Prostaglandins & other Lipid Mediators 124 (2016) 34–38

Contents lists available at ScienceDirect

Prostaglandins and Other Lipid Mediators

Original research article

Controlled moderate hypovolaemia in healthy volunteers is not associated with the development of oxidative stress assessed by plasma F_2-isoprostanes and isofurans

Tomas B. Corcoran[a,b], Emilie Mas[b], Anne E. Barden[b,*], L. Jackson Roberts II[c], Trevor A. Mori[b,1], Edmond O'Loughlin[b,d,1]

[a] Department of Anaesthesia & Pain Medicine, Royal Perth Hospital, Perth, Australia
[b] School of Medicine and Pharmacology, University of Western Australia, Perth, Western Australia, Australia
[c] Dept of Pharmacology, Vanderbilt University, Nashville, USA
[d] Department of Anaesthesia, Fremantle Hospital, Fremantle, Western Australia, Australia

ARTICLE INFO

Article history:
Received 26 February 2016
Received in revised form 23 June 2016
Accepted 1 July 2016
Available online 16 July 2016

Keywords:
Venesection
Human
Injury
Surgery
Lipid peroxidation

ABSTRACT

Hypovolaemia can be associated with substantial morbidity, particularly when it occurs in the setting of trauma and in patients with comorbid diseases. Hypovolaemia and inflammation such as occur in the setting of trauma and surgery, are associated with systemic oxidative stress and free-radical injury. Free-radical injury that results from hypovolaemia-induced organ reperfusion may further augment inflammatory processes. It is unknown exactly what proportion of free-radical injury is associated with isolated hypovolaemia as opposed to the contribution from inflammation from surgery or trauma. In the first human study of its kind, we exposed 8 adult male volunteers to venesection-induced hypovolaemia in progressive aliquots of 5% of total blood volume until 20% had been removed. This blood was subsequently reinfused. Plasma F_2-isoprostanes and isofurans, markers of *in vivo* lipid oxidation, were measured by gas chromatography-mass spectrometry at each 5% aliquot venesected and at each 5% reinfused. Between baseline and maximal blood loss there was a minor fall in haemoglobin concentration from 143.9 g/l to 138.8 g/l ($p = 0.004$, 95% CI 2.2, 8.0 g/L). No significant change from baseline occurred in the concentrations of either plasma F_2-isoprostanes or isofurans during venesection ($p = 0.116$ *and* $p = 0.152$, respectively) or blood reinfusion ($p = 0.553$ *and* $p = 0.736$, respectively). We can conclude that in healthy adult volunteers, isolated hypovolaemia to 20% total blood volume loss is not associated with detectable systemic oxidative stress. The free-radical injury identified in surgical and trauma patients may represent the effects of tissue damage and inflammation, with an uncertain contribution from tissue ischemia as may occur with hypovolaemia.

1. Introduction

Hypovolaemia due to bleeding is a common cause of major morbidity and mortality, particularly in young trauma patients and those patients who are taking anticoagulants for medical conditions [1,2]. There are no biochemical markers of hypovolaemia and clinical signs are very poor at detecting blood loss [3]. Much of the morbidity associated with hypovolaemia in patients who survive, is attributable to the consequences of ischemia and the subsequent reperfusion injury.

Ischemia-reperfusion injury (IRI) is one of the best studied pathologies at a cellular and subcellular level. It is a complex process characterised by the elaboration of micromolar concentrations of free radical compounds which are a central cause of many of the adverse sequelae [4]. It is a consequence of the temporary reduction or complete deprivation of blood supply (ischemia) to a tissue or organ followed by the re-establishment of blood flow. In most cases it is tolerated without consequences, and on an individual organ level (e.g. organ transplantation) is a necessary component of therapeutic processes. However, in extreme circumstances, such as in the case of haemorrhagic shock, or in individuals with significant medical comorbidities, it has major adverse consequences not only for the organ or tissue directly affected by the reduction in

* Corresponding author at: University of Western Australia, School of Medicine and Pharmacology, Royal Perth Hospital, Box X2213 GPO, Perth 6847, Western Australia, Australia.
E-mail address: anne.barden@uwa.edu.au (A.E. Barden).
[1] *Joint senior authors.

http://dx.doi.org/10.1016/j.prostaglandins.2016.07.001
1098-8823/

perfusion, but also because it can invoke a systemic inflammatory response. This response is at least in part amplified by the activation of nuclear transcription factors [5], increased nitric oxide production and inducible nitric oxide synthase gene expression [6] and endothelial and complement activation [4]. These processes can cause organ injury remote from the reperfused tissues which may result in multiorgan failure and death [7,8]. The same processes can occur during hypovolaemia without shock. Reperfusion injury has been characterised in virtually all organs with deleterious consequences [9–11], and particularly in vulnerable organs such as brain [12], kidney, lung [13] and the myocardium.

F_2-Isoprostanes (F_2-IsoPs) and isofurans (IsoFs) are sensitive lipid biomarkers of human *in vivo* oxidative stress [14] particularly in the setting of reperfusion as occurs following hypotension/hypovolaemia [15]. They have pathological biological activity and may be both markers and mediators of oxidative stress [16]. In particular, their concentrations may reflect ongoing endothelial injury, platelet activation and vasoconstriction [17], which is important in the context of reperfusion injury in hypovolaemic states. They have an exaggerated vasoconstrictive effect when the endothelium has been injured [18], as happens in reperfusion injury. Trauma patients have been shown to have elevated levels of urinary F_2-IsoPs [19] but the relative importance of hypovolaemia, tissue injury and hypotension in causing this rise has not yet been defined. It is also unknown whether hypovolaemia, such as may occur during surgical procedures with significant blood loss, contributes to free-radical production. The purpose of this study was to determine whether isolated hypovolaemia (controlled progressive hypovolaemia in healthy conscious human volunteers), without concomitant systemic inflammatory insults, produces detectable changes in plasma F_2-IsoP and IsoF levels. We hypothesised that hypovolemia would result in an increase in plasma F_2-IsoP and IsoF levels.

2. Methods

This study was performed in compliance with the principles of the Declaration of Helsinki. It is a biochemical substudy of a trial that received ethics approval from the South Metropolitan Health Service (10/421) and was prospectively registered with the Australian and New Zealand Clinical Trials Registry in May 2011 (ACTRN12611000707965). The process of blood sampling was approved by the Ethics committee in addition to the measurement of clinical parameters. Written informed consent was obtained from all study participants prior to the study. The haemodynamic data which was the primary outcome of the study has been submitted for publication elsewhere [20]. Eight healthy male anaesthetists who were not taking any medications were recruited from the anaesthesia departments of Fremantle and Royal Perth Hospitals, Western Australia. Participant's blood volumes (Nadler Formula) [21] and weights of the 5% blood volume increments to be venesected were calculated. Subjects were instructed to fast from solids for at least 6 h pre-study but to maintain clear fluid intake until 2 h pre-participation in order to be as close as possible to euvolaemic for the start of the study.

Blood was venesected from a 14 gauge antecubital fossa cannula in 5% (of total estimated blood volume) aliquots, with each aliquot drawn over 10 min. The venous sample (5 ml) was collected into tubes containing ethylene diamine tetra-acetic acid (EDTA) and reduced glutathione and butylated hydroxytoluene (BHT), for measurement of F_2-IsoPs and IsoFs. The sample was centrifuged at 4 °C and the plasma stored at −80 °C. The remainder of the aliquot was collected into a Citrate-Phosphate-Dextrose bag weighed using electronic scales tarred to the weight of the bag. Total venesection duration was 40 min, with samples taken at 0, 5, 10, 15 and 20% blood loss. Re-infusion of venesected blood was commenced immediately following 20% blood loss, and each 5% volume of blood was returned over 10 min. Arterial blood gas samples were taken at baseline and at maximum blood loss to assess haemoglobin and PaO_2.

2.1. Measurement of plasma F_2-IsoPs and IsoFs

2.1.1. Chemicals, reagents and chromatography

15-F_{2t}-IsoP (8-isoPGF$_2\alpha$), and 15 F_{2t}-IsoP-d_4 (8-isoPGF$_2\alpha$-d_4) were purchased from Cayman Chemicals (Ann Arbor, MI) and used without further purification. Pentafluorobenzylbromide (PFBBr) and N,N-diisopropylethylamine (DIPEA) were purchased from Sigma Chemicals (St Louis, MO). The silylating agent *N,O-bis*-(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (BSTFA-TMCS, 99:1) (BSTFA) was purchased from Pierce Chemicals (Rockford, IL). Certify II cartridges were from Varian (Lake Forrest, CA). All solvents were of HPLC grade. IsoFs standards were provided by Prof LJ Roberts II, Department of Pharmacology, Vanderbilt University, Nashville, USA [22].

F_2-IsoPs and IsoFs were measured by gas chromatography-mass spectrometry (GC–MS) using electron capture negative ionization and using a modification of our previously reported method [23]. Briefly, internal standard (15-F_{2t}-IsoP-d_4) was added to plasma (200 μl) and samples were hydrolysed with KOH in methanol, acidified and applied to pre-washed Certify II cartridges (Varian). After washing with methanol/water (1:1) and hexane/ethyl acetate (75:25) the F_2-IsoPs and IsoFs were eluted with ethyl acetate/methanol (90:10), dried and derivatised. The F_2-IsoPs and IsoFs were quantitated by GC–MS using 15-F_{2t}-IsoP-d_4 (5 ng) as an internal standard and monitoring ions at *m/z* 569, 573 and 585, for F_2-IsoPs, 15-F_{2t}-IsoP-d_4 and IsoFs, respectively. The intra-assay and inter-assay coefficients of variation were 8% and 5.6% for plasma F_2-IsoPs, and 9%, and 10% for IsoFs.

2.2. Statistical analysis

Participant demographic data are summarised as mean (standard deviation). The observed changes in venous blood composition with venesection was assessed using paired *t*-tests. The effects of hypovolemia and replacement of blood volume on plasma F_2-IsoPs and IsoFs were examined over time after log transformation using a linear mixed model in STATA® with the effects of blood removal and blood replacement examined separately over time. A P value < 0.05 was taken to indicate statistical significance.

3. Results

All participants completed the venesection and reinfusion protocol. Mean age was 36.5 (2.2) years and mean height and weight were 1.79 (0.03) m and 77.1 (7.3) kg respectively. Mean estimated blood volume was 5.20 (0.34) L with an average of 1.04 l (20%) venesected. Most participants described warmth in their chest and some peri-oral tingling associated with the reinfusion of the blood.

The changes in the composition of blood as measured by arterial blood gas analysis are listed in Table 1 with a fall in haemoglobin concentration being the only significant change.

There were statistically, but not clinically, significant changes observed in physical signs with hypovolaemia in this study. Mean heart rate was 7 beats per minute higher by 20% blood loss; 71 vs. 78 beats per minute (p = 0.004, 95% CI of difference 3.5, 11.6 beats/min) and mean systolic blood pressure was higher only at the first time point compared with all levels of blood loss; e.g. 153.5 vs. 144 mmHg (20% blood loss) (p < 0.001, 95% CI of difference 5.3, 13.2 mmHg). More detailed description of the clinical signs and monitoring of this blood loss are previously published [20].