

A new favourable effect of cocoa on atherosclerosis?

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This editorial refers to 'Cocoa procyanidins inhibit expression and activation of MMP-2 in vascular smooth muscle cells by direct inhibition of MEK and MT1-MMP activities' by K.W. Lee *et al.*, pp. 34–41,⁵ this issue.

Numerous epidemiological studies have indicated a role of different nutrients and phytochemicals in reducing the risk of cardiovascular disease, although the mechanisms are far from clear. Cocoa is one of the most concentrated sources of flavonols, a subgroup of the natural antioxidant compounds called flavonoids, also found in tea and red wine. Flavonoids, in turn, are a subgroup of compounds called polyphenols. Animal as well as human studies have shown beneficial changes in biological phenomena related to cardiovascular health after the consumption of polyphenol-rich foods such as cocoa, red wine, tea, and berries.^{1–3} Evidence that flavonol-rich cocoa contributes to health is convincing among Kuna Indians living off the coast of Panama, who have no increase in blood pressure with age and very low incidence of hypertension. The major chemical cocoa mediators have been identified, including the monomers epicatechin and catechin, and possibly procyanidins and metabolites.⁴ In a carefully controlled series of *in vitro* experiments, Lee *et al.*⁵ present an article in this issue of *Cardiovascular Research* describing new protective effects of cocoa on cardiovascular disease and atherosclerosis, adding to the list of cocoa-related cardiovascular protective mechanisms.

Polyphenols affect pathways related to cardiovascular health by several different antioxidation-dependent and -independent mechanisms. For instance, beneficial effects have been observed in humans after ingestion of cocoa on blood pressure, HDL-cholesterol, platelet aggregation, and endothelial function.^{6–8} The beneficial effects of cocoa on vascular function may be mediated through an antioxidant action, mainly related to improved nitric oxide bioavailability via either enhanced nitric oxide production or decreased nitric oxide inactivation.⁹ Furthermore, cocoa is able to increase the vasodilatory prostacyclin and to decrease leukotrienes through the modulation of the endothelial cell eicosanoid system pathway by inhibiting 5-lipoxygenase.

The reduction in the plasma leukotriene/prostacyclin ratio, a measure of the proinflammatory/anti-inflammatory eicosanoid balance, indicates that cocoa may have an anti-inflammatory effect. Because inflammation is associated with increased aortic stiffness, this anti-inflammatory effect of cocoa may also play a role in its beneficial effect on arterial function.¹⁰

It is now widely accepted that atherosclerosis is an inflammatory disease of the arterial vascular wall. Inflammation is a complex process that involves distinct cellular players and requires the occurrence of several steps including cell migration as well as synthesis of extracellular matrix (ECM) leading to the atherosclerotic fibrous plaque; in later stages, ECM degradation results in plaque disruption responsible for the clinical atherothrombotic syndromes. ECM degradation is tightly regulated within the normal vessel wall through a balance between proteinases and their endogenous inhibitors. However, within the atherosclerotic plaque, the balance may become shifted towards matrix degradation, particularly at the rupture-prone shoulder regions of the fibrous cap where accumulating macrophages and phenotypically altered vascular smooth muscle cells (VSMC) secrete a plethora of proteinases, including matrix metalloproteinases (MMPs)^{11,12}. It has been proposed that these enzymes contribute to plaque rupture; thus, inhibition of MMPs within the atherosclerotic plaque may prevent disruption and its clinical sequelae.

Lee *et al.*⁵ show that a procyanidin-rich cocoa fraction (CPF) and procyanidin B2 exerted a strong inhibitory effect on the thrombin-induced expression and activation of the latent zymogen form of MMP-2, pro-MMP-2, as well as thrombin-induced migration and invasion of VSMC by directly inhibiting activity of a membrane type of MMP, MT1-MMP, via the kinase MEK1. This effect was even greater than that achieved with red wine polyphenols, known to afford beneficial changes in pathways related to cardiovascular health.² Thrombin is a multifunctional serine protease generated at the site of vascular injury that transforms fibrinogen to fibrin, activates platelets and MMPs, and elicits multiple effects on endothelial cells and VSMC including proinflammatory activity¹²; in fact, thrombin is a potent inducer of MMP-2 in both cell types. Among MMPs, MT1-MMP was the first shown to be membrane anchored. It can degrade a variety of ECM components, including fibrillar collagen 1, and is particularly suited for

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pericellular proteolysis; therefore, it has been shown to be involved in cell migration and participates in distinct processes relevant to the inflammatory context. In particular, it is important for chemokine and nitric oxide-induced angiogenesis and relevant for human monocyte endothelial transmigration.¹³ It is interesting to note that constitutive activation of MEK1 results in induction of angiogenesis and atherosclerosis, whereas, as reported by Lee *et al.*,⁵ both CPF and procyanidin B2 inhibited the expression of pro-MMP-2 by directly inhibiting MEK1, likely providing atherosclerotic protection.

Numerous studies have tried to dissect the roles of MMPs in atherosclerotic plaque development and instability with the aid of a broad spectrum of MMP inhibitors. However, results have been contradictory. For instance, doxycycline treatment reduces the activity and/or expression of MMP-9 and MMP-1; however, in preliminary studies, no effect on morphologic characteristics or clinical end-points was observed in patients with atherothrombotic syndromes.¹⁴ It is becoming evident that MMPs can play distinct roles in different cell types and, therefore, to dissect MMP functionality in distinct cell types may help to approach cell-type specific inhibition of MMPs as a potential therapy for atherosclerosis.¹⁵

There are still questions to be resolved. Does cocoa intake achieve similar effects on MMP activation and cellular migration *in vivo*? If so, what is the amount of cocoa required to obtain anti-atherosclerotic effects and will these be maintained over time? In conclusion, the authors found favourable changes in cellular migration and expression of MMPs in cocoa- and procyanidin-treated VSMC. Provided their findings can be confirmed *in vivo* they may partly explain the cardiovascular-protective role of cocoa and other procyanidin derivatives. If cocoa ingestion indeed reduces coronary events, this issue will become important as future studies are designed. It could be inferred that adding modest amounts of dark chocolate, which is derived from cocoa, to the diet can help to improve cardiovascular health.

Conflict of interest: none declared.

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