Thrombosis Research 140 (2016) 110–117

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Full Length Article

Aspirin has limited ability to modulate shear-mediated platelet activation associated with elevated shear stress of ventricular assist devices

Lorenzo Valerio [a,e], Phat L. Tran [b], Jawaad Sheriff [d], William Brengle [b], Ram Ghosh [d], Wei-Che Chiu [d], Alberto Redaelli [a], Gianfranco B. Fiore [a], Federico Pappalardo [e], Danny Bluestein [d], Marvin J. Slepian [b,c,d,*]

[a] Department of Electronics, Information, and Bioengineering, Politecnico di Milano, Milan, Italy
[b] Department of Biomedical Engineering, University of Arizona, Tucson, AZ, USA
[c] Department of Medicine, Sarver Heart Center, University of Arizona, Tucson, AZ, USA
[d] Department of Biomedical Engineering, Stony Brook University, Stony Brook, NY, USA
[e] Department of Cardiothoracic Anesthesia and Intensive Care, Istituto Scientifico San Raffaele, Milan, Italy

ARTICLE INFO

Article history:
Received 30 August 2015
Received in revised form 25 January 2016
Accepted 31 January 2016
Available online 1 February 2016

Keywords:
Aspirin
Thrombosis
Platelets
Shear
Ventricular assist devices
Mechanical circulatory support

ABSTRACT

Continuous flow ventricular assist devices (cfVADs) while effective in advanced heart failure, remain plagued by thrombosis related to abnormal flows and elevated shear stress. To limit cfVAD thrombosis, patients utilize complex anti-thrombotic regimens built upon a foundation of aspirin (ASA). While much data exists on ASA as a modulator of biochemically-mediated platelet activation, limited data exists as to the efficacy of ASA as a means of limiting shear-mediated platelet activation, particularly under elevated shear stress common within cfVADs. We investigated the ability of ASA (20, 25 and 125 μM) to limit shear-mediated platelet activation under conditions of: 1) constant shear stress (30 dynes/cm^2 and 70 dynes/cm^2); 2) dynamic shear stress, and 3) initial high shear exposure (70 dynes/cm^2) followed by low shear exposure – *i.e.* a platelet sensitization protocol, utilizing a hemodynamic shearing device providing uniform shear stress *in vitro*. The efficacy of ASA to limit platelet activation mediated via passage through a clinical cfVAD system (DeBakey Micromed) *in vitro* was also studied. ASA reduced platelet activation only under conditions of low shear stress (38% reduction compared to control, $n = 10$, $p < 0.004$), with minimal protection at higher shear stress and under dynamic conditions ($n = 10$, $p > 0.5$) with no limitation of platelet sensitization. ASA had limited ability (25.6% reduction in platelet activation rate) to modulate shear-mediated platelet activation induced via cfVAD passage. These findings, while performed under "deconstructed" non-clinical conditions by utilizing purified platelets alone *in vitro*, provide a potential contributory mechanistic explanation for the persistent thrombosis rates experienced clinically in cfVAD patients despite ASA therapy. An opportunity exists to develop enhanced pharmacologic strategies to limit shear-mediated platelet activation at elevated shear levels associated with mechanical circulatory support devices.
© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Mechanical circulatory support (MCS) devices have emerged as vital life-saving therapeutic systems for failing patients with advanced and end-stage heart failure [1]. Despite their efficacy, MCS systems remain limited by post-implantation thrombotic complications [2]. Continuous flow ventricular assist devices (cfVADs), in particular, are plagued by intra-device thrombus buildup, reduced pump output with recurrent heart failure, and thromboembolic events - *e.g.* stroke, pump stop, and potential death [3]. The high incidence of thromboembolic events in cfVADs is largely due to non-physiological flows within these devices, where platelets, the principal cellular clotting elements in blood, are exposed to extremely elevated shear stresses. In an attempt to limit MCS thrombosis, patients are burdened with complex, life-long anti-thrombotic pharmacologic regimens [4]. The foundation of all regimens employed today is aspirin [5].

Aspirin, *i.e.* acetylsalicylic acid (ASA), has been utilized for over sixty years as a clinical antithrombotic agent [6], with initial use described by Craven [7]. ASA has been shown to inhibit platelet function by permanently acetylating cyclooxygenase (COX), both COX-1 and COX-2 isoforms, responsible for prostaglandin and thromboxane synthesis [8, 9]. Over the years, ASA has been increasingly employed as a vital agent in limiting intra-arterial thrombosis related to atherosclerotic coronary disease, acute coronary syndromes, vulnerable plaque, carotid artery disease and intra-arterial stent use [8, 10–13]. While all of these

* Corresponding author at: Departments of Medicine and BioMedical Engineering, Sarver Heart Center, University of Arizona, 1501 North Campbell Avenue, Tucson, AZ 85721, USA.
E-mail address: chairman.syns@gmail.com (L. Valerio).

http://dx.doi.org/10.1016/j.thromres.2016.01.026
0049-3848/© 2016 Elsevier Ltd. All rights reserved.

conditions impart shear stress to platelets, the intensity of stress, the time of exposure to stress, and the number of platelets exposed is significantly less than the stress accumulation (total dose of shear stress and exposure time) experienced by platelets exposed to cfVADs. Furthermore, the increase in stress accumulation is generally linked with an increase in platelet activation [14, 15].

In the present study we hypothesized that ASA, while an effective antiplatelet agent targeting a biochemical, *i.e.* thromboxane A_2 pathway of platelet activation, will have limited efficacy as an agent modulating shear-mediated platelet activation, particularly at significantly elevated or "hyper-shear" levels common in VADs. As such, utilizing gel-filtered platelets, we first examined the efficacy of ASA to modulate shear-mediated platelet activation *in vitro* – examining exposure to both constant and dynamic shear stress, extracted from platelet flight trajectories obtained from a clinical VAD [16, 17]. Secondly, we examined the ability of ASA to modulate shear-mediated "sensitization," *i.e.* continued platelet activation over time following initial rapid shear exposure – a phenomenon described previously by our group [18]. Finally, we examined the efficacy of ASA to limit VAD-mediated platelet activation *in vitro*, utilizing platelets obtained from normal human volunteers following *in vivo* aspirin ingestion and exposure.

2. Materials and methods

2.1. Platelet preparation

Whole blood (30 ml) was drawn via venipuncture into 3 ml acid-citrate dextrose (ACD-A) from consenting healthy adult volunteers of both sexes who had not taken aspirin or ibuprofen for two weeks, in accordance with a University of Arizona IRB-approved protocol. Whole blood was centrifuged at 500*g* for 15 min to obtain platelet-rich plasma (PRP), which was filtered through a column of Sepharose 2B beads (Sigma-Aldrich, St. Louis, MO, USA) to collect gel-filtered platelets (GFP) [18, 19]. GFP were diluted to a count of 20,000/μl in HEPES-modified Tyrode's buffer, with 3 mM $CaCl_2$ added 10 min prior to experiments [18, 20].

2.2. Exposure of gel-filtered platelets to aspirin

Platelets were treated with ASA dissolved in sodium bicarbonate solution (324 mg ASA, 965 mg citric acid, and 1744 mg sodium hydrogen carbonate in 50 ml double-distilled H_2O), diluted to 20, 25 or 125 μM final concentration, and incubated at 37 °C for 10 min prior to shear exposure. For each ASA-treated platelet sample, a paired control experiment with platelets exposed to solvent vehicle alone (control) was performed on the same day. To verify the consistent reactivity of ASA, GFP were pre-incubated with ASA (125 μM) and then treated with arachidonic acid (AA, 25 μM). Samples for platelet activation measurements were taken at 0, 10 and 30 min.

2.3. Exposure of aspirin-treated platelets to constant and dynamic shear stress

Platelets were exposed to shear stress in the hemodynamic shearing device (HSD), a computer-controlled cone-plate-Couette viscometer that replicates dynamic shear conditions found in blood recirculating devices [14, 15, 21]. GFP were pre-treated with ASA (25 or 125 μM) 10 min prior to shear exposure, which included either constant or dynamic conditions. In the constant shear stress experiments, platelets were exposed to 30 dynes/cm^2 or 70 dynes/cm^2 for a total exposure time of 10 min, with samples taken at 0, 2, 5 and 10 min (Fig. 1a). At each time point, the HSD was slowed down to 1 dyne/cm^2 for 30 s for sampling. For the dynamic experiments, the waveforms utilized were extracted from the probability density function (PDF) of the shear stress conditions found in the DeBakey VAD [16]. This function describes the stress accumulation, or product of shear stress and exposure time, experienced by the platelets and is calculated along thousands of simulated platelet trajectories [16]. The PDF represents the device thrombogenicity "footprint" and highlights potential thrombotic "hotspot" trajectories. For the purpose of the study, we exposed platelets to waveforms corresponding to the 30th and 50th percentiles of the PDF (Fig. 1b). The magnitude of the waveforms was scaled by a factor of 52.5 due to the limitation of the HSD (maximum shear stress of 108 dynes/cm^2 at 1 cP). Shear stress exposure was repeated at 110 passages per min for 10 min. Platelets were sampled at 0, 2, 5 and 10 min. Platelet activation was measured as detailed below.

2.4. Shear-induced sensitization of in vitro aspirin-treated platelets

GFP, prepared as above, were pre-treated with 20 μM ASA 10 min prior to shear experiments. Control GFP were prepared with the addition of the solvent vehicle alone 10 min prior to exposure. Both forms of GFP were then exposed to shear stress in the HSD. Platelets were sheared at 70 dynes/cm^2 for 40 s, followed by a subsequent low shear period of 1 dyne/cm^2, for a total experimental duration of 15 min. Exposure to 1 dyne/cm^2 for 15 min served as the negative shear control. Samples for activation measurements were taken at time 0, 40 s, 3 min, and every 3 min thereafter.

2.5. In vitro flow loop study with platelets pre-treated with ASA in vivo

Healthy adult volunteers were utilized for this study after providing informed consent in accordance with a Stony Brook University IRB-

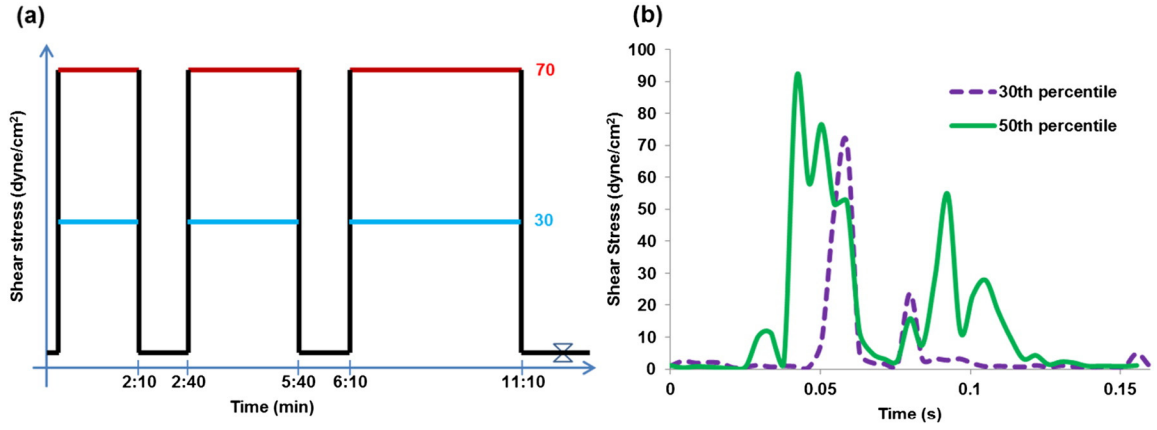

Fig. 1. Constant and dynamic shear stress waveforms. Gel-filtered platelets (GFP) were exposed to a) variable magnitudes of constant shear stress for variable durations and b) dynamic shear stress waveforms representing a single exposure in the DeBakey VAD.

Download English Version:

https://daneshyari.com/en/article/6000565

Download Persian Version:

https://daneshyari.com/article/6000565

Daneshyari.com