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Research Paper

Alditols and monosaccharides from sorghum vinegar can attenuate platelet aggregation by inhibiting cyclooxygenase-1 and thromboxane-A_2 synthase

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ABSTRACT

Q4 *Ethnopharmacological relevance:* Sorghum vinegar is an excellent source of physiological substances with multiple health benefits. We hypothesize that alditols and monosaccharides extracted from sorghum vinegar may inhibit platelet aggregation.

Methods and results: Alditol and monosaccharide extract (AME) from sorghum vinegar was first evaluated for antiplatelet activity using a turbidimetric method. AME showed significant induction of antiplatelet activity by arachidonic acid (AA), collagen, adenosine diphosphate (ADP) and thrombin in a concentration-dependent manner ($p < 0.05$). AME (0.5 mg/mL) reduced the AA-induced aggregation rate to $10.35 \pm 0.46\%$, which was comparable to acetylsalicylic acid (aspirin, ASA) (0.5 mg/mL, $6.35 \pm 0.58\%$), a medical standard. Furthermore, AME strongly inhibited cyclooxygenase-1 (COX1) and thromboxane-A_2 synthase (TXS), and subsequently attenuated thromboxane-A_2 (TXA_2) production. These findings indicated that AME attenuates platelet aggregation through the AA metabolism pathway. Computational docking showed that alditols (L-erythritol, L-arabitol, xylitol and D-sorbitol), monosaccharides (D-glucopyranose, D-fructofuranonse, D-xylopyranose, D-galactopyranose and D-ribose), ethyl glucoside and 3,4-(methylenedioxy)mandelic acid could dock directly into the active site of COX1.

Conclusion: Alditols and monosaccharides from sorghum vinegar inhibit multiple steps in the platelet aggregation pathway, and may be beneficial for the treatment of cardiovascular diseases.

1. Introduction

The aberrant activation of platelets and subsequent platelet aggregation caused by adhesive proteins (such as collagen) and soluble agonists (such as adenosine diphosphate (ADP) and thrombin) play essential roles in the pathogenesis of physiological hemostatic and pathological thrombotic processes, which are commonly associated with cardiovascular diseases such as stroke, unstable angina and reocclusion after angioplasty (Lee et al., 2006; Furie and Furie, 2008). Hence, a promising antiplatelet therapy is critical to prevent the progress of cardiovascular diseases (Warkentin, 2012). Although the use of specific pharmacological agents that modulate platelet activity; e.g., clopidogrel and acetylsalicylic acid (aspirin, ASA), can be used to prevent cardiovascular diseases (Wang et al., 2013), existing therapies have significant drawbacks, including issues related to limited efficacy and safety (Yu et al., 2011). Plant-derived food has a nature-friendly image and various safety merits. Recently, efforts have been made to identify bioactive compounds from food for the prevention of thrombosis and atherosclerotic cardiovascular diseases (Ryu et al., 2009; Endale et al., 2012).

Saccharides are important bioactive components that not only provide carbon and energy to cells but also act as important signaling molecules (Yin et al., 2010). Polysaccharides from algae

Abbreviations: AA, arachidonic acid; ADP, adenosine diphosphate; AME, alditols and monosaccharides extract; ASA, acetylsalicylic acid; COX1, Cyclooxygenase-1; GC–MS, gas chromatography–mass spectrometry; NS, normal saline; PGG_2, prostaglandin G_2; PGH_2, prostaglandin H_2; PPP, platelet-poor plasma; PRP, platelet-rich plasma; TXA_2, thromboxane-A_2; TXB_2, thromboxane-B_2; TXS, Thromboxane-A_2 synthase; WP, washed platelet

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in an in vivo study showed strong anticoagulant and antithrombotic effects (Athukorala and Lee, 2007; Wang et al., 2011). Polysaccharides from *Erigeron Canadensis L.* also significantly inhibited the formation of TXA_2, and thus attenuated platelet aggregation (Pawlaczyk et al., 2011). Recently, extracts from *Laminaria japonica*, garlic and Chuanxiong showed antiplatelet activity in vivo, and these activities were significantly correlated with specific polysaccharides (Wang et al., 2008; Liu et al., 2011; Jin et al., 2013). However, whether the low-molecular-weight saccharides, including monosaccharides and alditols, have effects on platelet aggregation remains unknown.

Vinegar is derived from grain fermentation, and has been used as both a common seasoning and a traditional Chinese medicine (Xu et al., 2003). It has been reported that vinegar contains various substances, including saccharides, organic acid, protein, amino acids, minerals, phenolics, alkaloid and saponin. The saccharide content ranges from 1.1–2.38% (Fan et al., 2011). Vinegar has a wide spectrum of physiological effects, and can affect serum triglyceride levels (Kondo et al., 2009), act as an antiglycemic agent (Johnston and Gass, 2006), have antioxidant activity (Xu et al., 2007; Xiang et al., 2013) and decrease allergenic responses (Armentia et al., 2010). Recently, it was shown that vinegar improves blood fluidity (Yamagishi et al., 1998).

The methanolic extract of aged sorghum vinegar has potent antithrombotic activity and antithrombotic activity in vitro, and this activity is highly correlated with polyphenols and low-molecular-weight substances (Fan et al., 2009; He et al., 2012). In this study, we extracted alditols and monosaccharides from vinegar, and for the first time evaluated the antiplatelet effect of alditol and monosaccharide extract (AME) using agonist-induced platelet aggregation and investigated its mechanism of action, focusing on cyclooxygenase-1 (COX1), thromboxane-A_2 synthase (TXS) inhibition and thromboxane-A_2 (TXA_2) production. In addition, computational docking was used to characterize the binding modes of alditols and monosaccharides with COX1.

2. Materials and methods

2.1. Materials

Blood was collected from healthy volunteer donors aged from 20 to 25 years, with approval from the Beijing Forestry University ethical review committee (Permission number: 2013XL001-3) and the informed consent of all volunteer donors. The volunteer donors fasted overnight before collecting blood from the vein in the morning, and the fresh blood was collected into plastic tubes containing 3.8% sodium citrate and used within 3 h. Sorghum vinegar was obtained from Shanxi Ziyuan Microorganism R&D Co., Ltd. (Shanxi, China) in 2012.

Arachidonic acid (AA), collagen, ADP, thrombin, and bovine serum albumin (BSA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acetylsalicylic acid (aspirin, ASA) was purchased from Alfa-aesar Co. (Tianjin, China). Human cyclooxygenase-1 (COX1) EIA kit, thromboxane-A_2 synthase (TXS) EIA kit and thromboxane-B_2 (TXB_2) EIA kit were purchased from Feng-xiang Biological Co. (Shanghai, China). Macroporous resin was purchased from Nankai University chemical Co. (Tianjin, China). Bis(trimethylsilyl)trifluoroacetamide (BSTFA) and pyridine were purchased from Anpel Scientific Instrument Co. (Shanghai, China). All other chemicals and reagents were of analytical grade.

2.2. AME extraction

Sorghum vinegar (25 mL) was condensed by rotary evaporation to 5% water content, and 50 mL of 60% (v:v) ethanol was added to the viscous residue. After ultrasonication at 60 °C for 60 min, the ethanol was removed from the suspension by rotary evaporation. Subsequently, the crude extract was completely dissolved in deionized water and the total volume was adjusted to 50 mL. AB-8 macroporous resin column (50 cm × 2 cm) was used for the separation and purification of crude extract. The column was first preconditioned with deionized water for 1 h at a flow rate of 1 mL/min, and the crude extract was loaded onto the resin column and washed thoroughly with 100 mL of 60% ethanol (v:v) at a flow rate of 1 mL/min. Desorption solution was used to remove any ethanol by rotary evaporation, followed by freeze-drying. The dried extract was named alditols and monosaccharides extract (AME) and stored at −20 °C for further study.

2.3. AME compositional analysis

AME composition was analyzed using gas chromatography–mass spectrometry (GC–MS). AME (1 mg) was silylated by dissolving in BSTFA (100 μL) and pyridine (50 μL), after which the mixture was ultrasonicated at 60 °C for 30 min. After silylation, AME was analyzed using QP2010A GC–MS (Shimadzu, Shimane, Japan) with a RTX-5 fused silica capillary column (30 m × 0.25 mm × 0.25 μm, Shimadzu, Shimane, Japan). The column temperature was initially held at 80 °C for 1 min, after which it was increased to 220 °C at a rate of 10 °C/min and from 220 °C to 310 °C at a rate of 20 °C/min and held for 6 min. Ultrahigh purity helium with an inline Alltech oxygen trap was used as carrier gas. The carrier gas was set at 40 psi with a column head pressure of 8 psi. Injector temperature was maintained at 280 °C, and the injection volume was 1.0 μL in splitless mode. The interface temperature was held at 280 °C. Mass spectra were scanned from *m/z* 50–650 at a rate of 1.5 scans/s. Electron impact ionization energy was 70 eV.

Compounds in AME were identified based on the gas chromatographic retention times and mass spectra, and by referring to the NIST11 MS Library (SIS, USA). Compounds were quantified based on the peak areas of the identified compounds.

2.4. Preparation of platelet-rich plasma

Platelet-rich plasma (PRP) was prepared as described previously with slight modification (Kim et al., 2011; Yu et al., 2011). Fresh blood was collected in plastic tubes containing 3.8% sodium citrate. The ratio of blood to sodium citrate was 1:9 (v:v). PRP was obtained by centrifugation of blood at 150*g* for 10 min at room temperature (25 °C). Supernatants (PRP) were used for the aggregation study. To prepare washed platelets (WP), blood was directly collected into plastic tubes containing anticoagulant citrate dextrose (ACD: 2.5% trisodium citrate, 2% dextrose, and 1.5% citric acid) and adjusted to a ratio of 1:9 (v:v). After centrifugation at 150*g* for 10 min at room temperature, the platelets were isolated and centrifuged again at 150*g* for 10 min in washing buffer (Tyrode's solution containing 10% ACD buffer and 0.3% BSA). Platelets were diluted with Tyrode's solution (NaCl 11.9 mM, KCl 2.7 mM, $MgCl_2$ 2.1 mM, NaH_2PO_4 0.4 mM, $NaHCO_3$ 11.9 mM, glucose 11.1 mM, and BSA 3.5 mg/mL, pH 7.2) containing 0.3% BSA. To make platelet-poor plasma (PPP), PRP was centrifuged at 1,200*g* for 10 min at room temperature and supernatants were obtained as PPP.

2.5. Antiplatelet activity of AME

Antiplatelet activity of AME was examined as described previously with slight modification (Seo et al., 2012). PRP was adjusted with PPP to obtain a platelet count of 2×10^8 platelets/mL. AME and ASA were dissolved in 0.9% normal saline (NS) to the appropriate concentrations. PRP was pre-incubated with AME

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