JOURNAL OF SURGICAL RESEARCH 195 (2015) 390–395

Available online at www.sciencedirect.com

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Association for Academic Surgery

Shock releases bile acidinducing platelet inhibition and fibrinolysis

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ARTICLE INFO

Article history:
Received 9 December 2014
Received in revised form
15 January 2015
Accepted 23 January 2015
Available online 29 January 2015

Keywords:
Taurocholic acid
Bile salt
Thrombelastography
Fibrinolysis
Clot strength
Platelet dysfunction

ABSTRACT

Background: Metabolites are underappreciated for their effect on coagulation. Taurocholic acid (TUCA), a bile acid, has been shown to regulate cellular activity and promote fibrin sealant degradation. We hypothesize that TUCA impairs whole blood clot formation and promotes fibrinolysis.

Methods: TUCA was exogenously added to whole blood obtained from volunteers. A titration from 250 μM–750 μM was used due to biologic relevance. Whole blood mixtures were assayed using thrombelastography for clot strength (maximum amplitude [MA]) and fibrinolysis (LY30) quantification. Tranexamic acid was used to block plasmin-mediated fibrinolysis. Platelet microfluidics were performed. A proteomic analysis was completed on citrated plasma obtained from a shock and resuscitation rat model.

Results: Fibrinolysis increased when 750-μM TUCA was added to whole blood (median LY30 0.08–5.7, $P = 0.010$) and clot strength decreased (median MA of 53.3–43.8, $P = 0.010$). The addition of tranexamic acid, to a 750-μM TUCA titration, partially reversed the induced fibrinolysis (LY30: without 7.7 *versus* with 2.7) and the decrease in clot strength (MA: without 48.2 *versus* with 53.2), but did not reverse the effects to whole blood levels. Platelet function reduced by 50% in the presence of 100-μM TUCA. Rats had a median 52-fold increase in TUCA, after a shock state that stayed elevated after resuscitation.

Conclusions: TUCA reduces clot strength and promotes fibrinolysis. The clot strength reduction is attributable to platelet inhibition. This metabolic effect on coagulation warrants further investigation, as localized areas of the body, with high levels of bile acid, may be at risk for postoperative bleeding.

Presented at the 10th Annual Academic Surgical Congress in Las Vegas, Nevada, February 4, 2015.

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0022-4804/$ – see front matter © 2015 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jss.2015.01.046

1. Introduction

Bile salts are unique amphipathic molecules that have been found to enhance tissue plasminogen activator-mediated fibrinolysis [1]. Normal levels of bile acids in systemic circulation are on the micromolar level [2]. However, bile acids such as taurocholic acid (TUCA) have been found to be elevated by a 1000-fold during pathologic conditions [3]. *In vitro* TUCA causes angiogenesis and cell proliferation [4]. Elevated blood TUCA levels have also been implicated with heart arrhythmias [5], suggesting that this is a highly active biologic molecule. In rat models, it has been shown that serum TUCA levels markedly rise after acute liver injury [6,7].

Previous studies evaluating the effect of TUCA on coagulation have not used whole blood assays. Hoffman and Monroe [8] have elucidated the cell-based model of hemostasis, emphasizing the key role of platelets. Because of the many receptors on platelets and biologic activity of TUCA, we hypothesize that this bile acid impairs whole blood clot formation via platelet inhibition, promotes fibrinolysis, and that this metabolite can be released after hemorrhagic shock due to oxygen sensitivity of the liver.

2. Methods

2.1. Subjects

After obtaining informed consent under an institutional review board approved protocol (COMIRB # 14-0366), blood samples were obtained from healthy volunteers with no known abnormalities in the coagulation or fibrinolytic system; none of these individuals were taking salicylic acid or other nonsteroidal anti-inflammatory medications within 120 h of the experiment. All subjects were male with a median age of 28 y, range 20–37.

2.2. Taurocholic acid

Lyophilized TUCA, purchased from Sigma–Aldrich (Product # 86,993, St. Louis, MO), was reconstituted to 0.125 M in normal saline. Aliquots of 40 μL of this solution were stored at 4°C until use.

2.3. Thrombelastography TUCA challenge

Blood was collected in 3.3-mL citrated blood tubes from venous puncture. Citrated samples were kept at room temperature and assayed between 20 min and 2 h after blood draw, in accordance with manufacturer recommendations. Whole blood was mixed with TUCA to reach a final volume of 500 μL in individual Eppendorf tubes (Hamburg, Germany). The concentration of TUCA ranged from 0–750 μM. TUCA was added to whole blood mixtures within 5 min of initiating the assay. Citrated native thrombelastography (TEG) assays were recalcified and run according to the manufacturer's instructions on a TEG 5000 Thrombelastograph Hemostasis Analyzer (Haemonetics, Niles, IL). The following parameters were recorded from the tracings of the TEG: R time (s), angle (α, °), maximum amplitude (MA, mm), and lysis 30 min after MA (LY30, %). To detect the presence of plasmin-mediated fibrinolysis, tranexamic acid (TXA) at a concentration of 20 mg/mL was mixed with samples.

2.4. Platelet function assay

A previous validated polydimethlysiloxane (Dow Corning, Midland, MI) flow device [9] was used to create eight evenly spaced flow channels (250 μm in width, 60 μm in height) that are perpendicularly deposited over pre-patterned collagen matrix (250 μm in width). Pro-Arg-chloromethylketone (Haematologic Technologies, Essex Junction, VT) (irreversible thrombin inhibitor, 100 μM) treated (+/- TUCA) whole blood was perfused over collagen fibrillar at a venous shear rate (100 s^{-1}). Dynamic change of platelet (labeled with anti-CD 61 Ab) deposition on collagen was monitored with fluorescence microscopy (Olympus IX81, Olympus America Inc., Center Valley, PA).

2.5. Animal shock resuscitation model

Sprague–Dawley rats (Harlam Laboratories, Indianapolis, IN) were subjected to hemorrhagic shock (IACUC # 90814), and citrated plasma samples were collected for proteomic and metabolic analyses. In brief, rats were anesthetized with pentobarbital and underwent tracheostomy and femoral artery cannulation. Blood was drawn through the femoral artery. After baseline blood was obtained, animals were hemorrhaged to a mean arterial pressure of 25 mm Hg for 30 min, followed by an additional blood draw. Animals were then resuscitated with normal saline, and blood samples were obtained 15 min after animals obtained a mean arterial pressure >30 mm Hg. The volume of resuscitation was limited to <20% of estimated blood volume. A *post hoc* analysis to assess TUCA was preformed on previously run samples corresponding to baseline, shock, and reperfusion plasma.

2.6. TUCA quantification via mass spectroscopy

Plasma samples (10 μL) were immediately extracted in ice-cold lysis and/or extraction buffer (methanol:acetonitrile:water 5:3:2) at 1:25 dilutions. Samples were then agitated at 4°C for 30 min and centrifuged at 10,000*g* for 15 min at 4°C. Protein and lipid pellets were discarded, whereas supernatants were stored at −80°C before metabolomics analyses. Ten microliters of sample extracts were injected into a UPLC system (Ultimate 3000; Thermo, San Jose, CA) and run on a Kinetex XB-C18 column (150 × 2.1 mm internal diameter, 1.7 μm particle size–Phenomenex, Torrance, CA) using a 9-min gradient at 250 μL/min (mobile phase: 5% acetonitrile, 95% 18 mΩ H_2O, 0.1% formic acid). The UPLC system was coupled online with a Q Exactive system (Thermo), scanning in Full MS mode (2 μscans) at 70,000 resolution in the 60–900 m/z range, 4 kV spray voltage, 15 sheath gas and 5 auxiliary gas, operated in negative, and then positive ion mode (separate runs). Calibration was performed before each analysis against positive or negative ion mode calibration mixes (Piercenet; Thermo Fisher, Rockford, IL) to ensure sub parts-per-million error of the intact mass. Taurocholate assignments were performed using the software Maven [10] (Princeton, NJ) on conversion of