REVIEW

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Possible antithrombotic effects of *Angelica keiskei* (Ashitaba)

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Angelica keiskei Koidzumi (Ashitaba) is a large perennial herb that is native to the Pacific coast of Japan, and it has recently become popular as herbal medicine, dietary supplement and health food in Asian countries. The structures of various constituents isolated from Ashitaba such as chalcones, flavanones and coumarins have been precisely characterized, and many of them have bioactivities. A recent study clarified that *Angelica keiskei* exerts actions that lead to the prevention of thrombosis. Here, we introduce the possibility that ingesting Ashitaba could help to prevent thrombotic diseases.

1. Introduction

The number of people with metabolic syndrome is increasing in Japan along with westernization of the diet and change of life styles. Myocardial infarction, cerebral infarction and venous thromboembolism are classified as thrombotic diseases that are caused by blood flow stasis due to thrombus formation and the numbers of such conditions are increasing concomitantly. Evidence has accumulated for an association between atherothrombosis and metabolic syndrome (Goldhaber 2010; Grundy et al. 2005). This is because decreased blood fluidity caused by elevated blood sugars and lipids, platelet activation, increased activities and amounts of blood coagulation factors, suppression of the blood fibrinolysis system and other processes are enhanced in metabolic syndrome. The risk of thrombotic diseases due to lifestyle habits can be reduced not only by pharmacotherapeutics but also by dietary improvement and moderate exercise. In addition, risk for thrombotic diseases should be further reduced if healthy blood and blood vessels can be brought about by the consumption of health foods or dietary supplements. Since health foods and dietary supplements are already popular, the science behind those that might prevent blood clots should be investigated.

We have recently focused on herbal medicines and health foods that might prevent thrombosis, and determined that *Angelica keiskei* exerts antithrombotic actions. Here, we introduce the possibility of preventing thrombotic diseases by ingesting *Angelica keiskei* (Ashitaba).

2. Ashitaba and chalcones

Ashitaba (*Angelica keiskei* Koidzumi) is a large perennial herb of the Apiaceae genus *Angelica* (Shiidida genus) that is native to the Pacific coast of Japan (Izu Islands and the Izu, Bōso, and Miura peninsulas). Ashitaba is a very vigorous plant that might have been germinated from volcanic ash during the eruption of the Miyake-jima Islands in 2000. Ashitaba has been consumed as a vegetable and used as a folk medicine since ancient times. It is now popular as herbal medicine, health food and dietary supplement in forms such as Ashitaba powder and Ashitaba exudate powder.

Ashitaba leaves, stems and roots contains abundant nutrients such as vitamin A, vitamin K and dietary fiber, as well as various flavonoids such as chalcones, flavanones and coumarins. The exudate from the cut ends of stems notably contains large amounts of a yellow substance that is unique to Ashitaba. Ashitaba is presently cultivated and consumed in Asian countries including Korea, China and Taiwan. Various constituents of Ashitaba including chalcones, flavanones, and coumarins have been isolated and structurally characterized, and several bioactivities have been described (Kil et al. 2017; Caesar et al. 2016). Baba et al. isolated 10 chalcones from Ashitaba and revealed their structures (Kozawa et al. 1977) (Table). The chalcones, xanthoangelol (XA) and 4-hydroxydelicin (4-HD), account for > 90% of all chalcones identified in Ashitaba. The remainder comprises trace amounts of xanthoangelols B, C, D, E, F, G, H, isobavachalcone and several other compounds. Chalcones are found in leaves, stems and roots and are particularly abundant in yellow exudates from cut ends of stems.

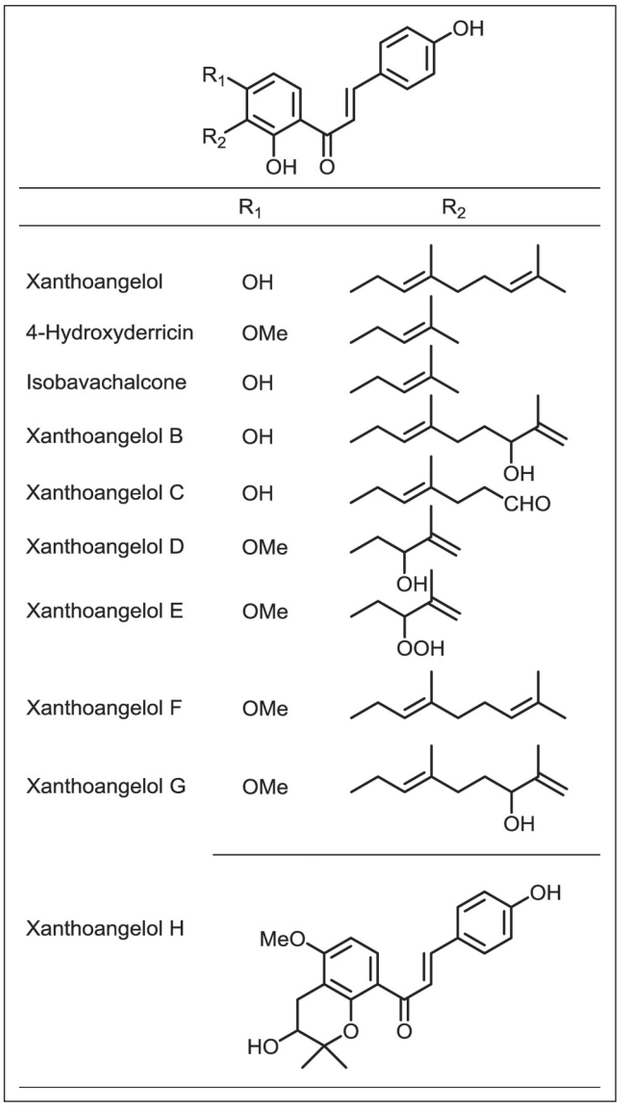
Studies of the physiological activities of Ashitaba were initially started based on folklore transmitted from the Izu Islands. The first finding was that chalcones from Ashitaba might have anti-tumor activity and then, gastric acid secretion suppressive effects and antimicrobial activities were identified (Okuyama et al.1991; Murakami et al. 1990; Inamori et al. 1991). Thereafter, the physiological activities of the major chalcones, XA and 4-HD were determined (Inamori et al. 1991; Murakami et al. 1990; Kimura et al. 2003; Kimura et al. 2004; Shin et al. 2011; Ogawa et al. 2005; Ogawa et al. 2007). After it was found that Ashitaba might be able to suppress high blood glucose and exert anti-obesity effects (Enoki et al. 2007), it received attention as a health food and dietary supplement that might improve lifestyle diseases such as obesity and diabetes.

3. Does Ashitaba exert antithrombotic effects?

Xanthoangelol E inhibits thromboxane B_2 (TXB_2) synthesis in rabbit platelets, suggesting that it might also inhibit platelet aggregation (Fujita et al. 1992). This finding seems to be the source of the popular belief that Ashitaba has antithrombotic activity; that is, it can reduce blood clot formation by regulating the hemostatic system in humans.

However, the hemostatic system comprises platelets, coagulation factors and the fibrinolytic system. Antithrombotic activity generally refers to antiplatelet action and the anticoagulant action of plasma, and occasionally fibrinolytic activity. In general, antithrombotic substances comprise anticoagulants that halt the coagulation system and interfere with further clot expansion, antiplatelet substances that decrease platelet aggregation and inhibit thrombus formation, and fibrinolytic enzymes that directly dissolve thrombus. Therefore, to determine whether or not natural products and synthesized substances exert anti-thrombotic effects,

Table: Ashitaba and chalcones



their actions on blood coagulation factors, platelets, and the fibrinolytic system should be investigated *in vitro* and *in vivo*. However, Fujita et al. found that only xanthoangelol E, of which only trace amounts are found in Ashitaba, inhibited the production of TXB_2 and 12-hydroxy-5,8,10-heptadecatrienoic acid from exogenous arachidonic acid *in vitro*. Hence, whether or not this Ashitaba chalcone inhibits platelet aggregation, whether it exerts antithrombotic activity *in vivo* has remained unclear.

4. Suppression of platelet aggregation by Ashitaba chalcones

Compounds isolated from ethyl acetate extracts of Ashitaba roots were identified as the known chalcones, XA and 4-HD that had already been isolated from Ashitaba. Son et al. (2014) also found that XA and 4-HD inhibited platelet aggregation induced by collagen, platelet activating factor (PAF) and phorbol 12-myristate 13-acetate, but not that induced by thrombin using aggregation assays of washed rabbit platelets. This indicates that XA and 4-HD are more likely to inhibit PLCγ-related, rather than PLCβ-related activation pathway in platelets (Son et al. 2014).

Our blood platelet aggregation assays also concurrently showed that Ashitaba chalcones inhibit platelet aggregation (Ohkura et al. 2016). Platelet aggregation was inhibited in whole blood incubated with XA or 4-HD and stimulated by collagen. We investigated the ability of Ashitaba exudate to suppress mouse tail bleeding to determine platelet function *in vivo* (Ohkura et al. 2016). The hemostatic function of platelets *in vivo* can be determined by measuring the amount of time required to stop bleeding from a small wound in the mouse tail (Beviglia et al. 1993). Mice were orally administered with Ashitaba exudate for 1 week once a day followed by lipopolysaccharide (LPS) to induce a thrombotic tendency. The amount of time required to stop bleeding from the tip of the tail was measured after complete anesthesia was induced. Less time was required to stop the bleeding in mice stimulated with LPS than in control mice. This is because the LPS induced a thrombotic tendency and platelet aggregation activity was accelerated, which shortened the amount of time needed to achieve hemostasis. In contrast, the elapsed time to hemostasis recovered to the same level in mice that were given oral Ashitaba exudate as control mice. This indicated that the oral Ashitaba exudate inhibits platelet activation in the mouse.

Ashitaba exudate contains not only XA and 4-HD but also more than 10 other types of chalcones with structures that differ slightly from those of XA and 4-HD, and at least 100 additional compounds. Therefore, the effects of chalcones on mouse tail bleeding was investigated using XA, 4-HD and other Ashitaba chalcones. Xanthoangelol and 4-HD normalized the bleeding time shortened by LPS, but other chalcones did not exert this effect. These findings showed that differences in the side chain structures of chalcones are important for bioactivities. The side hydrocarbon chain played an important role in this process and small modifications to this chain, or the addition of a small functional group to the A ring influenced anti-platelet activity of chalcone. Considering that > 90% of the chalcones in Ashitaba yellow exudate are XA and 4-HD, the ability of oral Ashitaba exudate to inhibit platelet function seems to be due to the effects of these two chalcones.

4. Effects of Ashitaba chalcone on plasma coagulation

We investigated whether Ashitaba chalcones affect the plasma coagulation and blood fibrinolysis systems. The action of a natural anticoagulant on blood clotting was assessed by evaluating its anticoagulant activity by measuring prothrombin time (PT) and activated partial thromboplastin time (aPTT) (Chee 2014). Prothrombin time and aPTT are tests of the amount of time required for plasma to clot. That is, PT and aPTT are measurements of the amount of time that elapses until plasma coagulates after adding a coagulation initiator. Prothrombin time measures the integrity of the extrinsic system as well as factors common to both systems, and aPTT, measures the integrity of the intrinsic system and common components. These blood tests are used not only as screening assays to detect deficiencies of coagulation factors, but also as a means of exploring new anticoagulant materials. Factors that inhibit blood clotting when added to plasma will prolong PT and/or aPTT. Those that prolong PT inhibit the extrinsic coagulation reaction, those that prolong aPTT inhibit the intrinsic coagulation reaction and those that prolong both PT and aPTT inhibit common reactions. We added XA or 4-HD to human plasma followed by a coagulation initiator, and compared PT and aPTT with those of control samples without XA and 4-HD. However, coagulation times did not differ between the samples and controls (Ohkura et al. 2011). Therefore, the ability of XA and 4-HD to inhibit the plasma coagulation system could not be detected using these assays.

5. Ashitaba chalcones inhibit PAI-1 production

The fibrinolytic system is a process that modulates clot degradation as damaged vascular tissue is repaired and replaced. The fibrinolytic system removes fibrin from the vascular system, thus preventing hemostatic clot enlargement and vessel occlusion (Chapin et al. 2015). Abnormalities of fibrinolysis can lead to an increased risk of thrombosis. Fibrinolysis is regulated by plasminogen activator (PA) and its crucial inhibitor, plasminogen activator inhibitor-1 (PAI-1), a protein that is associated with thrombotic diseases accompanied by metabolic syndrome (Alessi et al. 2011).

The liver, adipose tissues, muscle, bone and hematopoietic cells express PAI-1, which inhibits tissue plasminogen activator (tPA) on thrombus (fibrin) and thrombolysis in blood fibrinolysis reactions (van den Craen et al. 2012). Thrombus becomes difficult to dissolve when plasma PAI-1 concentrations are elevated, and persisting blood clots lead to thrombosis.

Since plasma PAI-1 is elevated in patients with metabolic syndrome including obesity and diabetes, PAI-1 might be associated with a thrombotic tendency in this syndrome. Since adipose tissues produce large amounts of PAI-1, the elevated plasma PAI-1 in obesity is thought to originate from these tissues. Adipocytes synthesize PAI-1, and plasma PAI-1 levels are increased in obesity and reduced by weight loss (Cesari et al. 2010). That is, a link between PAI-1 and metabolic syndrome has been established and elevated plasma PAI-1 levels are now considered a true component of the syndrome (Alessi et al. 2011).

Chronic low-grade inflammation has been linked to the progression of obesity and related diseases (Kimura et al. 2003, 2004). Elevated plasma PAI-1 is closely associated with chronic inflammation in the adipose tissues of obese patients. Therefore, controlling the PAI-1 elevation associated with chronic low-grade inflammation is thought to lead to the prevention of thrombosis caused by lifestyle-related diseases. We used low-grade inflammation to induce a thrombotic tendency in an animal model and found that the oral intake of Ashitaba exudate inhibited plasma PAI-1 elevation during inflammation. These studies included mice with a thrombotic tendency induced by very low levels of lipopolysaccharide (LPS) (Ohkura et al. 2011). Consuming Ashitaba exudate suppressed plasma PAI-1 elevation in these mice. Ashitaba also suppressed PAI-1 production in adipose tissue, the liver and the heart. We also showed that chalcones suppress PAI-1 production in cultured endothelial cells stimulated with inflammatory cytokines such as TNF-α that increase PAI-1 release into the medium. We then investigated whether chalcones in the medium would suppress the increased PAI-1 release into the medium. Human umbilical endothelial cells (HUVEC) were cultured with various chalcones isolated from Ashitaba and then stimulated with TNF-α. We found that not only XA, but also XB and XD inhibited the release of PAI-1 into the medium induced by TNF-α (Ohkura et al. 2011).

However, as described above, ≥ 90% of chalcones in Ashitaba exudate comprise XA and 4-HD. We found that 4-HD did not inhibit PAI-1 production whereas XA suppressed PAI-1 production by the human vascular endothelial cell line, EA.hy926 (Ohkura et al. 2016). Considering these facts, the inhibitory action of PAI-1 production by oral Ashitaba exudate is thought to be due to XA action. We recently determined that elevated plasma PAI-1 levels in obese diabetic mice are suppressed by feeding with Ashitaba exudate to almost the same level as those of control lean mice (Ohta et al. 2018).

6. Conclusion

Various physiological effects of Ashitaba chalcones have recently been identified. Since a wide range of Ashitaba chalcone functions have been reported, Ashitaba is currently perceived as a versatile and healthy vegetable. However, thrombotic diseases cannot be prevented simply by Ashitaba intake. Previous reports have only shown the possibility that Ashitaba chalcones have antithrombotic activity. Whether or not specific amounts of Ashitaba intake are actually effective for preventing thrombotic diseases in humans remains to be elucidated. Current findings seem to indicate that Ashitaba intake will help to prevent thrombotic diseases and could maintain health in a real sense. We plan to further deepen understanding of the effectiveness of Ashitaba against lifestyle diseases.

Conflicts of interest: None reported.

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