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REVIEW

Antihypertensive effects and mechanisms of chlorogenic acids

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Chlorogenic acids (CGAs) are potent antioxidants found in certain foods and drinks, most notably in coffee. In recent years, basic and clinical investigations have implied that the consumption of chlorogenic acid can have an anti-hypertension effect. Mechanistically, the metabolites of CGAs attenuate oxidative stress (reactive oxygen species), which leads to the benefit of blood-pressure reduction through improved endothelial function and nitric oxide bioavailability in the arterial vasculature. This review article highlights the physiological and biochemical findings on this subject and highlights some remaining issues that merit further scientific and clinical exploration. In the framework of lifestyle modification for the management of cardiovascular risk factors, the dietary consumption of CGAs may hold promise for providing a non-pharmacological approach for the prevention and treatment of high blood pressure.
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INTRODUCTION

Chlorogenic acids (CGAs) are a family of polyphenolic compounds, which are esters between *trans*-cinnamic acid (such as caffeic, ferulic and coumaric acid) and quinic acid (1 l—1 [OH], 3, 4/5-tetrahydroxy cyclohexane carboxylic acid)(Figure 1).[1] Three major subclasses of CGAs are caffeoylquinic (CQA), feruloylquinic (FQA) and dicaffeoylquinic (diCQA) acids.[1] The principal CGA in coffee is 5-O-caffeoylquinic acid (5-CQA) and its isomers 3- and 4-CQA.[1] Coffee and its derivatives can be a major source of dietary CGA for humans. A regular cup of Arabica coffee (*Coffea arabica*) contains between 70 and 200 mg of CGA, and a cup of Robusta coffee (*Coffea canephora*) contains between 70 and 300 mg of CGA.[2] Daily CGA intake in heavy coffee drinkers is about 0.5–1.0 g, whereas in coffee abstainers the daily intake can be <100 mg.[3] Thus, dietary CGA intake can be heavily influenced by coffee consumption. Other dietary sources of CGA include tea, cocoa, pome fruits (such as apples and pears), berry fruits and citrus fruits.[2]

From a nutritional point of view, CGAs are potent dietary antioxidants.[1–3] In recent years, a number of health benefits associated with the consumption of CGA-abundant foods and drinks have been elucidated from epidemiology investigations. In certain settings, CGAs have been shown to reduce the relative risks of type 2 diabetes,[4,5] obesity,[6] Alzheimer's disease,[7,8] eclampsia and stroke.[9,10] In some animal studies, CGAs have also been shown to sensitize insulin action, and to improve glucose tolerance and lipid metabolism.[11–13] Of great interest, investigations during the last decade have demonstrated that the consumption of green coffee extracts produces an anti-hypertensive effect in both rats and humans,[14,15] as a result of improved endothelial or vascular function.[16,17] Such biological effects have been attributed to the presence of CGAs in green coffee. The purpose of this invited review is to highlight the findings pertinent to the effects and mechanisms of CGA on arterial blood pressure control and to discuss unanswered questions that warrant further research effort.

EFFECT OF GREEN COFFEE EXTRACTS ON BLOOD PRESSURE IN ANIMALS

The blood pressure lowering effect of green coffee bean extracts (GCE) was first reported in 2002.[18] In spontaneously hypertensive rats (SHR), a single oral administration of 180, 360 and 720 mg kg^{-1} GCE (CGA content: 28%), respectively, reduced blood pressure (measured by tail cuff for 25 h) in a dose-dependent fashion: Systolic blood pressures(SBP) were reduced by 10–15 mm Hg from baseline 10 h after the ingestion of GCE at 720 mg kg^{-1}, and the effect persisted through the end of the experiment. However, GCE had no discernable effect in normotensive counterparts (control rats WKY). Furthermore, chronic administration of GCE mixed in rodent chow to young SHR for 6 weeks reduced the magnitude of high blood pressure development in a dose-dependent manner (SBP was 211 on control diet *vs.* 199, 186 and 179 mm Hg on diets containing 0.25, 0.5 and 1% GCE, respectively).[18]

As mentioned previously, GCE are rich in CGAs, with the principal form being 5-CQA. Logically, the blood pressure reduction effect of GCE was hypothesized to be due to the presence of 5-CQA. As expected, both acute and chronic administration of 5-CQA showed almost identical results as seen with GCE administration in the same animal model.[18,19] The reduction of blood pressure was accompanied

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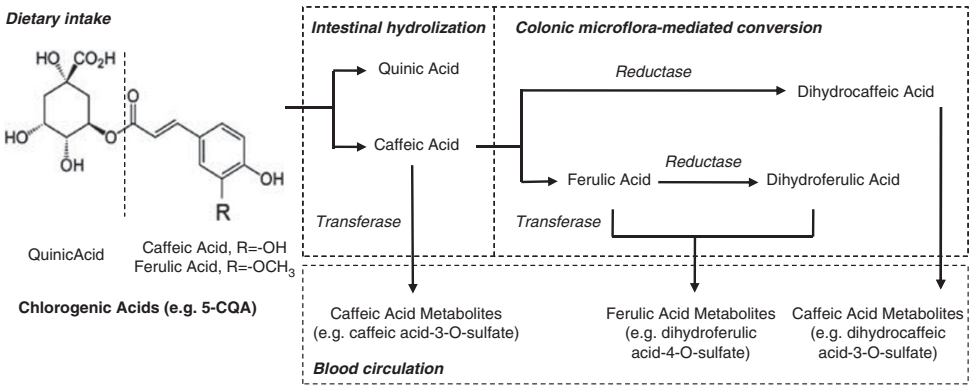

Figure 1 Simplified diagram of main metabolic pathway of chlorogenic acids (CGA). Dietary CGA is hydrolyzed into quinic acid and caffeic or ferulic acid after being taken. They are further metabolized in both small intestine and colon before entering into blood stream.

by an increase in plasma levels of phenolic compounds.[19] Aside from blood pressure reduction, chronic treatment of rats with either GCE or CGA did not show any discernable changes in food intake, body weight, heart rate, and urine output.[20]

EFFECT OF GREEN COFFEE EXTRACTS ON BLOOD PRESSURE IN HUMANS

These findings in animals were later confirmed by studies in human patients with mild hypertension. In a randomized, double-blind placebo-controlled study, 117 untreated patients with mild hypertension were assigned to four groups to receive 180 ml of fluid containing 0 (placebo), 46, 93 or 185 mg of GCE with 54% CGA content. After consumption of GCE for 28 days, SBP and diastolic blood pressure (DBP) were reduced in a dose-dependent manner by 1.3/0.8, 3.2/2.9, 4.7/3.2 and 5.6/3.9 mm Hg, respectively.[15] In another randomized, double-blind placebo-controlled clinical trial, when 480 mg per day of GCE (equivalent to 140 mg per day CGA) was given in a mixture of fruit and vegetable juice, SBP/DBP in the CGA-treated group decreased significantly by 8/7 mm Hg at week 4 and by 10/7 mm Hg at week 12 from baseline.[21] It should be pointed out that these two clinical trials were carried out in Far East Asia, and it is unknown at this time whether ethnicity or differences in dietary customs would affect the reproducibility of these results.

DIFFERENT ROLES OF COFFEE COMPONENTS IN BLOOD-PRESSURE REGULATION

With several studies explicitly demonstrating that GCEs and CGAs produce a moderate anti-hypertensive effect in SHR animals and a mild anti-hypertensive effect in patients with primary hypertension, the obvious question to ask is why drinking regular coffee does not seem to have such an effect. In fact, almost all epidemiologic studies and intervention trials have demonstrated that the consumption of regular roasted coffee does not reduce blood pressure,[22–25] even though the CGA content in roasted coffee falls within the range which produced anti-hypertensive effects in the previously mentioned studies.

The initial attention for this discrepancy was focused on the caffeine content in regular coffee for two main reasons: first, the molecule of caffeine is structurally similar to adenosine and capable of binding to adenosine receptors, thereby acting as non-selective adenosine antagonist; and second, caffeine inhibits phosphodiesterase non-selectively, thereby causing an accumulation of cAMP, which can mediate a vasoconstrictive response.[26] However, caffeine content *per se* does not seem to convincingly answer the question. A meta-analysis, which included 16 randomized controlled trails in 1010 subjects who were given either coffee or caffeine for at least a week, showed an average pressor response of 4.2/2.4 mm Hg in caffeine intervention trials (average caffeine dose 423 mg per day, dose range 295–750 mg) and only 1.2/0.5 mm Hg in coffee intervention trials (average caffeine dose 488 mg per day, dose range 225–798 mg).[24] More importantly, the extraction process for making GCE does not actually remove caffeine. As a matter of fact, decaffeinated coffee, in which caffeine was largely removed, still did not fully simulate GCE's effect on hemodynamics.[27] Finally, the pressor effect of caffeine was likely attenuated in chronic consumption, because a partial caffeine tolerance was developed.[28–30]

These findings are echoed in animal studies as well. In one animal experiment, a difference in hemodynamic effect between GCE and roasted instant coffee was observed despite both containing same amount of CGA (200 mg kg^{-1}). A single oral administration of GCE in SHR decreased SBP continuously until it was 12% lower than baseline at 10 h after treatment; by contrast, the roasted instant coffee did not induce any significant SBP reduction.[16] However, when caffeine was added to GCE to make the caffeine content (200 mg kg^{-1}) equivalent to that of the instant coffee, the same reduction in SBP was seen as compared with GCE alone.[16] Similarly, GCE reduced blood pressure in human hypertensive subjects despite a caffeine content equal to that in roasted coffee.[15] Thus, caffeine content alone does not convincingly explain the discrepancy in the hemodynamic effects of GCE *vs.* actual coffee.

The exclusion of caffeine's role in mediating the hypotensive effects of GCE resulted in further investigation to track down a culprit, which might explain this phenomenon. As GCE was manufactured from coffee beans without roasting, it was logical to postulate that other component(s) produced during the roasting process may be responsible for counteracting the anti-hypertensive effect of CGAs. Following repeated isolation steps using reverse phase high performance liquid chromatography (HPLC), hydroxyhydroquinone (HHQ), a particular fraction, which was derived from CGA and was not seen before high temperature treatment, was discovered in roasted coffee. In both acute and chronic studies,[19,20,31] the administration of HHQ (3 mg kg^{-1}) mostly eliminated the anti-hypertension effect of GCEs (containing 300 mg kg^{-1} of CGA) in SHR. Interestingly, HHQ alone had not been found to exert any effect on blood pressure and vascular function in both WKY and SHR models.

A method of producing HHQ-free coffee was to treat roasted coffee extracts with activated charcoal at room temperature followed by passage through a membrane filter, which removed nearly 97% of

HHQ.[16] When young SHR was orally treated with HHQ-free coffee for 8 weeks (approximately 300 mg kg^{-1} per day of CGA), the natural development of hypertension was significantly retarded compared with rats treated with regular coffee.[16] In addition, co-administration of 3 mg kg^{-1} HHQ along with HHQ-free coffee abolished the anti-hypertensive effect of HHQ-free coffee. In volunteer patients with mild hypertension who were not taking anti-hypertensive medication,[32] a combination treatment of 299 mg of CGA and 1.7 mg of HHQ had no effect on their BP. Consistent with the animal studies, after daily consumption of HHQ-free coffee (containing 82 to 299 mg per day of CGA and no more than 0.05 mg per day of HHQ) for 4 weeks, the BP were decreased in a marginally dose-dependent pattern.[32] The fact that in the presence of HHQ, GCE, CGAs and coffee no longer had any significant antihypertensive effect implicated that HHQ was the culprit in counteracting the anti-hypertensive effect of CGAs in regular coffee.

The current hypothesis for this finding was that the production of HHQ-derived superoxide[31,33,34] neutralized CGA-enhanced nitric oxide (NO) bioactivity,[35] therefore concealing the anti-hypertensive effects of CGA. However, the evidence for HHQ to produce superoxide is rather weak, because acetylcholine-induced vasorelaxation was not attenuated by HHQ in *ex vivo* experiments.[35] After 8 weeks of consumption, HHQ also failed to change the urinary excretion of nitrite/nitrate and hydrogen peroxide or to influence aortic nitrotyrosine (used to assess *in situ* oxidative/nitrosative stress) in both normotensive rats and SHR.[20] Long-term ingestion of HHQ alone had no effect on either SBP in intact animals *in vivo* or endothelium-dependent vasodilatation of aortic rings from SHR *ex vivo*.[20] Although the mechanism of HHQ is still unclear and the data relating to its function are still very preliminary, it appears that HHQ was inert alone because it had no effect on basal and endogenous NO status, but acted to modulate the NO pathway when the pathway was augmented by exogenous factors such as CGAs.

METABOLITES OF CHLOROGENIC ACID IN THE BODY

In various experimental protocols in animals and humans, CGA and its metabolites were detectable in systemic circulation.[18,36,37–40] The major CGA metabolites were ferulic, caffeic and isoferulic acid, which were seen in blood stream after CGA, GCE or regular coffee consumption. Physiologically, one-third of ingested CGAs in foods and beverages were absorbed by the small intestine (shown in subjects with ileostomy—a surgical procedure that brings a loop of small intestine, that is, ileum, out onto the surface of the abdominal skin) and was measured by high performance liquid chromatography in the forms of 3-CQA, 4-CQA and 5-CQA in plasma.[38,39] The remaining two-thirds passed to the large intestine,[1,38] where CGA was further metabolized by gut microflora and then absorbed. Biochemically, the small intestine is the site where cleavage of quinic acid from CQA and FQA, and then the release of caffeic and ferulic acid took place, whereas the colon was important for conversion of both ferulic and caffeic acid to dihydroferulic acid and for its absorption (Figure 1).[1]

The differential anti-hypertensive effects of these phenolic metabolites of coffee were compared by i.v. injection of 5 μmol kg^{-1} of ferulic acid, caffeic acid and quinic acid in anesthetized SHR. BP measurement from carotid artery indicated that ferulic acid had strongest hypotensive effect as SBP was reduced by 29.6%, whereas caffeic acid and quinic acid injection caused SBP to reduce by 3.2 and 1.7%, respectively.[18] Oral administration of 50 mg kg^{-1} of ferulic acid also induced anti-hypertensive effect in both SHR and stroke-prone SHR (that is, SHRsp) models.[14,41] After digestion of ferulic acid in doses ranging from 0.5 to 50 μM, plasma levels of ferulic acid were elevated, with negatively correlated SBP changes ranging from −5 mm Hg to −15 mm Hg.[14] This evidence suggests that the CGA metabolites ferulic acid and, to a lesser extent, caffeic acid are responsible for the hypotensive effect seen in oral administration of CGA.

MOLECULAR TARGETS OF CHLOROGENIC ACID IN BLOOD-PRESSURE REGULATION

Although the mechanisms by which CGAs exhibit cardiovascular protective benefit have not been fully elucidated yet, it has been ubiquitously proven that coffee and its derivatives are natural antioxidants. A positive relationship between oxidative stress and blood pressure were demonstrated in some models of experimental hypertension.[42] For example, an upregulation of reactive oxygen species appeared to precede the development of hypertension,[43,44] and an antioxidant regimen arrested the process.[44,45] In vasculature, the major source of reactive oxygen species was from a family of non-phagocytic NAD(P)H oxidases, which comprised a catalytic core (Nox/p22^{phox}) and several cytosolic subunits (p47^{phox}, p67^{phox}, p40^{phox}, Rac).[46] The core and the subunits heterodimerized in response to physiological and pathological stimulation such as angiotensin II (Ang II),[47] thereby facilitating superoxide (that is, O_2^-) production.[48,49] NAD(P)H oxidase-derived superoxide had a pivotal role in the regulation of vascular tone in health and disease.[48,49] It was found that continuous ingestion of CGAs for 8 weeks reduced NAD(P)H-dependent superoxide production in the aorta of SHR, which was directly linked to the inhibition of p22^{phox} gene expression.[16,19]

Caffeic acid in particular had been shown to attenuate the proliferation of vascular smooth muscle cells to Ang II stimulation in SHRsp and WKY through inhibiting intracellular superoxide anion generation and partially blocking both the JAK/STAT signaling cascade and the Ras/Raf-1/ERK1/2 pathway.[50] In vascular smooth muscle cells, caffeic acid decreased the protein and enzymatic activity levels of Rac1 GTPase, a partner of NAD(P)H oxidases, either in the presence or absence of Ang II.[51]

As discussed earlier, of the CGA metabolites, ferulic acid had greatest effect on BP reduction.[14,18] Interestingly, ferulic acid contained an ortho-methoxy-substituted-catechol structure (apocynin analogous) and had been implicated to work as a non-selective NAD(P)H oxidase antagonist by reacting with sulfhydryl groups.[52,53] In aortic rings pre-contracted with phenylephrine (an alpha-adrenergic neurotransmitter agonist), the administration of ferulic acid greatly increased NO bioavailability and enhanced acetylcholine-induced endothelial-dependent vasodilation,[35] but had no effect on sodium nitroprusside (a direct NO donor)-induced endothelium-independent vasodilation,[19,35] signifying that vascular integrity (in particular intact endothelium) is essential for CGAs to lower BP. *In vivo*, CGAs significantly increased levels of urinary NO metabolites (that is, the nitrite/nitrate ratio) in SHR,[19,20] but when non-selective nitric oxide synthase inhibitor Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME) was administered simultaneously, the hypotensive effect of CGAs disappeared.[16,19]

Additionally, CGAs might interact with the renin-angiotensin aldosterone system by inhibiting angiotensin-converting enzyme activity as shown both *in vitro* and *in vivo*.[12,41] In SHRsp, in response to single oral administration of ferulic acid at a dose of 9.5 mg kg^{-1}, SBP (measured by tail-cuff method) was about 30 mm Hg lower than baseline, corresponding with a simultaneous reduction of plasma angiotensin-converting enzyme activity from 20.6 to 16.9 mU ml^{-1} at 2 h.[41]

Taken together, several mechanisms by which CGAs or CGA metabolites cause hypotension have emerged: (1) inhibition of

NAD(P)H oxidase expression and activity, therefore reducing free radical production; (2) directly scavenging free radicals;[54] (3) stimulation of NO production by the endothelial-dependent pathway; and (4) inhibition of angiotensin-converting enzyme in the plasma, and possibly in the organs and tissues (for example, lungs) as well. The anti-inflammatory effect of CGAs, although less explored, is also likely to be relevant to the integrity of overall vascular function and BP regulation in the long run.[55,56]

Unlike many direct peripheral vasodilators, the hypotensive effect of GCE or CGA was not accompanied by reflex tachycardia, a relevant sign of sympathetic nerve activation.[57,58] Previous studies implicated that both ferulic acid and caffeic acid modulated NO pathways in the central nervous system.[59–61] Increased central NO had a role of restraining sympathetic outflow,[50,62,63] thereby attenuating reflex tachycardia when arterial baroreceptors were unloaded due to blood pressure reduction.[58]

Although the current data on CGAs are promising, gaps in knowledge remain to be filled.[64] For example, current studies indicated that the hypotensive effect of CGAs was endothelial-dependent, yet the gene expression of endothelial NOs was not upregulated and both the overall blood antioxidant status and the superoxide dismutase (that is, SOD) activity were not altered.[19] The mechanism of action of HHQ is also unclear, and it is puzzling that HHQ did not scavenge constitutive NO. Given the result that CGA antagonized the hypertensive effect of L-NAME, it is more interesting to know whether CGA neutralize asymmetric dimethylarginine (an endogenous inhibitor of NO synthases, which was elevated in some pathophysiological settings).[50,62] More scientific and clinical exploration in these areas is warranted.

In summary, dietary consumption of chlorogenic acids has demonstrated great potential for providing cardiovascular benefits. Mechanistically, CGAs (in particular their metabolites caffeic acid and ferulic acid) have shown anti-oxidative and anti-hypertensive effects, which are more pronounced in the absence of HHQ. As a source of antioxidants and as a potential lifestyle modification (that is, a non-pharmacological approach), CGAs hold great promise in the pursuit of a healthy blood pressure.

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