"Effects of coffee on the total plasma antioxidant capacity in humans and bioavailability of coffee polyphenols"

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Background

Polyphenols have been reported to exert a variety of biological actions, such as free radical scavenging, metal chelation, modulation of enzymatic activity and, more recently, to affect signal transduction, activation of transcription factors and gene expression (1-4). Epidemiological studies have suggested associations between the consumption of polyphenols-rich foods and beverages and the prevention of many human diseases.

Despite extensive literature describing the effects of polyphenols, our knowledge about their absorption from diet is scarce, one major question arising on the absorption of bound forms of phenolic compounds:

A number of beverages derived from vegetables have been tested for their in vitro and in vivo antioxidant activity (white and red wine, green and black tea, beer) (5-7). In particular, in the last years, a number of studies focused on the capacity of tea to elicit in vivo antioxidant protection in humans, giving pictures both contrasting or of largely different extent. However, a recent paper clearly demonstrated that consumption of a single dose of black or green tea induces a significant rise in plasma antioxidant activity *in vivo* (8). Black tea contains catechins, thearubigins and theaflavines, which are oxidation products of catechins formed during enzymatic oxidation by polyphenol oxidase in fresh tea leaves.

Although coffee is as rich as tea in phenolic antioxidants and is equally consumed in the world, its antioxidant activity in vivo has been never studied.

Coffee contains several phenolic components, other than tocopherols, endowed with antioxidant capacity, and the total polyphenols amount ranges from 200 to 550 mg per cup. Among the phenolic compounds identified are chlorogenic acids, a family of esters formed between quinic acid and several cinnamic acids such as caffeic, ferulic and p-coumaric acid, caffeoylquinic acid being by far the most abundant. Based on 10 g coffee per cup of brew, a cup content of chlorogenic acid (5'-caffeoyl quinic acid, the most abundant isomer) can range from 15 to 325 mg. A value of 200 mg/cup has been reported for coffee, brewed by drip filtering.

The aims of our studies were:

- To assess the capacity of coffee in affecting the plasma redox homeostasis in humans in fasting conditions, using tea as control. Total antioxidant capacity and the concentration of the main antioxidants were measured on plasma before and after the supplementation of a standard cup of coffee or black tea. Metabolic parameters in plasma were also measured to control the eventual effect of acute coffee and tea consumption on lipid metabolism.
- To determine the bioavailability of phenolic acids, with particular concern on the conjugated forms. The preliminary set up of a hydrolysis method was necessary to avoid the degradation of phenolic acids in the alkaline conditions commonly used.

Design of the study and brief description of methods utilized

In vitro study on detection of bound phenolic acids in coffee and compared analysis of antioxidant capacity and phenolic concentration in coffee and tea

Coffee brew was prepared using a commercial automatic brewing machine (60 g of roasted and ground coffee from an Italian brand per liter water) and used within 10 min from preparation. Coffee (non-hydrolyzed or subjected to alkaline hydrolysis in the presence of EDTA and ascorbic acid) was analyzed using an HPLC system, consisting of a Perkin-Elmer Series 4 Liquid Chromatograph with gradient pump, column thermoregulatory, auto sampling injector equipped with electrochemical coulometric detector

The total antioxidant capacity of coffee and tea (prepared by 5 min infusion of 20 g in 1 liter of water at 100°C) was measured using two different systems, the loss of fluorescence of r-phycoerithryn (TRAP test) and the competition kinetic with the bleaching of a carotenoid, the crocin (Crocin test), triggered by the peroxyl radicals generated by thermal decomposition of 2,2'-azobis(2-amidinopropane) HCl (AAPH).

Total phenols were measured by the Folin Ciocalteau method after deproteinization of samples with ammonium sulfate (9). Caffeine, theobromine and theophylline were detected by HPLC (10).

In vivo study on modulation by coffee and tea drinking of plasma antioxidant capacity in humans

A standard amount (200 ml) of brewed coffee was administered in fasting conditions to 10 healthy non-smoker moderate-coffee drinkers. In a different session (2 weeks apart) black tea was administered as control. Beverages were ingested within 10 min from brewing.

The total antioxidant capacity of plasma was measured using the same methods employed for the analysis of beverages. Single molecules with antioxidant capacity were individually measured (SH groups, ascorbic and uric acid, alpha tocopherol).

Plasma total cholesterol and triacylglycerols, HDL-cholesterol and LDL-cholesterol, total homocysteine were also measured to control any metabolic effect.

Bioavailability of phenolic acids from coffee in humans

Aliquots of plasma samples (0.5 ml) from each subject were thawed and treated according to one of the three following procedures: no treatment, to detect free phenolic acids; β-glucuronidase treatment (used to selectively hydrolyze glucuronidated forms of hydroxycinnamic acids) and alkaline hydrolysis treatment (used to liberate phenolic acids from bound complexes) to detect total (free + bound) phenolic acids. *o*-Coumaric acid was selected as internal standard due to the absence of detectable amounts of this compound in human plasma samples before and after coffee administration, with or without β-glucuronidase or alkaline hydrolysis treatments.

The presence of phenolic acids in treated and untreated samples was assessed by HPLC-ECD.

Results

In vitro study on detection of bound phenolic acids in coffee (11) and compared analysis of antioxidant capacity and total phenols concentration in coffee and tea (12)

Coffee brew was analyzed for phenolic acids composition, before and after hydrolytic treatment. Chlorogenic acid (5'-caffeoyl quinic acid) was present in non-hydrolyzed coffee at high concentration, while free caffeic acid, *p*-coumaric acid and ferulic acid were undetectable. After hydrolysis, ferulic acid, *p*-coumaric acid and high levels of caffeic acid were detected. The amount of caffeic acid released upon hydrolysis was higher than the

amount expected from hydrolysis of chlorogenic acid based on 1 to 1 stoichiometry. This result is explained by the fact that coffee also contains dicaffeoylquinic acid derivatives and different isomer of caffeoylquinic acids besides 5'-caffeoylquinic acid, the one detected in our experiments. From our data, we calculated that a cup of coffee (200 ml) contained 95.8±4.6 mg chlorogenic acid (5'-caffeoylquinic acid). After hydrolytic treatment, the total phenolic acids content of a cup of coffee was: caffeic acid, 166.0 ± 14.0 mg, *p*-coumaric acid 2.8 ± 0.2 mg, ferulic acid 28.6 ± 2.5 mg.

The measure of the antioxidant capacity of the two beverages, using both TRAP and Crocin test methods indicates that coffee is more powerful in scavenging peroxyl radicals than tea, at least in an hydrophilic environment. Total phenols, expressed as gallic acid equivalents, are still higher in coffee than in tea, but the difference is not as dramatic (+ 40%) as for the antioxidant capacity. Thus, the antioxidant capacity of the beverages cannot be explained by the mere measure of total phenols.

To further characterize the two beverages, we measured the concentration of 1,3,7-trimethyl xanthine (caffeine), 3,7-dimethyl xanthine (theobromine) and 1,3-dimethyl xanthine (theophylline). Caffeine in a cup of coffee (200 ml), as administered in the in vivo study, corresponded to 181 mg, while 200 ml of tea contained 130 mg of caffeine. The figures for theobromine were 28.9 and 5.9 mg/200 ml, respectively for coffee and tea. In both samples, theophylline was under the detection limit of our method.

As trimethyl xanthines don't have antioxidant activity against peroxyl radicals, we can postulate that the higher antioxidant activity of coffee in respect to tea is probably linked to its different pattern in antioxidant compounds. Alpha-tocopherol was present in negligible amount in coffee and it was absent in tea (data not shown). Thus, we can exclude a participation of α-tocopherol to the beverage's AC. Finally, the contribution of other compounds with antioxidant activity present in roasted coffee, namely Maillard products or melanoidins, can not be excluded.

In vivo study on modulation by coffee and tea drinking of plasma antioxidant capacity in humans (12)

The ingestion of 200 ml of coffee in bolus produced a statistically significant increase at t = 1 (5.5%, $P<0.05$) in the plasma antioxidant capacity, measured by the TRAP method, maintaining a 4% increase after two hours. The 4.7 % increase of TRAP 1 hour after tea administration did not reach statistical significance.

In the case of coffee, the Crocin test gave a similar trend in the modulation of antioxidant activity, even if the differences were not statistically significant. In the case of tea, the AC measured by the Crocin test, decreased significantly ($P<0.005$) after 2 hours, paralleling the decline of the reduced form of ascorbic acid.

The apparent lack of statistical significance in the increases of AC by TRAP test for tea and by Crocin test for coffee disappears when inter-individual differences are taken into account. In fact, analyzing individual data, we found that subjects did not reach the maximum value at the same time. Prevalently the peak time was 1 hour. However, in the case of the measurement of AC by Crocin test, 4 subjects reached the maximum value 2 hours after coffee drinking and in the case of the measurement of AC by TRAP, 3 subject reached the peak 2 hour after tea drinking. This event can be linked to differences in the efficiency of absorption and/or metabolism of antioxidant compounds. Comparing the individual AC at time 0 with the AC at the peak time (1 hour or 2 hours depending on the subjects), we observed a significant increase in plasma AC using both methods after coffee drinking. The increase in plasma AC after tea drinking reached statistical significance only when measured by TRAP method.

The two methods employed to measure AC differ for their capacity to be affected by uric acid: in fact plasma uric acid contribution to TRAP is about 60%, while its contribution to the Crocin test is equal to zero. Because coffee and tea drinking induced a significant increase of plasma uric acid, we can speculate that the increase in plasma AC measured by the TRAP method was largely affected by the increase of plasma uric acid concentration. After coffee drinking, we observed a significant increase of AC also using the Crocin test. As uric acid do not contribute to the Crocin test, we can speculate that molecules other than uric acid (probably phenolic acids) are responsible for the observed increase of antioxidant capacity.

Caffeic acid is the most abundant phenolic compound in coffee brew and it is endowed with strong antioxidant activity in vitro and in vivo. As caffeic acid is present in human plasma at μmolar concentration after coffee drinking (*13*), we can assume that it is at least in part directly responsible for the increase in plasma antioxidant capacity observed in this study.

Therefore, whilst the contribution of phenolic compounds from tea to the AC is essentially indirect, 'influencing' the plasma uric acid level (even if a slight direct contribution can not be ruled out) phenolic compounds from coffee could act both directly and indirectly.

Bioavailability of caffeic acid from coffee in humans (13)

In order to study the absorption of coffee phenolic acids, plasma samples collected before and after coffee administration were analyzed for content of both free and total (free + bound) phenolic acids, using two different procedures of hydrolysis to release phenolic acids from bound forms. In the first procedure, β-glucuronidase was used to selectively hydrolyze glucuronidated forms of hydroxycinnamic acids. In the second procedure, an alkaline hydrolytic treatment was used to liberate phenolic acids from bound complexes.

Less than 12.6±7.4 ng/ml of free caffeic acid (corresponding to 0.07 μM) was detected in the untreated control plasma samples taken immediately prior to coffee brew administration (time 0). A significant increase in free caffeic acid plasma levels was found in untreated plasma samples 1 h after coffee brew consumption in respect to time 0.

After β-glucuronidase treatment, total caffeic acid in plasma was significantly higher at both 1 h and 2 h after coffee administration than at time 0 with a maximum absorption peak at 1 h for all subjects. Alkaline hydrolysis treatment of plasma samples gave similar results, with significantly higher levels of total caffeic acid at 1 h and 2 h in respect to time 0 and maximum absorption peak at 1 h. Both β-glucuronidase and alkaline hydrolysis treatment released a considerable amount of caffeic acid at 1 h and 2 h after coffee consumption. Interestingly, the plasma levels of caffeic acid measured after both hydrolysis procedures were very similar and no significantly different by ANOVA.

Conclusions

1. Following our experimental conditions, coffee drinking increases plasma antioxidant capacity, probably due to bioavailability and antioxidant activity of its peculiar group of phenolic compounds (chlorogenic acids).
2. Coffee administration resulted in increased total plasma caffeic acid concentration, with an absorption peak at 1h. Caffeic acid was the only phenolic acid found in plasma samples after coffee administration, while chlorogenic acid was undetectable. Most of caffeic acid was present in plasma in bound form, mainly in the glucuronate/sulfate forms. Due to the absence of free caffeic acid in coffee, plasma caffeic acid is likely to be derived from hydrolysis of chlorogenic acid in the gastrointestinal tract.

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