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Research Paper

Effects of *Cydonia oblonga* Miller extracts on blood hemostasis, coagulation and fibrinolysis in mice, and experimental thrombosis in rats

Wenting Zhou [a,1], Adil Abdurahman [a,1], Anwar Umar [a,b,*], Guldiyar Iskander [a], Elzira Abdusalam [a], Benedicte Berké [b], Bernard Bégaud [b], Nicholas Moore [a,b,**]

[a] Department of Pharmacology, Xinjiang Medical University, 830011 Urumqi, Xinjiang, People's Republic of China
[b] Department of Pharmacology, Université Bordeaux Segalen, F-33076 Bordeaux, France

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ABSTRACT

Introduction: *Cydonia oblonga* Miller (COM) is traditionally used in Uyghur medicine for the prevention of cardiovascular disease. The present study is designed to explore the effects of COM extracts on models and markers of thrombosis and related biomarkers.

Materials and methods: 20, 40, 80 mg/kg/day COM aqueous extracts and 5 mg/kg/day aspirin, orally for 14 days were compared to untreated controls in mice on bleeding and clotting times, using the tail cutting and glass slide methods and for death rates in collagen–epinephrine pulmonary thrombosis, thrombolysis in vitro and euglobulin lysis time (ELT). In rats, common carotid artery $FeCl_3$-induced thrombus and inferior vena cava thrombosis occlusion time, plasma concentrations of thromboxane B_2 (TXB_2) and 6-keto-prostaglandine $F_1\alpha$ (6-keto-PGF1α) were measured.

Results and conclusion: Compared to controls, COM extracts dose-dependently prolonged bleeding by 2.17, 2.78 and 3.63 times, vs. aspirin 2.58, and the clotting time by 1.44, 2.47 and 2.48 times, vs. aspirin 1.91. COM reduced pulmonary embolus mortality by 27, 40 and 53%, vs. 47% for aspirin. COM dose-dependently increased thrombolysis by 45, 55 and 63%, vs. 56% for aspirin, and shortened ELT to 71, 61 and 43%, vs. 43% for aspirin. In rats, venous occlusion time was prolonged. Arterial and venous thrombus weights were dose-dependently reduced in COM groups. TXB_2 decreased and 6-keto-PGF1α increased with COM and aspirin, with an association between 6-keto-PGF1α/TXB2 and arterial or venous thrombus weight for all products, and for occlusion time with COM but not for aspirin.

Conclusion: We confirm the experimental effects of COM on hemostasis and thrombosis. Further exploration of putative clinical effects appear justified.

1. Introduction

Cydonia oblonga Mill. (COM), the common Quince, belonging to the Rosaceae Family, Cydonia Genus (Sadik, 1993), has been used in traditional medicine for a long time in Xinjiang. It is called "Biye" in Uygur, or Man Tan, and Quince in English.

The chemical composition of COM is rich. Ripe fruits contain fructose, tannin, protopectin, organic acids, amino acids and other ingredients; pulp contain tetradecylic acid, vaccenic acid glyceride, volatile oil, flavonoids, etc.; pericarps contain heptyl ethyl ether and nonyl ethyl ether, etc. Seeds contain mostly mucilage, amygdalin, fatty oil, etc.; leaves contain alkaloids, glycosides, tannins, mucilage, ketose, aldose, lipids, vitamin C, cyanogenetic glycoside, etc. (Nanjing, 1997; Kurban, 2004). COM fruit are used to make jam and jelly in the western world. In China, the use of COM for medicine or food has not been developed. In traditional Uyghur medicine, COM leaves are used as a decoction to reinforce the individual's defenses, and prevent cardiovascular diseases among other virtues (Xakir et al., 2006). Interestingly, it is also commonly used in Turkey (Kultur, 2007) to treat or prevent diabetes and seems to have antidiabetic

Abbreviations: COM, *Cydonia oblonga* Mill.; ELT, euglobulin lysis time; TXB2, thromboxane B2; SPF, specific pathogen-free; RIA, radioimmunoassay; SD, standard deviation

* Corresponding author at: Department of Pharmacology, Xinjiang Medical University, 830011 Urumqi, Xinjiang, People's Republic of China. Tel./fax: +86 991 43 62 421.
** Corresponding author at: Department of Pharmacology, University of Bordeaux Segalen, 33076 Bordeaux, France. Tel.: +33 557571560; fax: +33 557574671.
E-mail addresses: Anwar.umar@126.com (A. Umar), nicholas.moore@pharmaco.u-bordeaux2.fr (N. Moore).
[1] Both are equal contributors to this work.

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and antioxidant effects (Aslan et al., 2010). In previous studies we found an effect of COM on lipid metabolism in hyperlipemic rats (Abliz et al., 2014), and on blood pressure in a renal hypertension model in rats (Zhou et al., 2014).

Because of its traditional use in the prevention of cardiovascular disease, and the societal importance of such diseases, and having shown the effects of COM on the other main risk factors for cardiovascular diseases, lipids and hypertension we decided to test the effects of COM on coagulation and thrombosis, the endpoint of coronary disease. This was tested in vitro for coagulation and fibrinolysis in vivo, and on models of thrombosis and related biomarkers in mice and rats (Jiang et al., 2007), using common models (Umar et al., 2003b; Umar et al., 2004; Tohti et al., 2006), compared to the standard antiplatelet agent aspirin.

2. Materials and methods

2.1. Apparatus, materials, animals

2.1.1. Drugs and reagents

Leaves of *Cydonia oblonga* Mill. were collected in October 2010 from the Kashgar area in Kargilik County, Xinjiang, PRC, dried and crushed then set aside. Aqueous extract of COM (Department of Pharmacology, Xinjiang Medical University, China) was obtained by steeping the dried and crushed fresh *Cydonia oblonga* Mill. leaves in water at 60 °C three times, once for two hours and twice one hour before first drying and then freeze-drying the extract thus obtained. One gram powder was equivalent to about 1.6 g crude leaves. *Cydonia oblonga* Mill. leaves were identified by Prof. Parida Abliz, and deposited in the herbarium of the Traditional Chinese Medicine Ethnical Herbs Specimen Museum of Xinjiang Medical University under number NO.TCMEHSM2013_100.

Other drugs and reagents were Aspirin, collagen (Sigma Co, USA), Adrenaline Hydrochloride Injection (Xinzheng Pharmaceutical Co., Ltd., Tianjin, China), urethane, barbital (Miura Chemical Co., Ltd., Shanghai, China), sodium citrate (Beijing Chemical Plant, Beijing, China), 6-keto-PGF 1α RIA kit, and TXB_2 RIA kit (Puerwei biotechnology Co., Ltd., Beijing, China).

2.1.2. Instruments

RE-52 Rotary Evaporator (Yarong Biochemical Instrument Factory, Shanghai, China), DK-S24 digital electric thermostat water bath (Jinyun Medical Instrument Factory, Jiangsu, China), SK8210HP ultrasonator (Kedao Instrument Factory, Shanghai, China), BL1500 electronic balance (Sartorius Instrument Co., Ltd., Beijing, China), KH-120M desktop high-speed centrifuge (Anting Science Instrument Factory, Shanghai, China), analytical balance (Mettler – Toledo Instrument Factory, USA), and Automatic RIA analyzer (USTC affiliated Zhongjia Co., Ltd., Anhui, China).

2.1.3. Animals

Male ICR mice weighing 18–22 g and male Wistar rats weighing 300–350 g, both specific pathogen free (SPF), were provided by the Experimental Animal Center of the Xinjiang Medical University (certificate number SYXK2003-0001).

2.2. Methods

All animals received care in compliance with the Chinese Convention on Animal Care, and the study was approved by the Institutional Ethics Committee (No. IACUC – 20130129016).

2.2.1. Experiments in mice

2.2.1.1. Treatment groups. In each of the following experiments, male ICR mice, weighing 18–22 g were randomly divided into groups of 10–15 animals each, treated as follows:

One group of mice received only neutral saline preparation (control group).
Three groups received COM extract at the dose of respectively 20, 40 and 80 mg/kg (low, medium, high doses).
One group received aspirin 5 mg/kg.
All drugs were given orally once daily for 14 days, dissolved in water.
After the last administration, mice were anesthetized with urethane.

2.2.1.2. Bleeding time. One hour after the last administration, the tail was cut 5 mm from the end. Timing was started when the blood flowed. The tail end was blotted every 15 s until the end of bleeding (natural stop of blood flow) which was the bleeding time (McKay et al., 1971; Li, 2004; Xu et al., 2005; Furie and Furie, 2008; Stoll et al., 2008).

2.2.1.3. Clotting time in vitro. One hour after last treatment administration, an eye was rapidly removed with ophthalmic elbow tweezers, two drops of blood were dropped at the end of a slide and timing immediately started with a stopwatch. One of the drops of blood was pricked lightly with a pin every 30 s. When a blood streak appeared, timer was stopped to record the whole blood clotting time. The other drop of blood was used for retesting (Li, 1991).

2.2.1.4. Collagen–epinephrine induced pulmonary thrombosis. Thirty minutes after the last drug administration, acute pulmonary thromboembolism was induced by 0.2 ml of collagen and epinephrine 10:1 mg/kg injected into the tail vein of the mice (Umetsu and Sanai, 1978; DiMinno and Silver, 1983; Yu and Hu, 1997; Zhou et al., 2006). The number of the deaths was counted within 15 min. The inhibitory rate was computed using the following formula: Inhibition = ((number of deaths in treated − number of deaths in controls)/number of deaths in controls) × 100, eventually adjusted for different number of animals per groups.

2.2.1.5. Thrombolysis in vitro. One hour after last treatment administration, an eye was rapidly removed with ophthalmic elbow tweezers, the blood was dropped in a plastic tube with a precisely weighed 3 cm-long No. 4 surgical silk thread, left standing for 10 min, then the blood was coagulated. The silk thread was then pulled out with the thrombus sticking on gently, cut into 2 cm sections and placed in a tube, 2 ml of a solution of normal saline, containing COM 4 g/L, 2 g/L, or 1 g/L, aspirin 0.25 g/L, or Ginkgo biloba 2 g/L, were added and left standing for 24 h at 37 °C, before measuring the thrombolytic rate (Pang et al., 1996).

2.2.1.6. Euglobulin lysis time (ELT). One hour after the last drug administration, 1 ml blood was drawn from the retro-orbital venous plexus. 3.8% sodium citrate was added in the test tube and the ratio of anticoagulant and blood was strictly controlled to 1:9. The blood was centrifuged at 3000 rpm for 10 min, then 0.25 ml plasma was separated, 4.5 ml distilled water and 0.05 ml 1% acetic acid were added in turn, then adjusted to pH = 9. After it was fully mixed, the mixture was placed in a refrigerator at 4 °C for 10 min. The mixture was centrifuged at 3000 rpm for 10 min and the euglobulin was precipitated. The supernatant was removed and the tube placed upside down on filter paper to absorb