The Science of Cocoa Flavanols: Bioavailability, Emerging Evidence, and Proposed Mechanisms[1,2]

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ABSTRACT

Over the past 20 y, evidence derived from in vitro experiments, animal models, observational studies, and clinical interventions have suggested that cacao (cocoa) flavonoids act through a variety of mechanisms to modify a number of risk factors associated with chronic conditions, including cardiovascular and neurodegenerative diseases. Recent studies have elucidated the synthesis of flavonoids by plants, making available for research specific flavonoids and their metabolites. The body of evidence suggesting that cocoa flavanols may play a role in reducing the risk of cardiovascular disease has been sufficient to generate several systematic reviews and meta-analyses. Studies are now being directed to identify the molecular pathways underlying the effect of cocoa flavanols, and clinical trials are being planned to test their impact on disease endpoints. *Adv. Nutr. 5: 547–549, 2014.*

Introduction

Among the broad array of dietary polyphenols, research has stimulated particular interest in the flavonoids, which comprise approximately two-thirds of the total of this class of bioactive compounds. Flavonoids are largely present in fruits, chocolate, and beverages such as coffee and tea. Cocoa contains the highest flavan-3-ol (flavanol) content of all foods on a per-weight basis and can contribute substantially to the total intake of dietary flavonoids. A growing body of basic research, observational studies, and clinical interventions strongly suggests that flavonoids, particularly the flavanols, from cocoa may affect multiple risk factors for chronic diseases, including elevated blood pressure, dyslipidemia, inflammation, insulin resistance, and vascular reactivity. Our knowledge of the mechanisms underlying these actions is limited but is steadily emerging to reveal interactions with signaling proteins, cell membranes, microRNA expression, and other targets. The goal of this symposium was to provide a brief update of recent research, from the farm to pharmacokinetics to physiology, exploring the putative role of cocoa flavonoids on cardiovascular and cognitive functions.

Biosynthesis of Flavanols within Plants

The session was opened by Dr. Dixon who described the biochemical pathways used by plants for the biosynthesis of the major flavanols, catechin and epicatechin. Two important model systems used in this research are *Arabidopsis thaliana* (thale cress, a common weed) and *Medicago truncatula* (barrel medic, a forage legume in the Southern hemisphere and a close relative of alfalfa). Both species accumulate proanthocyanidins derived from flavanols in their seed coats. The genomes of both plants have been sequenced, and extensive collections of mutants are now available. Loss of proanthocyanidins in the seed coat leads to a transparent testa phenotype (clear seed coat), and understanding the genes affected in transparent testa mutants has greatly aided in the elucidation of the flavanol pathway. Catechin and epicatechin differ at the stereochemistry around the 2 and 3 positions of the central heterocyclic ring: catechin is 2,3-*trans* whereas epicatechin is 2,3-*cis*. The flavonoid precursors of both catechin and epicatechin are of the 2,3-*trans* configuration, established earlier in the pathway by the enzyme chalcone isomerase. Catechin is formed by direct reduction

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[10] Abbreviations used: AD, Alzheimer disease; COSMOS, Cocoa Supplement and Multivitamins Outcomes Study; CVD, cardiovascular disease; GATA1, erythroid transcription factor or GATA-binding factor 1; MI, myocardial infarction; RCT, randomized controlled trial.

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of 2,3-*trans*-leucoanthocyanidin by leucoanthocyanidin reductase. Epicatechin is formed via the reduction of an achiral anthocyanidin by the enzyme anthocyanidin reductase, allowing for introduction of 2,3-*cis* stereochemistry. Leucoanthocyanidin is the immediate precursor of anthocyanidin, formed by the action of anthocyanidin synthase, highlighting the close relation between the flavanol and anthocyanin pathways in plants.

Recent studies of the regulatory genes that control the biosynthesis of flavanols and anthocyanins reveal pathways that are regulated by ternary complexes of transcription factors, each containing a myeloblastosis family protein, a basic helix-loop-helix protein, and a β-transducin repeat domain protein. So, it is now possible to engineer plant tissues to accumulate increased amounts of flavanols and proanthocyanidins through overexpression of ≥1 of these types of transcription factors (1). Interestingly, naturally occurring mutants of some plants exist in which the fruits lack astringency due to loss of function of such regulatory genes.

After their biosynthesis in the plant, flavanols can be stored in the central vacuole of the cell as glycosides (epicatechin as the 3′-*O*-glucoside, at least in *Medicago* and *Arabidopsis*) or further converted to proanthocyanidins by mechanisms yet unknown. It is important to contrast the plant profile of these phytochemicals with their bioavailability and metabolism in vivo. For example, when mice are fed fractionated polymeric, oligomeric, and monomeric flavanol preparations, only the monomers (catechin and epicatechin) appear to be bioavailable. These monomers are recovered in plasma and, at much lower concentrations, in brain tissue as glucuronides of the parent molecule or of *O*-methylated derivatives. Importantly, these flavanol metabolites can now be synthesized and used in studies of their mechanisms of action.

Cocoa Flavanols in Alzheimer Disease Pathology: Experimental Approaches and Clinical Applications

Dr. Pasinetti noted that interest in developing polyphenols for treatment of dementia is warranted due to findings from basic research studies as well as a clinical trial showing that dietary supplementation with cocoa polyphenol preparations improve cognitive function in the elderly. It is pertinent that a new trial is now testing the cognitive benefits of pomegranate polyphenols in the elderly. However, our understanding of the mechanisms by which polyphenols benefit cognition is limited. Recent and ongoing studies of the role of brain-penetrating polyphenols on synaptic plasticity can provide important information supporting their potential application for Alzheimer disease (AD) prevention as well as treatment.

The profiles of flavanols in cocoa and grapes are similar, with catechin, epicatechin, catechin gallate, and epicatechin gallate as the principal flavonoids. Pasinetti and colleagues characterized the pharmacokinetics of these flavanols in Sprague-Dawley rats and found that they accumulate at submicromolar concentrations in brain. By using electrophysiology, some of these metabolites, such as 3′-*O*-methyl-epicatechin-5-*O*-β-glucuronide, were found to prevent acute oligomeric amyloid-β-induced long-term potentiation impairments and improve basal synaptic transmission and long-term potentiation through activation of the calmodulin kinase II-cAMP response element-binding protein signaling pathway in hippocampal slices from a genetically modified AD mouse model (2). Investigations of molecular pathways with the use of Luminex xMAP® multiplexed immunoassays revealed that, by modulating neuroplasticity mechanisms, flavanols may be able to promote synapse growth and increase receptor density. A minimally processed cocoa extract ("Lavado") was found to attenuate erythroid transcription factor or GATA-binding factor 1 (GATA1)–mediated repression of presynaptic genes in the AD mouse model. Because exogenous GATA1 expression increases amyloid-β content through mechanisms influencing β-secretase 1, GATA1 presents an additional target through which cocoa flavanols may beneficially influence AD neuropathology. Thus, cocoa flavanols may attenuate the onset and progression of AD neuropathology through molecular mechanisms influencing GATA1 expression in the brain, a molecular index of major depressive disorders.

Synaptic alterations and dysfunction are among the earliest events in the development of AD-type cognitive decline, preceding neuronal loss and likely contributing to the progressive failure of neuronal systems leading to clinical dementia. Targeting the molecular mechanisms that contribute to synaptic dysfunction in the brain with cocoa flavanols and other dietary polyphenols should provide useful insight into the development of novel primary and secondary prevention strategies to preserve cognitive function as well as the rational design of future clinical studies.

Evidence-Based Assessment of Cocoa Flavanol Effects on Cardiovascular and Metabolic Risk Factors

In addition to laboratory experiments and case series reports suggesting benefits of cocoa flavonoids, epidemiologic and clinical evidence have been mounting in support of their action to reduce the risk of cardiovascular disease (CVD). Dr. Ding characterized the body of evidence for the clinical effectiveness of cocoa flavanols from short-term randomized trials as strong for traditional risk factors for CVD, such as metabolic mediators like blood pressure, lipids, and insulin resistance. However, long-term randomized controlled trials (RCTs) with hard CVD endpoints are necessary before recommendations for cocoa flavonoid can be proffered.

Systematic reviews of short-term cocoa flavanol trials and meta-analyses of cohort studies are now available and are largely consistent in their conclusions regarding metabolic endpoints. For example, Shrime et al. (3) analyzed studies with an average cocoa flavonoid dose of 400–600 mg/d and with isocaloric comparison to controls and found supplementation or fortification to improve multiple CVD risk factors, including lowered blood pressure, lower LDL and higher HDL cholesterol, improved insulin resistance, and enhanced flow-mediated dilation; however, total cholesterol,

TGs, pulse rate, BMI, and C-reactive protein were unchanged by the intervention. Although limited by their short duration (2–18 wk), these trials testing intermediate endpoints do provide evidence for an impact of cocoa flavonoids on metabolic factors associated with primary and secondary prevention of CVD.

Clinical evidence of hard endpoints from prospective cohort studies also supports the putative CVD benefits of cocoa flavonoids. For example, the Zutphen Elderly Study, a prospective cohort of 470 men in The Netherlands followed for 15 y with highly detailed assessment of cocoa intake, found a significant inverse association between cocoa intake and reductions of 50% and 47% in CVD mortality and total mortality, respectively, in the highest versus the lowest tertile. Overall, a systematic review and meta-analysis of 7 cohort studies showed that higher versus lower amounts of calorie-adjusted chocolate consumption were associated with a 37% reduction in the relative risk of total CVD (4). Recently, Larsson et al. (5) reported a 19% reduction in the risk of stroke in their analysis of calorie-adjusted chocolate and cocoa consumption.

To solidify the body of evidence for a role of cocoa flavonoids in CVD prevention, long-term RCTs with hard endpoints are needed to build on the results from cohort studies and short-term trials. The Cocoa Supplement and Multivitamins Outcomes Study (COSMOS), an RCT to test cocoa flavonoid supplementation with such hard CVD endpoints led by investigators from Brigham and Women's Hospital and Harvard Medical School, will commence enrollment in 2015. COSMOS will enroll 12,000 women aged ≥65 y and 6000 men aged ≥60 y who are free of CVD to determine the effect of high-quality cocoa flavanol supplementation at 750 mg/d (containing 75 mg epicatechin). This RCT has a 2 × 2 factorial design with multivitamin and placebo controls and a planned mean treatment duration of 4 y. At completion, this study is expected to yield strong evidence for or against cocoa flavonoid supplementation for the reduction in risk of CVD.

Flavanol Enhancement of Mitochondria Structure-Function as a Cardioprotective Mechanism

Central to the control of cell and organ bioenergetics are the mitochondria, organelles mainly responsible for the provision of cellular energy as ATP. Proper mitochondrial function is also necessary to minimize cell damage associated with the production of reactive oxygen species during ATP generation. Oxidative stress produced during myocardial ischemia can induce calcium overload in cardiac muscle, leading to the swelling of the mitochondria. Mitochondrion swelling is an established trigger of cell death, releasing from the inner membrane factors that activate cell apoptosis. Thus, mitochondria play a central in maintaining optimal cellular bioenergetics, so that loss of organelle structure or function is considered a critical aspect of the pathophysiology of many chronic diseases, including type 2 diabetes, AD, Parkinson disease, fibromyalgia, and various cardiovascular pathologies. Myocardial infarction (MI) is a major cause of worldwide morbidity and mortality, and pharmacologic strategies designed to limit heart damage are an important target for therapy. Dr. Villarreal noted that evidence on the association between consumption of modest amounts of cocoa products high in flavanols (specifically, epicatechin) and reductions in cardiometabolic risk has stimulated new research in this area.

Yamazaki et al. (6) implemented preclinical studies in rodents with the use of (−)-epicatechin to ascertain its potential to prevent or treat MI. In their pretreatment studies, 2 modalities of MI were tested: an ischemia-reperfusion injury in which blood flow to myocardium was transiently interrupted and a permanent obstruction of flow to the heart. In both cases, daily pretreatment with 1 mg/kg (−)-epicatechin for 10 d was able to reduce MI size by ~50% at 48 h after transient or permanent coronary occlusion. A second study was conducted to evaluate the participation of mitochondria in (−)-epicatechin–induced protection via assessment of organelle volume (density) and cristae abundance. After an i.v. dose of (−)-epicatechin, rats were subjected to a transient occlusion of the coronary artery. A dose-response effect on infarct size reduction was observed, with a single dose providing ~35% reduction in MI size and doubling the dose yielded a reduction of ~80%. Similarly, an acute dose-dependent benefit of (−)-epicatechin was obtained in assays of calcium-induced mitochondrial swelling. These preclinical studies indicate that (−)-epicatechin provides a cardioprotective action by limiting mitochondrial swelling, reducing apoptosis, and preventing cell death. Future studies are now warranted to evaluate the cardioprotective potential of (−)-epicatechin in other animal models to move closer to the potential translation of dietary flavanols for disease prevention and treatment.

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