Antithrombotic potential of plant products

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Received 23 Oct. 2014
Accepted 3 Dec. 2014
ePublished 19 Jan. 2015

Abstract
Atherosclerosis is a silent and chronic inflammatory process at the artery wall. A procoagulant state may occur after atherosclerotic plaque rupture and provoke an acute atherothrombotic event, which are among the first causes of death in many countries. Antiplatelet agents are prescribed for antithrombotic therapy; however, the efficacy of currently available drugs is limited, thus, other effective, safe and low-cost therapeutic options are needed. Antithrombotic agents could emerge from a herb product with platelet inhibitory activity, however “antiplatelet” is not a popular term, a limitation of the Ethnomedical studies. This review is focused on the association of some traditional uses and the antiplatelet effects of plant products that could provide a basis to select the botanical species to be included in research protocols to discover antithrombotic agents. The analysis of the characteristic features of the antiplatelet effects of different products derived from Allium sativum L., Ginkgo biloba L., Sacharum officinarum L., Theobroma cacao L. and Vitis vinifera L. suggest that antithrombotic potentials could be found in medicinal plants traditionally used to treat inflammation, bronchitis, bronchial asthma and vascular disorders. The reductions of thromboxane A2 formation and calcium uptake, the increases of cyclic adenosine monophosphate and nitric oxide levels, besides the inhibition of oxidative reactions in platelets could be among the possible mechanisms of action of the new products.

Keywords: Platelet, Traditional medicine, Drug discovery

Introduction
Atherosclerosis is a silent and chronic inflammatory process at the artery wall that reduces the arterial lumen, the blood flow inside it and the oxygen supply to the affected organs. A procoagulant state may occur after an atherosclerotic plaque rupture and provoke an acute atherothrombotic event (myocardial infarction, stroke or lower limb occlusive disease), which are among the first causes of death in many countries.1 Blood platelets play a key role in atherogenesis and arterial thrombosis,2 therefore antiplatelet agents are prescribed for antithrombotic therapy. However the efficacy of the currently used drugs is limited due to the inter-individual differences of the responses to the treatments3 and new effective, safe and low-cost therapeutic options are needed for primary and secondary prevention of thrombosis.

A great number of drugs have been generated from plants,4 thus, one can hypothesize that new antithrombotic agents could emerge from herbal products with platelet inhibitory activity. The correlations between the Ethnomedical information on plants and the biological actions or the uses of their chemical components or related plants have proved to be appropriate for drug discovery,4 however, “antiplatelet” is not a popular term, a limitation to use this approach in this case. Identifying the common characteristics of plant products with antithrombotic potential due to the inhibition of platelet reactivity could help to discover new drugs from botanical sources. It has been suggested that the extracts or active compounds derived from fruits of Solanaceae, Rutaceae, Cucurbitaceae, Rosaceae, Musaceae, Anacardiaceae, Vitaceae, Arecaceae, Ericaceae and Lauraceae botanical families could be raw materials to manufacture oral preparations for the prevention of atherothrombotic diseases.5 This could be a guide for selecting plant species to be included in a pharmacological screening, but the need for additional elements is evident from the diversity of medicinal plants used in different countries. This review is focused on the association of some traditional uses and the antiplatelet effects of plant products that could provide a basis to select the botanical species to be included in research protocols to discover antithrombotic agents.

Pharmacological targets for antiplatelet action
Blood platelets are small cytoplasmic fragments from bone marrow megakaryocytes. They have a complex structure: an external membrane with different glycoproteins, receptors and enzymes and that mediates platelet adhesion to the artery wall and the interaction with other platelets and blood cells; membranous and
contractile systems the exchange of materials from inside/ outside the cell and platelet shape changes; alpha granules containing enzymes, adhesion proteins, coagulation and growth factors; dense granules where adenosine diphosphate (ADP), serotonin, epinephrine, calcium and magnesium are stored. They can be physiologically activated by different agonists, including ADP, thromboxane A2 (TXA2), epinephrine, serotonin and thrombin, the main coagulation enzyme, arachidonic acid (AA). An increase in free Ca2+ concentrations ([Ca2+]i) activates both the Ca2+- calmodulin-dependent phosphorylation of myosin light chain (20-kDa), diacylglycerol-dependent phosphorylation of cytosolic proteins (40- or 47-kDa) and GPIIb/IIIa activation that bind plasma fibrinogen to form aggregates and the exocytosis of alpha and dense granules constituents (release reaction). Ca2+ is under cyclic adenosine monophosphate (cAMP) and cyclic guanosyl monophosphate (cGMP) physiological control.6,7

The pharmacological manipulation of the mechanisms of platelet activation and inactivation has given rise to different types of antiplatelet agents. Some of them are currently used as antithrombotic drugs and other are under investigation. They include the inhibitors of AA cyclooxygenase 1 isoenzyme (COX-1) (aspirin) that prevent TXA2 formation after platelet stimulation with collagen, epinephrine, ADP and AA; antagonists of P2Y12 ADP receptors (Clopidogrel); GPIIb/IIIa antagonists (Abciximab), that reduces the platelet activation induced by all kinds of stimuli; inhibitors of cAMP phosphodiesterase enzyme, that enhance the intracellular levels of this biochemical mediator (Dipyridamol, Cilostazol); the increase of circulating adenosine levels, that stimulate the adenylyl cyclase enzyme that catalyses cAMP formation (Dipyridamol); the increase of the formation of prostacyclin (PGI2) an AA metabolite that inhibit platelet adhesion and aggregation (Dipyridamol) or simulation of its receptor on the platelet membrane (Iloprost, a PGI2 analogue). Other targets for the development of antiplatelet drugs are the inhibition of TXA2 synthase enzyme, antagionism of TXA2 receptor, as well as “protease –activated receptors” of high and low affinity antagonists for thrombin respectively.3,8 Some of these antiplatelet mechanisms are summarized in the Figure 1.

It is known that [Ca2+]i, cAMP and the oxidative reactions that participate in AA metabolism leading to the formation of cyclic endoperoxides and very active metabolites (TXA2, PGI2, prostaglandins or leukotrienes, according to the cell type) are involved in the activation and regulation of other cells with key roles in inflammation, bronchial asthma and cardiovascular diseases, like macrophages, mast cells airways and vascular smooth muscle cells, therefore it is not surprising the influence on platelet aggregation by non-steroidal anti-inflammatory analgesic and antipyretic drugs (NSAID), via COX 1 inhibition;9 prophylactic anti-asthmatic agents;10,11 vasodilator nitro compounds, that stimulate cGMP synthesis;12 calcium blockers with anti-hypertensive effect.13

Pre-clinical assessment of antiplatelet/antithrombotic activities consists of the determination of the reductions of platelet aggregation, release reaction or arterial thrombus formation in the presence of the test product during in vitro, ex vivo or in vivo experimental designs, based on the stimulation of platelets by adding an agonist (ADP, collagen, thrombin, AA, epinephrine, the TXA2 analogue U 46619) to platelet-rich plasma (PRP) or whole blood from untreated humans, untreated or pre-treated experimental animals, as well as the intravenous administration of any of these agents or the induction of thrombosis in pre-treated animals.14

Plant products with antiplatelet effect

The scientific evidence on the antiplatelet effect of anti-inflammatory, anti-asthmatic and antihypertensive drugs suggest that medicinal plants traditionally used for these purposes could have antithrombotic potentials. The following information could provide validation to this hypothesis.

*Allium sativum* L. (*A. sativum*; Garlic). Family: Liliaceae

The treatment of inflammation, fever, pain, bronchitis, bronchial asthma, hypertension and vascular disorders are among the traditional uses of this species.15-17

*A. sativum* bulbs (unprocessed material aqueous and organic extracts) inhibited the activation of platelets from different species stimulated by a variety of agonists.18 The pharmacological activity of natural bulbs was reduced after heating.19 Chronic intake of fresh or dried bulbs, garlic oil or hydro-alcohol extracts have produced this effect in humans.20

It has been stated that the antiplatelet effects of *A. sativum* preparations is associated to the inhibition of cyclooxygenase activity and thus thromboxane A2
formation by suppressing intraplatelet Ca2+ mobilization, increasing levels of cAMP and cGMP, besides its antioxidant properties as well as the stimulation of nitric oxide synthase (NOS), leading to an increase in platelet-derived NO. A reduction of fibrinogen binding to GPIIb/IIIa on platelets may also occur. Allicin (from natural and dried bulbs) and ajoene (from bulb oils and macerates), are the sulfur compounds responsible for garlic antiplatelet properties.  

**Ginkgo biloba** L. *(G. biloba; Ginkgo). Family: Ginkgoaceae*  

G. biloba seeds and leaves are traditionally used to treat different illnesses including inflammation, arthritis, rhinitis, bronchial asthma and vascular disorders.  

EGb 761, a standardized *G. biloba* aqueous leaf extract, inhibited the aggregation of human platelets induced by ADP, collagen, AA, PAF and oxidative stress in PRP, whole blood and gel-filtered platelets suspensions *in vitro*. On the other hand, it showed a synergistic effect on Cilostazol antithrombotic activities. Its antiplatelet effect has been demonstrated in healthy and diabetic subjects along with a reduced TXA2 formation.  

Moreover, it has been suggested that some beneficial effects of EGB might be due to its modulating influences on cellular cyclic AMP levels. Ginkgolides A, B, C, kaemferol and quercetin among the major chemical components of this extract, could be its active compounds.  

**Saccharum officinarum** L. *(S. officinarum; Sugar cane). Family: Gramineae*  

Domestic preparations from this species (root decoction, stem juice) are used as traditional remedies for the treatment of bronchial asthma and bronchitis.  

Oral administration of Policosanol, a Cuban standardized mix of high molecular weight alcohols (octacosanol, the major component) from *S. officinarum* wax have been able to *ex vivo* and *in vivo* inhibit ADP- and collagen-induced platelet aggregation in rats, respectively. Randomized-controlled clinical trials as well as a descriptive post-commercial study have demonstrated significant *ex vivo* reductions of platelet reactivity in human PRP.  

**Vitis vinifera** L. *(V. vinifera; Grape). Family: Vitaceae*  

Inflammation and bronchitis are two of the Ethnomedical uses of dried fruits and aguardiente from grapes.  

*V. vinifera* seed and fruit peel aqueous extracts, inhibited the activation of human platelets *in vitro* and dog platelets *ex vivo*. In addition, grape juice and red wine showed this effect on ADP- and thrombin-stimulated human PRP *in vitro*. Oral treatment with these products were effective against platelet aggregation in healthy subjects. The inhibition of phospholipase C enzyme, reduction of TXA2 formation, probably related to limitations of oxidative reactions and the augmentation of NO release from the vascular endothelium are the proposed mechanisms of the antiplatelet activity of grape products. Anthocyanide (juice), proanthocyanidin (seeds), resveratrol, transresveratrol, quercetin, epicatechin and catechin (wine) are the chemical constituents responsible for the pharmacological activity.  

**Concluding remarks**  

The analysis of the scientific evidence on five botanical species that have been broadly researched with respect to the antiplatelet effect, Allium sativum L., Ginkgo biloba L., *Saccharum officinarum* L., Theobroma cacao L. and *Vitis vinifera* L., shows that four of them are traditionally used for inflammation and bronchitis and three of them are popularly considered useful for the treatment of bronchial asthma and vascular disorders, thus suggesting that antithrombotic potentials could be found in other medicinal plants with these Ethnomedical uses. The reductions of thromboxane A2 formation and calcium uptake, the increases of cyclic adenosine monophosphate and nitric oxide levels, besides the inhibition of oxidative reactions in platelets besides other known pharmacological targets could be among the possible mechanisms of action of the new products. This research approach could be useful to discover new antithrombotic options according to the botanical richness and traditions of each country and could be improved with the results of future experimental studies.


Competing interests

The authors declare no competing interests.

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