FOOD
RESEARCH

Angelica keiskei (Ashitaba) has potential as an antithrombotic health food

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Abstract

Angelica keiskei (Ashitaba) is a large perennial herb that is native to the Pacific coast of Japan. It has recently become popular as a healthy food in Asian countries because it might have various physiological benefits including antithrombotic properties. Most studies of the bioactive constituents from Ashitaba have focused on the activities of the major chalcones, xanthoangelol and 4-hydroxyderricin. However, other chalcones, flavanones and coumarins have also been isolated from Ashitaba, precisely characterized, and investigated *in vivo*. Platelets play a key role in haemostasis and wound healing processes. Dysregulated platelet activity is associated with the progression of platelet aggregation and decreased venous blood flow, which results in thrombotic diseases. A minor chalcone, xanthoangelol E, inhibits TXB_2 synthesis in rabbit platelets, which seems to be the source of the belief that Ashitaba has antithrombotic properties. However, recent data showed that xanthoangelol and 4-hydroxyderricin inhibited the aggregation of rabbit platelets. Platelet aggregation stimulated by collagen was also inhibited in whole blood incubated with Xanthoangelol or 4-hydroxyderricin. Plasminogen activator inhibitor-1 is the primary physiological inhibitor of tissue type plasminogen activator, a key protease of the fibrinolytic system. An increase in plasma of this inhibitor is associated with thrombotic conditions. Ashitaba yellow exudate inhibited the elevation of plasma plasminogen activator inhibitor-1 in mice induced by obesity or chronic low-grade inflammation. These studies showed the yellow exudate from stem cuttings and chalcones isolated from Ashitaba roots and leaves might have antithrombotic activity. This article reviews the possible antithrombotic properties of Ashitaba.

1. Introduction

Metabolic syndromes such as diabetes, hypertension, obesity, and dyslipidaemia are associated with an increased risk of thrombotic diseases caused by the formation of blood clots inside blood vessels that obstruct blood flow through the circulatory system. Myocardial and cerebral infarctions and venous thromboembolism are classified as thrombotic diseases, the prevalence of which is increasing along with that of metabolic syndrome (Grundy *et al.*, 2005; Goldhaber, 2010; Previtali *et al.*, 2011). Elevated blood sugars and lipids decrease blood fluidity, which in turn lowers the threshold of platelet activation, increases amounts and

activities of blood coagulation factors, and suppresses the blood fibrinolytic system in metabolic syndrome; all of these changes are associated with thrombotic diseases (Russo, 2012).

The risk of thrombotic diseases due to lifestyle habits can be reduced not only by pharmacotherapeutics but also by exercise and consuming dietary supplements that might help blood and blood vessels regain healthy status. Although many health foods are considered effective against thrombotic diseases, the science behind the ingredients that might prevent blood clots should be investigated in more detail. We have recently focused on health foods that might prevent thrombosis, and suggest

that Ashitaba is an antithrombotic health food. Here, we introduce the possibility that ingesting Ashitaba or its constituents prevents thrombotic diseases.

2. Ashitaba and its unique components, chalcones

Ashitaba (*Angelica keiskei* Koidzumi) is a large perennial herb of the *Apiaceae* genus *Angelica* (Figure 1). Ashitaba is native to the Pacific coast of Japan (Izu Islands and the Izu, Bōso, and Miura peninsulas), and it is now cultivated in Southeast Asia and the Korean peninsula. Ashitaba is the common Japanese name for the *Angelica keiskei* cultivar Koidzumi, and the English translation of it is "tomorrow's leaf". This description reflects the vitality of Ashitaba, which can regenerate within one day of cutting (Baba, 2013). Ashitaba has been consumed as a vegetable and used as folk medicine since ancient times. The leaves, stems, and roots of Ashitaba are rich in nutrients such as vitamin A, vitamin K, and dietary fibre, as well as various unique polyphenols such as chalcones and coumarin. Powdered Ashitaba leaves, roots, and yellow exudates have become popular as societies have become increasingly health-conscious. Various compounds in Ashitaba such as chalcones, flavanones and coumarin have been isolated and their structures and bioactivity have been investigated (Caesar *et al.*, 2016; Kil *et al.*, 2017). The structures of 10 chalcones isolated from Ashitaba have been determined (Hata and Kozawa, 1961; Kozawa *et al.*, 1977; Kozawa *et al.*, 1978; Baba, Nakata, Taniguchi *et al.*, 1990, Baba, Kito, Yoneda *et al.*, 1990; Nakata *et al.*, 1999; Figure 2). The chalcones, xanthoangelol (XA) and 4-hydroxydelicin (4-HD) account for > 90% of all chalcones identified in Ashitaba. The remaining chalcones comprise trace amounts of xanthoangelols B, C, D, E, F, G, and H, and isobavachalcones. Chalcones are found in leaves, stems, roots and yellow exudates from cut stems. Ashitaba chalcone has a long history of human use as a dietary supplement with claims of multiple health benefits. Previously published animal studies were without toxic effects (Maronpot, 2015).

Studies of the physiological activities of Ashitaba were initiated based on the folklore of the Izu Islands (Baba, 2013). The initial discovery was that Ashitaba chalcones might inhibit gastric acid secretion, and have antitumor and antibacterial activities (Murakami *et al.*, 1990; Inamori *et al.*, 1991; Okuyama *et al.*, 1991). Thereafter, various physiological activities of the major chalcones, XA and 4-HD were determined (Murakami *et al.*, 1990; Inamori *et al.*, 1991; Kimura *et al.*, 2003; Kimura *et al.*, 2004; Ogawa *et al.*, 2005; Ogawa *et al.*, 2007; Shin *et al.*, 2011). After finding that Ashitaba can suppress high blood glucose and exert anti-obesity effects in mice (Enoki *et al.*, 2007), it attracted attention

as a health food that might improve lifestyle diseases such as obesity and diabetes, and animal and clinical studies were conducted (Zhang *et al.*, 2015; Ohnishi *et al.*, 2017, Kalman *et al.*, 2018; Ohta *et al.*, 2019; Oh *et al.*, 2019; Zhang *et al.*, 2019). Accumulating evidence supports the notion that Ashitaba could be a useful health food or dietary supplement.

Figure 1. A: *Angelica keiskei* Koidzumi (Ashitaba), B: Yellow exudate from stem cuttings
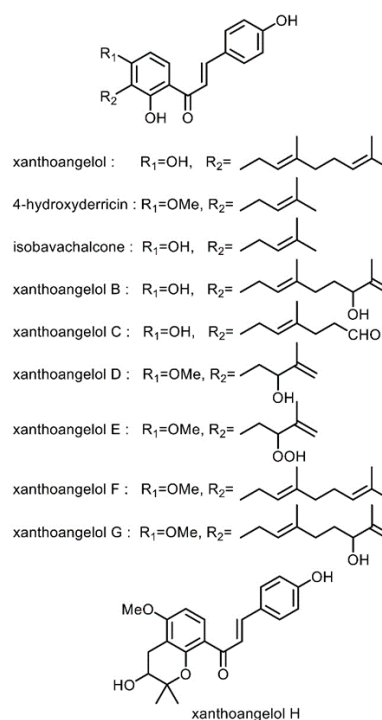

Figure 2. Ashitaba chalcones

3. How the belief that Ashitaba has antithrombotic properties developed

The haemostatic system comprises platelets, coagulation factors and the fibrinolytic system. Antithrombotic activity generally refers to antiplatelet action and anticoagulant action induced by plasma coagulation factors. In general, antithrombotic agents comprise anticoagulants that inhibit the coagulation cascade of blood coagulation factors that interfere with further clot expansion, and antiplatelet substances that decrease platelet aggregation and inhibit platelet thrombus formation. Therefore, to determine whether candidate substances exert anti-thrombotic effects, their actions on the blood coagulation system and platelets, and the fibrinolytic system should be investigated not only *in vitro* but also *in vivo*. The first study of possible antithrombotic effects showed that isolated xanthoangelol E, trace amounts of which are found in Ashitaba, inhibits thromboxane B_2 (TXB_2) synthesis *in vitro* (Fujita *et al.*, 1992). However, the authors did not describe whether xanthoangelol E inhibits platelet aggregation. Fujita et al. (1992) found that xanthoangelol E inhibited the production of TXB_2 and 12-hydroxy-5,8,10-heptadecatrienoic acid from exogenous arachidonic acid in rabbit platelets. However, they did not prove that xanthoangelol E inhibits platelet aggregation. Hence, whether or not xanthoangelol E inhibits platelet aggregation and exerts antithrombotic activity *in vivo* remains unclear. Regardless, this finding seems to be the source of the popular belief that Ashitaba has antithrombotic activity (Ohkura *et al.*, 2018).

4. Effects of Ashitaba chalcones on platelets

Platelets play a key role in haemostasis and wound healing processes to maintain the integrity of the circulatory system. Dysregulated platelet activity is associated with the progression of platelet aggregation and decreased venous blood flow, which results in thrombotic diseases (George, 2000). Son and co-workers. assessed the effects of XA and 4-HD on the aggregation of rabbit washed platelets. They found that XA and 4-HD, the main components of Ashitaba chalcones, inhibited platelet aggregation induced by stimulation with collagen, platelet-activating factor (PAF) and phorbol 12-myristate 13-acetate, but not by thrombin (Son *et al.*, 2014). This indicated that XA and 4-HD are more likely to inhibit the phosphoinositide phospholipase C gamma (PLCγ)-, rather than PLCβ-related activation pathway in platelets (Son *et al.*, 2014).

Although Son and co-workers investigated the effects of chalcones on isolated platelets, we assessed these effects on whole blood platelet aggregation. Our findings also showed that Ashitaba chalcones inhibit platelet aggregation (Ohkura *et al.*, 2016). Platelet aggregation stimulated by collagen was inhibited in bovine whole blood incubated with XA or 4-HD. Tail bleeding in mice is primarily due to platelet plug formation as a result of platelet aggregation, and platelet function *in vitro* is generally measured as tail bleeding time (Day *et al.*, 2004). The haemostatic function of platelets *in vivo* can be determined by measuring the amount of time required to stop bleeding from a small wound in mouse tails (tail bleeding assay; Beviglia *et al.*, 1993). Therefore, we investigated whether Ashitaba yellow exudate could suppress mouse tail bleeding to determine platelet function *in vivo* (Ohkura *et al.*, 2016). Mice were orally administered with Ashitaba yellow exudate once daily for 7 days, then lipopolysaccharide (LPS) was intraperitoneally administered to induce a thrombotic tendency. The tail tip was cut with a sharp knife under complete anaesthesia, and we measured the amount of time required to stop bleeding. We found that bleeding stopped sooner in LPS-stimulated than in control mice. This is because the LPS induced platelet aggregation, which shortened the amount of time needed to achieve haemostasis (MacIntyre *et al.*, 1977). In contrast, the elapsed time to haemostasis recovered to the normal level in mice given oral Ashitaba yellow exudate (Ohkura *et al.*, 2016). This indicated that the oral Ashitaba yellow exudate inhibits platelet activation *in vivo*. As described above, Ashitaba yellow exudate contains not only XA and 4-HD but also > 10 other chalcones with slightly different structures from those of XA and 4-HD, and many other compounds. Therefore, we investigated the effects of various isolated Ashitaba chalcones on mouse tail bleeding (Ohkura *et al.*, 2016). We injected XA, 4-HD, XB, XD, XE and XF into the mouse peritoneal cavity and found that only XA significantly recovered tail bleeding. Therefore, structural differences in the side chains of chalcones are important for their bioactivities. The hydrocarbon side chain of Ashitaba chalcones plays an important role and small modifications to this chain, or the addition of a small functional group to the A ring influences the anti-platelet activities of these chalcones. Only XA was effective in terms of tail bleeding time and platelet aggregation in mice. In contrast, both XA and 4-HD inhibited bovine and human platelet aggregation. The removal of prenylation in 4-HD decreases hydrophobicity and thus might decrease mouse platelet translocation or interactions. Regardless, considering that > 90% of the chalcones in Ashitaba yellow exudate are XA and 4-HD, the inhibition of platelet function seems to be the main effect of these two Ashitaba chalcones.

5. Effects of Ashitaba chalcones on coagulation factors in plasma

Ashitaba chalcones were examined for their effect on the coagulation pathway by measuring prothrombin time (PT) and activated partial thromboplastin time (APTT) that reflect the amount of time required for plasma to coagulate after adding a coagulation initiator (Ohkura *et al.*, 2011). Prothrombin time and APTT indicate the integrity of coagulation factors in the common system, and in the extrinsic and intrinsic systems, respectively. These tests are clinically applied to screen coagulation factor deficiencies. These tests are defined as the time (seconds) needed to clot plasma upon addition of coagulation triggers, such as tissue factor in complex with phospholipids and calcium chloride (in the PT), or negatively charged phospholipids-activators and calcium chloride (in the APTT). They are considered global coagulation tests sensitive to the amounts of coagulation factors. Prothrombin time and APTT are both sensitive to factor X, V, II and fibrinogen, whereas the PT is only sensitive to factor VII amounts and the APTT is sensitive to factor XII, XI, IX and VIII amounts. Furthermore, the PT and APTT are variably sensitive to the presence of antithrombotic agents directed against specific coagulation factors. These assays are also used to explore novel anticoagulant agents (Chee, 2014).

Adding coagulation inhibitors to plasma prolongs PT and/or APTT by inhibiting coagulation factors involved in the extrinsic and intrinsic coagulation pathways, respectively. Adding those that prolong both PT and APTT inhibit coagulation factors involved in a common pathway. We added XA or 4-HD to human plasma followed by each coagulation initiator, and compared PT or APTT with those of control samples without XA and 4-HD. Coagulation times did not differ between the samples and controls (Ohkura *et al.*, 2011), indicating that neither XA nor 4-HD inhibited the plasma coagulation system according to these assays.

6. Effects of Ashitaba chalcones on fibrinolysis

Fibrinolysis is a highly regulated enzymatic process that prevents unnecessary accumulation of intravascular fibrin and enables thrombus removal. The fibrinolytic system removes fibrin from the vascular system, thus preventing haemostatic clot enlargement and vessel occlusion (Chapin *et al.*, 2015). Like the coagulation cascade, fibrinolysis is tightly controlled by a series of proteases, protease inhibitors, and receptors. Plasmin is the key fibrinolytic protease, and it is activated from plasminogen by either tissue plasminogen activator (tPA) or urokinase (uPA) which are primary serine proteases (Chapin *et al.*, 2015). Fibrinolysis is regulated by these two PA and its crucial inhibitor, plasminogen activator inhibitor-1 (PAI-1).

Liver, adipose tissues, and muscle, bone, and hematopoietic cells express PAI-1 that inhibits thrombolysis in blood fibrinolytic reactions (Van Den Craen *et al.*, 2012). Thus, high plasma PAI-1 levels are causally related to attenuated fibrinolysis and increased risk for thrombosis. Since plasma PAI-1 levels are highly elevated under various pathological conditions including infection and inflammation, their fibrinolytic potential in plasma and on vascular endothelial cells is readily suppressed and persistent blood clots lead to thrombosis (Van Den Craen *et al.*, 2012). Other than this, plasma PAI-1 is elevated in patients with metabolic syndrome including obesity and diabetes. Thus, PAI-1 might be associated with a thrombotic tendency in this syndrome (Alessi *et al.*, 2011). 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 thrombotic tendency in this syndrome (Alessi *et al.*, 2011).

We recently analysed the effects of Ashitaba yellow exudate on enhanced PAI-1 levels in obese diabetic mice (Ohta *et al.*, 2019). Intake of Ashitaba yellow exudate significantly decreased food efficiency and plasma PAI-1 in obese diabetic mice but did not affect lean control mice. Ashitaba yellow exudate also decreased some parameters in plasma, such as glucose, insulin, tumour necrosis factor-alpha (TNF-α) and gains in body, subcutaneous, and mesenteric fat weight in TSOD mice but had little effect on these parameters in TSNO mice. These findings suggested that Ashitaba yellow exudate decreased plasma PAI-1 levels by suppressing both the adipose tissue retention of PAI-1 protein and liver PAI-1 production in obese diabetic mice (Ohta *et al.*, 2019). Thus, supplementing diets with Ashitaba might help to prevent or alleviate the risk of thrombotic diseases, and suppress dysfunctional metabolic states in obese individuals.

Chronic low-grade inflammation has been linked to the progression of obesity and related diseases (Kimura *et al.*, 2003; Kimura *et al.*, 2004). Therefore, controlling PAI-1 elevation associated with chronic low-grade inflammation is thought to lead to the prevention of thrombosis caused by not only inflammation but also lifestyle-related diseases. We used low-grade inflammation to induce a thrombotic tendency in a mouse model induced by extremely low levels of lipopolysaccharide (LPS) and found that oral Ashitaba yellow exudate inhibited plasma PAI-1 elevation

(Ohkura *et al*., 2011). Ashitaba also suppressed PAI-1 production in the liver, heart, and adipose tissues in this model (Ohkura *et al*., 2011) and in cultured endothelial cells. Human umbilical endothelial cells (HUVEC) stimulated with inflammatory cytokines such as TNF-α increase the amount of PAI-1 released into the medium. We cultured HUVEC with various Ashitaba chalcones then stimulated the cells with TNF-α. We found that XA, XB and XD inhibited PAI-1 release into the medium induced by TNF-α (Ohkura *et al*., 2011).

However, as described above, ≥ 90% of chalcones in Ashitaba comprise XA and 4-HD. We later discovered that 4-HD does not inhibit, whereas XA suppresses PAI-1 production by the HUVEC cell line, EA.hy926, (Ohkura *et al*., 2015). Considering these findings, the inhibitory action of PAI-1 production by oral Ashitaba yellow exudate is thought to be due to XA action. As described above, Ashitaba yellow exudate suppressed PAI-1 production in the heart, liver, and adipose tissues, and Nakamura *et al*. (2012) showed that XA and 4-HD are rapidly absorbed and distributed to various tissues. Thus, Ashitaba chalcones inhibit PAI-1 production in adipocytes and hepatocytes.

7. Conclusion

Dysregulated platelet activity is associated with the progression of platelet aggregation which results in thrombotic diseases. Two major Ashitaba chalcone, xanthoangelol and 4-hydroxyderricin, inhibited the platelet aggregation induced by various stimulants. Xanthoangelol was effective in terms of tail bleeding time that reflect platelet activity *in vitro* in mice and platelet aggregation in mice whole blood. On the other hand, Ashitaba yellow exudate inhibited the elevation of PAI-1, the primary physiological inhibitor of tissue type plasminogen activator, in mice induced by obesity or chronic low-grade inflammation. Whether intake of Ashitaba or Ashitaba chalcones confers health benefits upon humans remains to be elucidated. However, current findings indicate that dietary Ashitaba might help to prevent thrombotic diseases and maintain good health.

Conflict of interest

The authors have no conflicts of interest to declare. The authors alone are responsible for the content and writing of the article.

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