage for up to 20 days, allowing instant assessments of the hemodynamic profile and blood and urine sampling for hematological profile and biochemical measurements to assess hepatic, renal and metabolic functions. In addition, body weight, food and water intake, and diuresis were monitored daily. After a 7-day stabilization period, the rats underwent severe and acute hemorrhagic shock (HS) (removal of 50% of total circulating blood volume), kept in hypovolemic shock for an ischemic period of 50 min and then resuscitated over 10 min. Gr. 1 was re-infused with autologous shed blood (AB; $n=10$) whereas Gr. 2 was infused 1:1 with a solution of sterile saline-albumin (SA; 7% w/v) ($n=8$–12). Ischemic rats recovered much more rapidly following AB re-infusion than those receiving SA. Normal hemodynamic and biochemical profiles were re-established after 24 h. Depressed blood pressure lasted 4–5 days in SA rats. The hematological profile in the SA resuscitated rats was even more drastically affected. Circulating plasma concentrations of hemoglobin (−40%), hematocrit (−50%), RBC (−40%) and platelets (−41%) counts were still severely decreased 24 h after the acute ischemic event whereas WBC counts increased 2.2-fold by day 4. It took 5–9 days for these profiles to normalize after ischemia-reperfusion with SA. Diuresis increased in both groups (by 45±7% on day 1) but presented distinct electrolytic profiles. Hepatic and renal functions were normal in AB rats whereas altered in SA rats. The present set of experiments enabled us to validate a model of HS in conscious rats and the use of an integrated in vivo platform as a valuable tool to characterize HS-induced stress and to test new classes of blood substitutes in real time, post-event, over days.

Keywords: Autologous blood; Blood substitute; Clinical biochemistry; Hematology; Hemodynamic profile; Hemorrhagic shock; Ischemia reperfusion injury

1. Introduction

The need of blood for transfusion remains very important for "blood consuming" surgical procedures (Graves et al., 1989) and in trauma where hemorrhage is one of the major causes of morbidity and mortality (Faist et al., 1983). The risk of viral transmissions (HIV, CMV, hepatitis C, *Trepanosoma cruzii*, West Nile Virus) remains significant (Busch et al., 2005), even though it has been markedly reduced with their

Abbreviations: AII, angiotensin II; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CK, creatine kinase; HDL, high density lipoprotein; Hct, hematocrit; HR, heart rate; MABP, mean arterial blood pressure; NO, nitric oxide; Plts, platelets; RBC, red blood cell; TGs, triglycerides; WBC, white blood cell.

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detection albeith at an added cost. In addition, blood supply is reduced because of restrictions on certain groups of donors such as residents of countries associated with outbreaks of Creutzfeldt-Jacob disease (Ironside and Head, 2004). The use of intraoperative autologous blood (AB) transfusion is increasing, thus avoiding allergenic transfusion which may otherwise lead to cases of hemolytic or allergic reactions (transfusion-related immunomodulation or TRIM) (Carless et al., 2004). Consequently, sterile, stable artificial, or engineered, hemoglobin-based blood substitutes (HBBS), hemoglobin-based oxygen carriers (HBOCs), would constitute efficient synthetic substitutes (Chang, 2004; Kim and Greenburg, 2004), if they were to present limited post-transfusion side-effects (D'Agnillo and Alayash, 2000). Those blood substitutes would also provide blood banks and non-civilians (combat) users with important reserve of ready-to-use stock solutions (Nouwairi, 2004) for emergency use.

Present-day second generation blood substitutes presents a number of serious pathophysiological problems ranging from systemic hypertension (Olson et al., 2004) to nephrotoxicity and osmotic diuresis (Simoni et al., 1997). Thus, safety problems and irreversible damage to organs remain an unresolved issue. Therefore, the development of novel blood substitutes would greatly benefit trauma patients or those undergoing elective surgery. There are novel avenues being pursued some of which have recently entered clinical trials but failed. They include Baxter's Diaspirin cross-linked hemoglobin (Schubert et al., 2002, 2003) and Hemosol's Hemolink (Hill et al., 2002; Cheng et al., 2004). Others substitutes are still undergoing clinical trials (Pfizer/Pharmacia Corp./Northfields' PolyHeme; Jahr and Varma, 2004) or at the preclinical stages (Oxyglobin (OXY) and Hemopure (HMP); Guan et al., 2004).

Because of increasing cost for the development of blood substitutes, the need to limit their side-effects and to insure a strong safety profile, the research area of blood transfusion needs better pre-clinical experimental animal platforms to define and understand the physiopathological events that occur during transfusion studies with those new blood substitutes.

In search of such an in-vivo animal model system (Buehler and Alayash, 2004; Sakai et al., 2004a), we developed and validated a fully integrated pharmacological platform using rats undergoing severe, acute, short term, potentially lethal, ischemia-related hemorrhagic shock. Since a great number of hemorrhagic shock models exist in rats, we adapted one from the literature (Chang, 1997; Chang and Varma, 1992) that reflects and mimics at best the ischemia-reperfusion stress injury consecutive to that of a hemorrhage observed in patients surviving an initial severe blood loss (Garrioch, 2004). This model also allows the evaluation of subsequent responses triggered to preserve blood flow to vital organs and is associated to complications (renal and hepatic failures, intestinal infarction, sepsis; Stephan et al., 1987).

Such an attempt was developed with a clear rationale: to focus on identifying the mechanisms involved in mediating the complications of hemorrhagic shock and the side-effects of various perfusates in order to limit, or prevent at best, the toxicities of blood substitutes, and predict a better clinical outcome.

To achieve these aims, we took advantage of a platform that allows a constant daily evaluation of more than 60 parameters and analytes in chronically instrumented, unrestrained and conscious rats. These parameters include body weight-growth, food and water intake, diuresis, blood hematology, plasma biochemistry of electrolytes and lipids, hepatic, renal, cardiac and metabolic functions and the hemodynamic profile.

We observed that ischemic (50% blood loss over 50 min) rats ressuscitated with their own shed whole blood survived without presenting major alterations in their physiobiological parameters; would normalize within 24 h and remain stable for over 10 days. Conversely, hemorrhaged rats ressuscitated with an isovolumic (1:1) saline-albumin (SA, 7% w/v) solution revealed severe hemodynamic dysfunctions and hepatorenal failures for up to 5 days along with increased mortality. These sets of rats will serve as comparables to study the safety and efficacy profiles of novel blood substitutes.

2. Materials and methods

2.1. Pre-surgical setup, anaesthesia, surgical procedures and post-operative care

Adult Sprague-Dawley rats (430±20 g, 12–14 w, Charles River, St-Constant, Canada) were housed individually in their metabolic cage (cat# 650-0350, Nalgene, Missisauga, ON, Canada), according to the local (Ethic Committee of Sherbrooke University) and national (Canadian Council on Animal Care; CCAC) guidelines on animal welfare under a 12-h cycle of day/night, with free access to drinking water and fed ad libitum.

As previously described (Blouin et al., 2000; Daull et al., 2004), all surgical procedures were performed in a strictly aseptic environment and all materials [surgical tools, various connecting catheters of the upper part, the 22 G single channel swivel (0.016 in. i.d.; cat #61-0001; Instech-Solomon, Plymouth Meeting, PA) and urethane-coated anti-thrombogenic vascular catheter (PhysioCath, Data Sciences International, Saint Paul, MN)] were gas sterilized.

Briefly, rats were anaesthetized through the inhalation of isoflurane (Baxter Corp, Toronto, ON, Canada) kept at 2% throughout the surgical procedure. Body temperature was maintained at 37 °C using a homeothermic blanket while the vascular catheter was surgically inserted into the femoral artery up to the abdominal aorta. The catheter was tunneled under the skin from the left leg through the dorsal site at the neck to connect to the Covance infusion harness, passed through a stainless steel spring stock protector and connected to the swivel as mentioned above. A continuous infusion of sterile heparinized (4 U/ml) saline was initiated (250 μl/h=6 ml/day) to prevent the formation of blood clots within the vascular catheter (Fig. 1a).