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.

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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, Treponosoma cruzii, West Nile Virus) remains significant (Busch et al., 2005), even though it has been markedly reduced with their
detection albeit 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-Jakob disease (Ironside and Head, 2004). The use of intraoperative autologous blood (AB) transfusion is increasing, thus avoiding allergic 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 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 resuscitated 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 resuscitated 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, Mississauga, 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).
2.2. Measurements of physiological parameters

Physical examination, body weight (growth), food and water intake and diuresis were monitored every day to assess the growth and well being of the animals. The volume of fluid delivered by the low-flow peristaltic pump was also measured daily. Positioning, integrity and functionality of the vascular catheter inside the abdominal aorta was determined at euthanasia.

2.3. Blood and urine collection

Arterial blood was collected twice daily through the implanted vascular catheter, 12 h apart at 8:00 and 20:00 h. The sampled blood was replaced by an equal amount of sterile saline, and collected in heparin-lithium (am) and K2 EDTA (pm) vacutainers, for biochemical analyses. Urine samples were collected over 24 h periods starting prior to (day −1) and after hemorrhagic shock (days 1, 5 and 10).

2.4. Hemorrhagic shock and reperfusion

A severe and acute, short term ischemia-related hemorrhagic shock was used according to Chang and Varma (1992) and Chang (1997). Blood removal was initiated 7 days after catheter implantation. Briefly, $2 \times 25\% = 50\%$ of estimated total circulating blood volume (estimated at 70 ml/kg of body weight; e.g. $2 \times 7$ ml/400 g of rat body weight) was removed over $2 \times 5$ min periods through the arterial catheter. The second bleeding period was performed 20 min after the first one. The re-infusion sequence of the perfusates (Gr. 1, $n = 10$, autologous blood; Gr. 2, $n = 8–12$, saline-bovine serum albumin, (7% w/v; Sigma Chemical Co., St Louis, MO) was initiated 20 min after the second bleeding. The total ischemia-reperfusion period lasted $50 + 10$ min (Fig. 1b).

During the hemorrhagic process, the hemodynamic profile was monitored and 300 µl aliquots of blood were kept for biochemical analysis of analytes to further characterize the changes that take place in the first 24 h following a HS (manuscript in preparation).

2.5. Hemodynamic measurements

The hemodynamic profile was assessed daily at 13:00 h via direct measurement through the implanted vascular catheter over a 3-min recording on calm, resting, conscious rats using a blood pressure transducer (Harvard Apparatus, Montreal, QC, Canada) connected to a pre-calibrated computerized system (PowerLab, ADInstrument, Colorado Springs, CO). Heart rate (HR) in beat-per-min (bpm) was simultaneously derived from these data and recorded.

2.6. Biochemical and hematological analyses

Biochemical analyses include the measurement of plasma and urinary electrolytes (sodium, calcium, magnesium, potassium, phosphorus and chloride), total proteins, total bilirubin, creatinine, glucose, triglycerides (TGs), cholesterol, high density lipoprotein (HDL), blood urea nitrogen (BUN), aspartate aminotransferase (AST), alanine aminotransferase.
(ALT), alkaline phosphatase (ALP), lipase and creatine kinase (CK; also named creatinine (phospho)kinase or CPK) activities via a Vitros 750 XRC analyzer (Johnson–Johnson Clinical Diagnostics, Rochester, NY).

Hematological analyses included measurement of blood hematocrit (Hct), total white blood cell (WBC), red blood cell (RBC) and platelet (Plt) counts assessed using a Coulter GEN*S station (Beckman Coulter, Fullerton, CA). All biochemical measurements were obtained through standardized procedures developed and validated by the Department of Clinical Biochemistry of the Sherbrooke University Hospital (CHUS, Sherbrooke, QC, Canada).

Fig. 2. Hemodynamic profile. (a–b) Mean arterial blood pressure (MABP; mm Hg), (c–d) systolic blood pressure (SBP; mm Hg), (e–f) diastolic blood pressure (DBP; mm Hg) and (g–h) heart rate (bpm) profile in instrumented, unrestrained and conscious Sprague-Dawley rats alongside and following a severe and acute hemorrhagic shock procedure and reperfusion process. Profile over the first 24 h (left panel) and over 10 days (right panel). Gr. 1 (○; n=10), transfused with autologous whole blood. Gr. 2 (◆; n=8–12), re-infused with an isovolumic solution of saline-bovine serum albumin (7% w/v). The hemorrhagic shock was performed on day 7 post-surgical implantation of the arterial vascular catheter and studied for 10 days. *, significant difference between Gr. 1 and Gr. 2 on a given day; †, significant difference within Gr. 1 over time; #, significant difference within Gr. 2 over time.
2.7. Renal clearance

The clearance of creatinine and urea was calculated according to the following formula, based on measurement of each marker in the blood and urine on a given day: 

$$\text{Clearance} = \frac{\text{Urine concentration} \times \text{Urine volume}}{\text{Blood concentration} \times \text{Time}} = \frac{\text{mmol/l} \times \text{ml}}{\text{mmol/l} \times \text{min}} = \text{ml/min}.$$ 

2.8. Statistical analyses

Results of representative measurements were expressed as mean ± S.E.M. Because some observations were sometimes missing for a given time-point, a repeated measurement analysis of variance was inapplicable to the data to compare measures at different time period. Thus, for comparisons within groups, a randomized block design was applied using two factors defined for the analysis: the subject effect and the time period effect.

Comparison between groups was performed by a three-way ANOVA with a blocking factor representing subjects. Interaction between the time period factor and one used to compare groups was added to the model. When interaction was significant for parameters, comparisons at different time period were analyzed using Student’s paired t-tests. The normality and variance assumptions were met for almost all data. All analyses were conducted using the statistical package SAS (SAS Institute Inc, Cary, NC, USA).

3. Results

3.1. Pre- and post-hemorrhagic shock and reperfusion

3.1.1. Hemodynamic profile

The hemodynamic profile was assessed during the first 24 h following HS and reperfusion and subsequently for 10 days in both groups (Fig. 2a–h). The first period of hemorrhage is associated with a >60% decrease (100 ± 3 to 36.5 ± 1.7 mm Hg) in MABP within 5 min (Fig. 2a). During the resting period, the MABP bounced back by about 20% until the second identical period of hemorrhage brought back MABP to 40 mm Hg. Thereafter, the MABP remained stable until the re-infusion period (after a second 20 min ischemic period). A similar profile was observed for SBP and DBP (Fig. 2c,e). Interestingly, the first hemorrhage sequence induced a cardiodepression (32% decrease from 370 to 250 bpm) that paralleled MABP in the first 5 min, followed by a slow and steady recovery during the next 20 min. The second hemorrhagic phase did not reverse the recuperating effect but rather sustained the positive chronotropic effect toward stabilizing HR to pre-hemorrhage baseline values within 50 min (Fig. 2g).

Autologous blood transfusion after 50 min of central ischemia restored blood pressures in hemorrhagic rats within 1–2 h (Fig. 2a,c,e). HR was already normalized by the time of blood reperfusion. Conversely, isovolemic (1:1) infusion of saline-albumin was unable to normalize blood pressures toward baseline (MABP: 66.9 ± 3.7 mm Hg vs. baseline 97.4 ± 3.1 mm Hg)
Hg, 1 h after HS; Fig. 2a,c,e). It took approximately 4–5 days for the blood pressure to return to baseline. Over the next days, blood pressure remained stable in SA reperfused rats (Fig. 2b,d,f) except that HR started to decrease from day 7 onwards (Fig. 2h).

3.1.2. Physiological profile

Food and water intake were not affected by severe and acute HS in rats transfused with AB (Fig. 3b–c). Consequently, body weight increased, gaining 23 g (5.5%) within 10 days, reflecting a normal growth profile (Fig. 3a). At the opposite, SA reperfused rats lay amorphous in their cage which is reflected by decreases in both water (−57%) and food (−83%) intake that peaked within 1 day (Fig. 3b–c). It took those rats 4–5 days to recover. Consequently, they lost body weight (max on day 2; −6%) and were not able to regain the weight lost over the next ten days, maintaining a 5.5% difference with AB-reperfused rats (Fig. 3a). Unlike the other three endpoint parameters, diuresis was not significantly different between the two groups of rats: A 50% increase in urine excretion was observed 24 h post-HS and reperfusion, which was unrelated to water intake (no immediate compensation). Diuresis stabilized thereafter in both groups up to day 10 (Fig. 3d).

3.1.3. Hematological profile

The blood concentrations of Hb, the profile of the Hct and various blood cells’ count (RBCs, WBCs, platelets) remained mostly stable in rats transfused with AB following the ischemic-hemorrhagic shock period, from 1 to 10 days (Fig. 4a–e). Only the RBCs count was reduced by 14% from days 1 to 3 (Fig. 4b). An isovolumic perfusion (1:1) of SA greatly affected these parameters. Hct, [Hb], and RBCs’ count dropped by 55%, 56% and 59%, respectively, within 1 day to slowly recover over 7, 7

Fig. 4. Hematological profile. (a) Hematocrit (Hct; L/L), (b) red blood cells count (RBCs; $\times 10^{12}$/l), (c) circulating blood concentration of hemoglobin ([Hb]; g/l), (d) white blood cells count (WBCs; $\times 10^{9}$/l), (e) platelets count ($\times 10^{9}$/l) and (f) rat survival rate (% of pre-hemorrhage baseline), in instrumented, unrestrained and conscious Sprague-Dawley rats alongside and following a severe and acute hemorrhagic shock procedure and reperfusion process. Gr. 1 (●; $n$= 10), transfused with autologous whole blood. Gr. 2 (○; $n$=8–12), re-infused with an isovolumic solution of saline-bovine serum albumin (7% w/v). The hemorrhagic shock was performed on day 7 post-surgical implantation of the arterial vascular catheter and studied for 10 days. *, significant difference between Gr. 1 and Gr. 2 on a given day; †, significant difference within Gr. 1 over time; ‡, significant difference within Gr. 2 over time.
Fig. 5. Profile of cationic electrolytes. Circulating plasma (right panel; mmol/L) and urinary (left panel; mmol/day) concentrations of (a–b) calcium, (c–d) sodium, (e–f) magnesium, (g–h) and potassium, in instrumented, unrestrained and conscious Sprague-Dawley rats alongside and following a severe and acute hemorrhagic shock procedure and reperfusion process. Gr. 1 (●; n = 10), transfused with autologous whole blood. Gr. 2 (○; n = 8–12), re-infused with an isovolumic solution of saline-bovine serum albumin (7% w/v). The hemorrhagic shock was performed on day 7 post-surgical implantation of the arterial vascular catheter and studied for 10 days. *, significant difference between Gr. 1 and Gr. 2 on a given day; †, significant difference within Gr. 1 over time; #, significant difference within Gr. 2 over time.
and 9 days, respectively (Fig. 4a–c). Platelets also decreased by 35% within 1 day and bounced back within 4 days (Fig. 4e). WBCs were also greatly affected, increasing from day 2 to peak at day 4 (243%), before returning to baseline values within 7 days (Fig. 4d).

3.1.4. Circulating plasma and urinary concentrations of electrolytes

AB transfusion following severe, acute HS did not affect the electrolytic circulation profile (Figs. 5a,c,e and 6a,c), except for potassium, which increased by 114% within 1 day and remained elevated (5.6–5.7 mM between 1 and 5 days) (Fig. 5g). Two cations rapidly increased at day 1 (by 7%; calcium; Fig. 5a) and remained elevated for up to 3 days (by 36%; magnesium; Fig. 5e) in SA-perfused rats. Two anions were also affected in this group of rats with chloride levels increasing after 1 and 2 days (by 4%; Fig. 6a) whereas phosphate levels were significantly decreased from days 1 to 3 by up to 11% (Fig. 6c).

As shown in Fig. 3d, diuresis was increased in both groups of hemorrhaged rats only on day 1. The profile of urine electrolytes is complex (Figs. 5b,d,f,h and 6b,d). Only potassium is unaffected by HS and reperfusion of either solutions (Fig. 5h). The urinary concentrations of calcium and magnesium that were normal on day 1 after HS and reperfusion, were increased from day 5 onward by 3- and 1.5-fold, respectively, regardless of the reperfusion fluids (Fig. 5b,f). However, clear discrepancies were observed between AB transfusion and SA reperfusion regarding sodium, chloride and phosphate, but only on day 1. Both sodium and chloride urinary concentrations decreased by 22% and 52%, respectively, following SA reperfusion (Figs. 5d and 6b) whereas phosphate increased by 2.2-fold (Fig. 6d).

3.1.5. Profile of hepatic functions

Autologous blood transfusion had no effect on hepatic functions (Fig. 7a–e). Circulating plasma concentrations of bilirubin, AST, ALP, ALT and CK remained at baseline values. Reperfusion with SA significantly increased all hepatic biomarkers (1.7-, 15-, 2.6-, 12- and 1.2-fold, respectively) by days 1–2, and remained elevated only up to day 3 (Fig. 7a–e). Survival rates dropped to 73% in SA-reperfused rats between days 3–4 (Fig. 4f) and morbidity was associated with elevated liver enzyme activities.

3.1.6. Profile of renal functions

Autologous blood transfusion following HS protected the rats from renal dysfunctions (Fig. 8a–f) as creatinine, urea and their clearance were unaffected. Conversely, SA reperfusion caused havoc by increasing circulating plasma concentrations of both creatinine (1.2-fold; Fig. 8a) and urea (2.2-fold; Fig. 8b). Those effects were evident for 3 days but decreasing clearance of both markers were observed over a much longer

Fig. 6. Profile of anionic electrolytes. Circulating plasma (right panel; mmol/l) and urinary (left panel; mmol/day) concentrations of (a–b) chloride and (c–d) phosphate, in instrumented, unrestrained and conscious Sprague-Dawley rats alongside and following a severe and acute hemorrhagic shock procedure and reperfusion process. Gr. 1 (n = 10), transfused with autologous whole blood. Gr. 2 (n = 8–12), re-infused with an isovolumic solution of saline-bovine serum albumin (7% w/v). The hemorrhagic shock was performed on day 7 post-surgical implantation of the arterial vascular catheter and studied for 10 days. *, significant difference between Gr. 1 and Gr. 2 on a given day; †, significant difference within Gr. 1 over time; #, significant difference within Gr. 2 over time.
The urinary excretion of urea was first markedly increased (129%) in the SA group of rats on day 1 but reduced (−20%) on day 10 (Fig. 8d). The urinary excretion of total proteins was increased (3.6-fold) only on day 1 by SA reperfusion (Fig. 9f).

### 3.1.7. Profile of lipids, proteins and metabolic functions

Autologous blood transfusion following HS did not affect LPL activity (Fig. 9a) but was associated with a transient increase in circulating plasma concentrations of cholesterol (days 1–5; 115%; Fig. 9b), HDL (days 1–3; 120%; Fig. 9c), TGs (days 3–7; 137%; Fig. 9d) and total proteins (days 3–10; 106%; Fig. 9e). Conversely, SA reperfusion caused a 45-fold increase in LPL activity that lasted 48 h (Fig. 9a). Both circulating plasma concentrations of cholesterol and HDL acutely decreased within 1 day (40% and 33%, respectively) to normalize by day 3 (Fig. 9b–c), correlating with reduced food intake (Fig. 3c). As for the post-hemorrhage and reperfusion profiles of TGs and total proteins, they shadowed those of rats transfused with AB (Fig. 9d–e).

### 4. Discussion

In-vivo animal model systems aimed at assessing blood substitutes are limited (Buehler and Alayash, 2004; Sakai et al., 2004a). The present set of experiments was undertaken to develop, evaluate and validate the use of chronically instrumented, unrestrained and conscious Sprague-Dawley rats as an integrated in-vivo platform allowing hemorrhagic/hypovolemic shock and reperfusion of physiological fluids. This new platform would allow the evaluation of the plasma volume-expanding effect of colloid and crystalloid solutions and to test the safety and
efficacy of novel blood substitutes. Hemorrhagic shock and resuscitation models using conscious small animals are even more limited in number [rats (Schaefer et al., 1983; Darlington and Tehrani, 1997; Lu et al., 1999) and hamster (Sakai et al., 1999)]. These platforms did not integrate as many parameters and analytes as the one profiled here. To complicate matters, these conscious and other anaesthetized models present various degrees of severity (20–30 ml/kg) of hemorrhage (Persson and Grande, 2005) and/or presented, graded, shorter versus longer, and single versus multiple periods of ischemia (5–60 min) and/or reperfusion (10–60 min; Waschke et al., 1996; Carrillo et al., 1998; Lu et al., 1999; Leonov et al., 2002).

Here, we used two groups of rats: transfused with shed autologous blood serving as controls versus isovolumic (1:1) reperfused SA (7% w/v) rats, as previously described (Chang and Varma, 1992, 1994; Buehler et al., 2000). The concentration of albumin solutions usually varies in the range of 5–8.3% (Schultz et al., 1994; Mayer et al., 1998; Persson and Grande, 2005). The present experimental model of severe, acute but controlled hemorrhagic shock was adapted from Chang and Varma (1992, 1994).

The present results show that transfusion of autologous shed blood fully and immediately restored the central hemodynamic profile and prevented post-ressuscitation tissue/organ failure alongside mid-term recovery (10 days). These observations are similar to those reported in other models published elsewhere (anaesthetized, conscious, restrained, and/or unrestrained rats) (Paes-da-Silva et al., 2003; Onen et al., 2003; Sakai et al., 2004b). Conversely, isovolumic reperfusion with SA failed to normalize blood pressures, which took 4–5 days to recover, although it did immediately normalize HR (Yoshizu et al., 2004; Chang and Varma, 1992, 1994).
However, a steady decreased was noted from 7 to 10 days revealing signs of post-HS and ischemia-reperfusion cardiodepression that can be anticipated by early transient (from days 1 to 3, back to normal by day 4) plasma elevation in total CK indicative of injury to the heart and deterioration of cardiac function (Zhong et al., 1999). Elevation in total CK may not be solely reflective of myocardial tissue injury (CK-MB) but also from skeletal muscle as a sign of neuromuscular injury (McDonald et al., 1999). A subsequent vagally mediated, reversible decrease in HR has been documented during central hypovolemia in man (Sander-Jensen, 1991).

The very limited capabilities of SA as a compensatory fluid for volume restoration with no oxygen-carrying ability revealed rapid, transient, hepatocellular dysfunction through elevation of all four hepatic biomarkers measured. The liver is thus very vulnerable to hypoxic insult after trauma-HS and is known to be the first organ to display the signs of injury during HS (Dart et al., 1993; McDonald et al., 1999; Portella et al., 2004; Sharma et al., 2005) and to correlate with increased mortality within the first 4 days post ischemia.

Circulating plasma concentrations of most electrolytes (4 out of 6 but with distinctive profiles) were affected by SA reperfusion: $\text{Mg}^{2+} > \text{Ca}^{2+} = \text{Cl}^{-}$ were increased; $\text{K}^{+}$ was increased whereas $\text{Na}^{+}$ decreased as it did in AB transfused rats. Urinary excretions of $\text{Mg}^{2+} = \text{Ca}^{2+}$ were increased in both groups of rats and only after 5–10 days whereas $\text{K}^{+}$ excretion was unaffected. It reflects a complex balance as previously described (Rocchio et al., 1973). Clearly, the kidney is a very sensitive organ system modulated by HS as diuresis was acutely increased, but only within the first 24 h. Both plasma concentrations of creatinine and urea were also increased (up to 3 days), in both groups of rats. An acute increase in urinary excretion of total proteins was observed only at 24 h post-HS and only in SA reperfused rats. Clearance of these two makers...
were subsequently decreased in both AB (only after 10 days) and SA (within 24 h onward) reperfused rats clearly showing that HS affects renal function (Yu et al., 1992).

In AB transfused rats, RBCs count was slightly reduced probably due to hemolysis after a single 14 ml, 10-min transfusion. The rapid transient increase in circulating WBCs count (intravascular leukocyte accumulation) may be the reflection of compensatory fluids redistribution from the interstitial compartment to the blood, as reported (Keel and Trentz, 2005). Such elevation may lead to polymorphonuclear leukocytes-endothelial cell interactions involved in an inflammatory cascade following HS (Pruefer et al., 2003) but may also be involved in cardiovascular depression (Kapoor and Prasad, 1996), as discussed above. In addition, elevated WBC count may mediate hepatocellular injury through their accumulation, primarily within the centrolobular sinusoids. SA reperfusion was also associated with a drastic but transient decrease in platelet counts for 3 days. Thrombocytopenia, accompanying the hemoconcentration (drop in hematocrit), has not been reported to our knowledge, as platelet adhesion and thrombus formation in the micro-vessels may occur and affect the blood flow of the microcirculation.

Finally, only SA reperfused rats became amorphic, which translated in a transient reduction in food and water intake, weight loss, and concomitant decreased in circulating plasma concentrations of cholesterol, HDL and TGs within 24 h, but not in total proteins, and also a 2-day acute rise in LPL activity that may be related to pancreatic injury. Interestingly, exogenous administration of HDL was reported to attenuate ischemia and reperfusion injury after hemorrhagic shock in rats (Cockerill et al., 2001). The less active AB-transfused, unrestrained and conscious rats inside their metabolic cage did show small fluctuations in circulating plasma concentrations of such lipids (see above) and total proteins between days 1 through 10.

In conclusion, these experiments profiled the versatility and reproducibility of the integrated in vivo platform with chronically instrumented rats as a valuable tool to characterize potential complications associated with severe, acute but controlled HS on a day-to-day basis for up to 30 days (Dalil et al., in press; Nantel et al., in press). Such insight will be helpful for testing the safety and efficacy of volume-expanders and novel blood substitutes.

Thus, the previous hemophysiological responses will be addressed with other diverse resuscitation regimens using this platform. In addition, it remains to be determined what kind, and over what periods of time (acute versus chronic sustained release), a number of vasoactive/pro-inflammatory mediators and other neurohormonal agents are released leading to an unbalance upon hemorrhagic shock and reperfusion, affecting central cardiovascular regulation and peripheral (microcirculation) blood flow, vascular smooth muscle tone, fluid exchange and permeability, and cell (e.g. leukocyte) activation, thus global homeostasis. These elements constitute keys to develop effective and safe HBBS/HBOC. The scaling of active-counteractive physiological responses using the present platform may lead to new approaches for treating hemorrhagic shock (new resuscitation fluids, targets for therapy to reduced side-effects of existing fluids, novel strategies of vaso/immunomodulation).

References


