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Antiplatelet effects of *Angelica dahurica* extracts in rat platelets

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Introduction

Platelets play an important role to hemostasis and repair at the site of vascular damage lead to platelet aggregation and forms platelet plugs that stopping hemorrhage.[1] However, the excess platelet plug are the development of thrombus formation and will progress to cardiovascular disease including coronary artery disease (CAD), stroke and peripheral artery disease (PAD). The World Health Organization (WHO) report in 2557 found that heart disease and stroke are the number one of result of death worldwide, about 17.3 million people and tend to increase. While Thailand Ministry of Health found 54,530 people died from cardiovascular disease in 2556, which is the second inferior to cancer. Platelets mediate the initiation of thrombosis through platelet adhesion, activation and aggregation. [2] Thus, the inhibition of platelet function has the potential to treat circulatory diseases, spurring the development of many antiplatelets and prompting an examination of their effects in antiplatelet aggregation.

Angelica dahurica (Fisch. Ex Hoffm.) Benth. & Hook.f.ex Franch & Sav. (AD) is named as Bai Zhi in China and Kot Sor in Thailand. AD is used for the treatment of headache, toothache, cold and neuralgia in Chinese traditional medicine.[3] In Thai traditional medicine Kot Sor is used for circulatory disorder.[4] Therefore, the aim of this study was to investigate the antiplatelet aggregation activity of AD extract in rat platelets.

Methods

Angelica dahurica extract (ADE)

Dried root of *A. dahurica* was purchased from a traditional drugstore in Bangkok, Thailand during 2015. Its ground powder (1 kg) was extracted with 50% EtOH (20 L) for 2-3 hours for 2 times. The combined extract was evaporated to dryness to obtained ADE for 15%. ADE was dissolved in DMSO for stock solution and diluted to the final concentration of 0.1, 0.25, 0.5 and 1 mg/ml with normal saline.

Animals

Male Sprague-Dawley rats, weight 260 - 340 g, were purchased from the National Laboratory Animal Center, Mahidol University, Salaya, Thailand. Animals were acclimatized to standard laboratory conditions (25±2°C, 55- 60% humidity and 12 h light/dark cycle) for 5 days in the animal facility in Faculty of Pharmaceutical Sciences, Chulalongkorn University. Food and water were given ad libitum. All animal protocol received approval from committee of the Faculty of Pharmaceutical Sciences, Chulalongkorn University.

In vitro antiplatelet aggregation activity [5]

Rats were anesthetized using pentobarbital (i.p., 50 mg/kg body wt.). Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were prepared as follows: rat blood from Heart was anti-coagulated with 3.8% sodium citrated (9:1, v/v), and centrifuged at 170 × g for 15 minutes at 21 °C to get PRP. The precipitate was further centrifuged at 350 × g for 10 minute at 21 °C to yield PPP. PRP was incubated with 0.5% DMSO or aspirin at the concentration of 0.5 and 1 mM (positive control) or ADE at the concentration of 0.1, 0.25, 0.5 and 1 mg/ml at 37 °C for 3 minutes. Then the aggregation-inducing agent, ADP, was added. Aggregation was measured using a platelet aggregometer. The percentage of inhibition of platelet aggregation was calculated by using the following equation:

$$\% \text{ inhibition} = [(A-B) \div A] \times 100$$

A is the maximum aggregation rate of vehicle-treated PRP, whereas B is the maximum aggregation of ADE-treated PRP.

In vitro COX-screening assay [6]

ADE were incubated directly with COX-1 or COX-2 in assay buffer for 5 min. Then, AA was added as substrate and ADHP (10-acetyl-3,7-dihydroxyphenoxazine) for 2 min. The reaction between prostaglandin G_2 (PGG_2) and ADHP

produces the highly fluorescent compound resorufin. Resorufin fluorescence can be analyzed with an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm. The effect of ADE on COX activity were evaluated by Inhibition rate and calculated as follow:

Inhibitory rate (%) = (Initial activity – sample activity) x 100 / Initial activity.

Statistical analysis

All data were expressed as the means ± standard errors of mean (S.E.M). Differences between groups were assessed by one-way analysis of variance. If there were significant differences among group means, then each group was compared using Tukey method using the SPSS software (Version22). P-values less than 0.05 were accepted as statistical significance.

Results

***In vitro* antiplatelet aggregation activity**

To assess the effect of ADE on platelet aggregation, the platelet aggregation induced by ADP in rat platelet was measured (Figure 1) and expressed as % inhibition (Figure 2). ADE at concentration of 0.1, 0.25, 0.5 and 1 mg/ml showed % inhibition of ADP-induced platelet aggregation by -3.63±4.70%, -0.15±1.76%, 8.36±2.36% and 25.51±3.84% ($p<0.05$), respectively. The results indicated that ADE at 1 mg/ml significantly inhibited platelet aggregation and comparable to 0.5 or 1 mM ASA (14.71±1.91% and 34.53±2.57%) respectively.

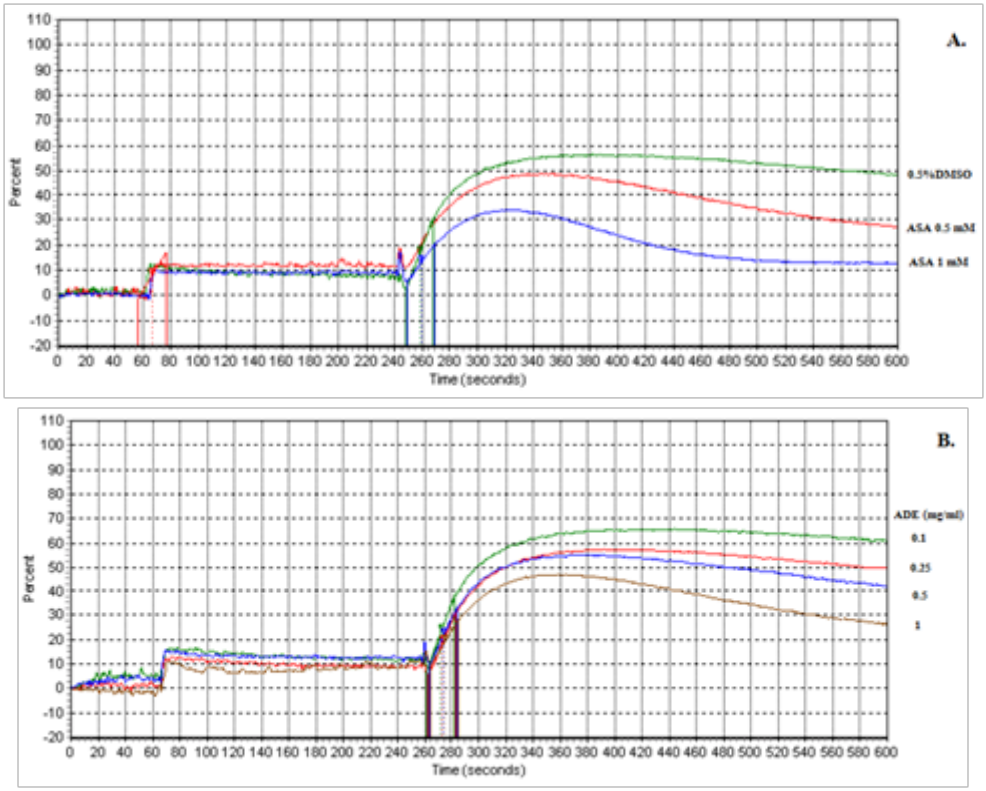

Figure 1. Aggregram showed the effect of 0.5% DMSO, ASA at 0.5-1 mM (A) and ADE at 0.1-1 mg/ml (B) on ADP-induced platelet aggregation.

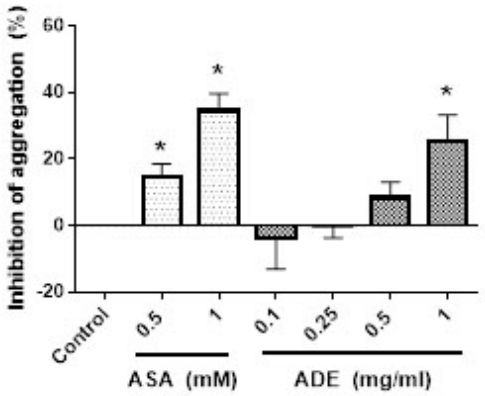

Figure 2. Inhibitory effect of ADE on ADP-induced platelet aggregation. Results showed as mean±SEM (n=4).* Significant difference from vehicle control at $p<0.05$.

***In vitro* COX-screening assay**

ADE has an Inhibitory effect against both isoforms of cyclooxygenase (COX). But inhibitory effect on COX-2 is more potent than COX-1 enzyme as shown in figure 3.

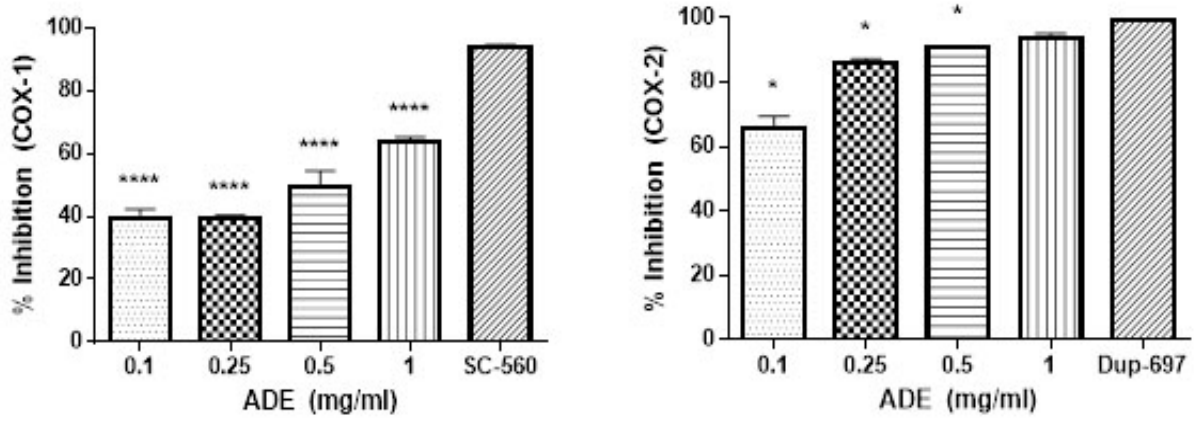

Figure 3. Direct effect of various concentration of ADE on the enzymatic activities of COX-1 and COX-2. Results showed as mean±SEM (n=3). (* P ≤ 0.05 ** P ≤ 0.01 *** P ≤ 0.001 **** P ≤ 0.0001 *vs* positive control)

Discussion

Many life-threatening diseases, such as atherosclerosis, cerebrovascular, coronary artery disease, stroke and tumour metastasis are related with platelet dysfunction.[7] Many available antiplatelet agents interfere with platelet function at various levels of activation, which results in several clinical side effect including gastrointestinal side effect and hemorrhage. For this reason, a search for safer and more effective antiplatelet agents without these adverse effects is still explored.

In this study, we examined the antiplatelet effect of ADE on platelet function by measuring ADP-induced platelet aggregation *in vitro*. The results show that ADE 1 mg/ml has antiplatelet activity on ADP induced aggregation. Thus ADE might inhibit platelet aggregation through ADP-mediated signaling pathway. Since ADE has antiplatelet activity, then it might be a potential Thai traditional medicine to use as antiplatelet therapy. However, in case of using antiplatelet drug such as aspirin for secondary prevention of coronary artery disease or stroke, it should be aware of herb-drug interaction between Thai traditional medicines and antiplatelet or anticoagulant.

In addition, this study also evaluated the effect of ADE on COX-enzyme activity. The result indicated that ADE could inhibit both COX-1 and COX-2 enzyme. The COX-1 is responsible for the synthesis of thromboxane A_2 (TXA_2) which is the potent aggregating substance.[8] COX-1 is constitutively expressed in most tissue including platelet and gastrointestinal mucosa. it is thought to exert homeostatic properties that are crucial for vascular and gastric physiologic functions. COX-2 is absent from most healthy tissue but it is induced by proinflammatory. It has been reported that ADE has antiinflammatory activity by suppressed COX-2 expression.[9]

Conclusion

The ADE can inhibit platelet aggregation induced by ADP in rat platelets. The inhibitory effect on platelet aggregation might be in part acted *via* ADP-mediated signaling pathway. ADE can inhibit both COX-1 and COX-2 activity. The *in vivo* model in rat and the mechanism of action of antiplatelet aggregation should be further investigated.

Acknowledgements

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