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Original Article

Antithrombotic potential of plant products

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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 treatments[3] 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 herb 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 *Solnaceae, 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 grow 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, serotonine 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+]i 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 $P2Y_{12}$ 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 adenyl 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, antganonism 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

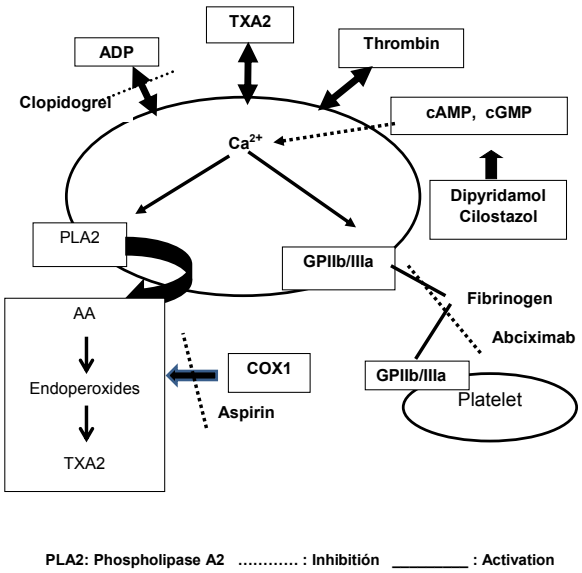

Figure 1. Pharmacological targets for antiplatelet action

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-asthma tic 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.[20,21] Allicin (from natural and dried bulbs) and ajoene (from bulb oils and macerates), are the sulfur compounds responsible for garlic antiplatelet properties.[18]

***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.[22]

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*,[23] On the other hand, it showed a synergistic effect on Cilostazol antithrombotic activities.[24] Its antiplatelet effect has been demonstrated in healthy and diabetic subjects along with a reduced TXA2 formation.[25,26] Moreover, it has been suggested that some beneficial effects of EGB might be due to its modulating influences on cellular cyclic AMP levels.[27] Ginkgolides A B, C, kaemferol and quercetin among the major chemical components of this extract, could be its active compounds.[24]

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.[15,17]

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, respectivelly.[28] Randomized-controlled clinical trials as well as a descriptive post-commercial study have demonstrated significant *ex vivo* reductions of platelet reactivity in human PRP.[29]

D003, a standardized mix of octacosanoic, triacontanoic, dotriacontanoic and tetratriacontanoic acid from *S. officinarum* wax was effective against ADP, collagen and AA in rat guinea pig and rabbit PRP *ex vivo* intravascular platelet aggregation and arterial thrombosis in rats.[30] Randomized-controlled studies with healthy volunteers showed its *ex vivo* effect in humans.[31] In addition, D003 prevented showed and anti-inflammatory activity in an experimental model of arthritis with mice.[32]

Policosanol and D003 antiplatelet effects are associated to reductions of TXA2 formation.[31,33] In addition, they have antioxidant effects.[34]

***Theobroma cacao* L.** (*T. cacao;* Cacao). Family: Sterculiaceae

T. cacao seed decoctions and infusions are folk remedies for inflammation, fever, bronchitis and vascular disorders.[16,35]

Controlled clinical studies with healthy volunteers demonstrated reduced platelet reactivity to ADP- and epinephrine in whole blood and to collagen in PRP after the intake of standardized *T. cacao* seed water extracts[36-38] that is parallel to reduced TXA2 formation.[39] On the other hand, peripheral vasodilation, via activation of the vascular endothelium nitric oxide system, was demonstrated in healthy volunteers who consumed flavanol-rich cocoa beverages[40,41], Furthermore, a cocoa extract inhibited the secretion of inflammatory mediators from macrophages *in vitro*,[42] providing another plausible mechanism for the antiplatelet effect. Catequin, epicatequin, rutin and procyanidin are major active principles associated to cacao antiplatelet activity.[36]

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

Inflammation and bronchitis are two of the Ethnomedical uses of dried fruits and aguardiente from grapes.[43]

V. vinifera seed and fruit peel aqueous extracts, inhibited the activation of human platelets *in vitro*[43] and dog platelets *ex vivo*. In addition, grape juice and red wine showed this effect on ADP- and thrombin-stimulated human PRP *in vitro*.[44] Oral treatment with these products were effective against platelet aggregation in healthy subjects.[45] 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:[43] Anthocyanide (juice), proanthocyanidin[46] (seeds), resveratrol),[47] transresveratrol, quercetin, epicatechiin and catechin (wine)[48] 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[3,8] 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.

References

1. George J. Mechanisms of disease: the evolving role of regulatory T cells in aterosclerosis. Nat Clin Practice Cardiovasc Med 2008; 5: 531-40.
2. Davì G, Patrono C. Platelet activation and atherothrombosis. N Engl J Med 2007; 357: 248-94.
3. Varon D, Spectre G. Antiplatelet agents. Hematology 2009; 1: 267-72.
4. Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. Environ. Health Perspect 2001; 109 (suppl 1): 69-75.
5. Duttaroy A. Antithrombotic agents. European Patent EP1083912, I0 P Research and Communities, 2003. Available from: http://www.freepatentsonline.com/EP1083912.html
6. Offermanns S. Activation of platelet function through G protein–coupled receptors. Circulation Res 2006; 99: 1293-304.
7. Varga-Szabo D, Pleines I, Nieswandt. B. Cell adhesion mechanisms in platelets. Arteriosclerosis ThrombVasc Biol 2008; 28: 403-12.
8. Hall R, Mazer CD. Antiplatelet drugs: a review of their pharmacology and management in the perioperative period. Anesth Analg 2011; 112: 292-318.
9. Dugowson CE, Gnanashanmugam P. Nonsteroidal anti-inflammatory drugs. Phys Med Rehabil Clin N Am 2006; 17 : 347-54.
10. García Mesa M, Arruzazabala ML. Effect of ketotifen and disodium cromoglycate on human platelet aggregation. Allergol Immunopathol 1989; 17: 33-4.
11. García Mesa M, Casacó A, Carbajal D, Arruzazabala ML, Friman M. [Effect of N- (3,4-dimethoxycinnamoyl) anthranilic acid (Tranilast) on intravascular platelet agrtegación induced in experimental animals]. Rev Iberoamer Tromb Haemostasia 1990; 1: 31-3.
12. Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev 1991; 43: 109-43.
13. Blache D, Ojeda C. Comparative Inhibitory Effects of Dihydropyridines on Platelet Aggregation, Calcium uptake and cyclic AMP concentration. Pharmacology 1992; 45: 250-9.
14. Just M, Laux V. Activity on blood constituents. In: Vogel H G, editor. Drug Discovery and Evaluation: Pharmacological Assays. 2nd ed. Berlin: Springer; 2002. p. 289-307.
15. Roig JT. [Plantas medicinales, aromáticas o venenosas de Cuba]. 2nd ed. La Habana: Editorial Científico Técnica; 1988.
16. TRAMIL. Farmacopea Vegetal Caribeña. 2nd ed. L. Germosén Robineau. (ed.). León: Editorial Universitaria UNAN; 2005.
17. García Valido PE, Pérez Alejo JL. Especies medicinales. en el Delta Orinoco: aspectos promisorios para la medicina tradicional cubana. La Habana: eCiMED; 2011.
18. Hiyasat B, Sabha D, Grotzinger K, Kempfert J, Rauwald JW, Mohr FW, et al. Antiplatelet activity of Allium ursinum and Allium sativum. Pharmacology 2009; 83: 197-204.
19. Cavagnaro PF, Camargo A, Galmarini CR, Simon PW. Effect of cooking on garlic (Allium sativum L.) antiplatelet activity and thiosulfinates content. J Agric Food Chem 2007; 55: 1280-8.
20. Banerjee SK, Maulik SK. Effect of garlic on cardiovascular disorders: a review. Nutr J 2002; 1: 4.
21. Bhandari PR. Garlic (*Allium sativum* L.): A review of potential therapeutic applications. Int J Green Pharmacy 2012; 6: 118-29.
22. World Health Organization. Zhang X. (ed). WHO monographs on selected medicinal plants, Vol. 1. Geneva: WHO; 2002.
23. Akiba S, Kawauchi T, Oka T, Hashizume T, Sato T. Inhibitory effect of the leaf extract of Ginkgo biloba L. on oxidative stress-induced platelet aggregation. IUBMB Life 1998; 464: 1243-8.
24. Ryu KH, Han HY, Lee SY, Jeon SD, Im GJ, Lee BY, et al. Ginkgo biloba extract enhances antiplatelet and antithrombotic effects of cilostazol without prolongation of bleeding time. Thromb Res 2009; 124: 328-34.
25. Kudolo GB, Dorsey S, Blodgett J. Effect of the ingestion of *Ginkgobiloba* extract on platelet aggregation and urinary prostanoid excretion in healthy and Type 2 diabetic subjects . Thromb Res 2002; 108: 151-60.
26. Kudolo GB, Wang W, Barrientos J, Elrod R, Blodgett J. The ingestion of ginkgo biloba extract (EGb 761) Inhibits arachidonic acid-mediated platelet aggregation and thromboxane B2 production in healthy volunteers. J Herb Pharmacother 2004; 4: 13-26.
27. Macovschi O, Prigent AF, Nemoz G, Pacheco H. Effects of an Extract of *Ginkgo biloba* on the 3′ ,5′ -Cyclic AMP phosphodiesterase activity of the brain of normal and triethyltin-intoxicated rats. J Neurochemi 1987; 49: 107-14.
28. Arruzazabala ML, Carbajal D, Más R, García M, Fraga V. Effect of policosanol on platelet aggregation in rats. Thromb Res 1993; 69: 321-7.
29. García Mesa M, Díaz Batista A, Fernández Montequín José I, Hernández Carretero J, Coma Alfonso C. Natural anti-platelet agents. Pharmacologyonline 2006; 3: 625-32.
30. Molina V, Arruzazabala ML, Carbajal D, Más R, Valdés S. Antiplatelet and antithrombotic effect of D-003. Pharmacol Res 2000; 42: 137-43.
31. Arruzazabala ML, Mas R, Molina V, Carbajal D, Fernández L, Illnait J, et al. Effects of d-003, a new substance purified from sugar cane wax, on platelet aggregation and plasma levels of arachidonic acid metabolites in healthy volunteers. Int J Clin

Pharmacol Res 2004; 24: 55-63.
32. Ledón N, Casacó A, Remirez D, González A, Cruz J, González R, et al. Effects of a mixture of fatty acids from sugar cane (Saccharum officinarum L.) wax oil in two models of inflammation: Zymosan-induced arthritis and mice tail test of psoriasis. Phytomedicine 2007; 14 : 690–5.
33. Carbajal D**,** Arruzazabala MJ, Valdés S, Más R. Effect of policosanol on platelet aggregation and serum levels of arachidonic acid metabolites in healthy volunteers. Prost Leukot Essent Fatty Acids 1998; 58: 61-4.
34. Pérez Y, Mas R, Gonzalez RM, Jimenez S, Molina V. Effects of D-003 a mixture of very long chaín saturated fatty acids and policosanol on *in vivo* lipid peroxidation in rats. Arzneim Forsch Drug Res 2008; 58: 126-30.
35. Lans CA. Ethnomedicines used in Trinidad and Tobago for urinary problems and diabetes mellitus. J Ethnobiol Ethnomed 2006; 2: 45.
36. Heptinstall S, May J, Fox S, Catherine KU, Lian Z. Cocoa flavanols and platelet and leukocyte function: recent in vitro and ex vivo studies in healthy adults. J Cardiovasc Pharmacol 2006; 47: S197-205.
37. Bordeaux B, Yanek LR, Moy TF, White LW, Becker LC, Faraday N. Casual chocolate consumption and inhibition of platelet function. Prev Cardiol 2007; 10: 175-80.
38. Hamed MS, Gambert S, Bliden KP, Bailon O, Anand S, Antonino MJ, et al. Dark chocolate effect on platelet activity, Creactive protein and lipid profile: a pilot study. South Med J 2008; 10: 1203-8.
39. Colombo ML, Pinorini-Godly MT, Conti A. Botany and Pharmacognosy of the cacao tree. In: Paoletti R, Poll A, Conti A (eds.). Chocolate and Health. Milan: Springer; 2012. p. 41-62.
40. Fisher ND, Hughes M, Gerhard-Herman M, Hollenberg NK. Flavanol-rich cocoa induces nitric-oxide-dependent vasodilation in healthy humans. J Hypertension 2003; 21: 2281-6.
41. Engler MB, Engler Marguerite M, Chen Ch Y, Malloy MJ, Browne A, Chiu EY, et al. Flavonoid-rich dark chocolate improves endothelial function and increases plasma epicatechin concentrations in healthy adults. J Am Coll Nutr 2004; 23: 197-204.
42. Ramiro E, Franch À, Castellote C, Pérez-Cano Francisco, Permanyer J, Izquierdo-Pulido M, et al. Flavonoids from *Theobroma cacao* down-regulate inflammatory mediators. J Agric Food Chem 2005; 53: 8506-11.
43. Freedman JE, Parker C 3rd, Li L, Perlman JA, Frei B, Ivanov V, et al. Select flavonoids and whole juice from purple grapes inhibit platelet function and enhance nitric oxide release. Circulation 2001; 103: 2792-8.
44. Shanmuganayagam D, Beahm MR, Osman HE, Krueger CG, Reed JD, Folts JD. Grape seed and grape skin extracts elicit a greater antiplatelet effect when used in combination than when used individually in dogs and humans. J Nutr 2002; 132: 3592-8.
45. Keevil JG, Osman HE, Reed JD, Folts JD. Grape juice, but not orange juice or grapefruit juice, inhibits human J Nutr 2000; 130: 53-6.
46. Sano T, Oda E, Yamashita T, Naemura A, Ijiri Y, Yamakoshi J, et al. Anti-thrombotic effect of proanthocyanidin, a purified ingredient of grape seed. Thromb Res 2005; 115: 115-22.
47. Zhirong W, Jiangang Z, Yuanzhu H, Kejiang C, Yinan X, Wu JM. Effect of resveratrol on platelet aggregation in vivo and in vitro**.** Chinese Med J 2002; 115: 378-80.
48. De Lange DW, Van Golden PH, Scholman WL, Kraaijenhagen RJ, Akkerman JW, Van de Wiel A. Red wine and red wine polyphenolic compounds but not alcohol inhibit ADP-induced platelet aggregation. J Int Med 2003; 14: 361-6.