JPP
Journal of
Pharmacy and Pharmacology

JPP 2006, 58: 1139–1144
© 2006 The Authors
Received September 21, 2005
Accepted May 1, 2006
DOI 10.1211/jpp.58.8.0016
ISSN 0022-3573

Tea flavanols inhibit angiotensin-converting enzyme activity and increase nitric oxide production in human endothelial cells

Ingrid A.-L. Persson, Martin Josefsson, Karin Persson and Rolf G. G. Andersson

Abstract

A diversity of pharmacological effects on the cardiovascular system have been reported for *Camellia sinensis*: antioxidative, antiproliferative and anti-angiogenic activity, and nitric oxide synthase activation. The purpose of this study was to investigate if the connection between tea and angiotensin-converting enzyme (ACE) and nitric oxide (NO) might be an explanation of the pharmacological effects of tea on the cardiovascular system. Cultured endothelial cells from human umbilical veins (HUVEC) were incubated with extracts of Japanese Sencha (green tea), Indian Assam Broken Orange Pekoe (black tea) and Rooibos tea, respectively. The main flavanols and purine alkaloids in green and black tea were examined for their effects on ACE and NO. After incubation with green tea, black tea and Rooibos tea for 10 min, a significant and dose-dependent inhibition of ACE activity in HUVEC was seen with the green tea and the black tea. No significant effect on ACE was seen with the Rooibos tea. After 10-min incubation with (–)-epicatechin, (–)-epigallocatechin, (–)-epicatechingallate and (–)-epigallocatechingallate, a dose-dependent inhibition of ACE activity in HUVEC was seen for all four tea catechins. After 24-h incubation, a significantly increased dose-dependent effect on NO production in HUVEC was seen for the green tea, the black tea and the Rooibos tea. After 24-h incubation with (–)-epicatechin, (–)-epigallocatechin, (–)-epicatechingallate and (–)-epigallocatechingallate, a dose-dependent increased NO production in HUVEC was seen. In conclusion, tea extracts from *C. sinensis* may have the potential to prevent and protect against cardiovascular disease.

Department of Medicine and Care, Division of Pharmacology, Faculty of Health Sciences, Linköping University, Sweden

I. A.-L. Persson, K. Persson, R. G.G. Andersson

National Board of Forensic Medicine, Department of Forensic Genetics and Forensic Toxicology, Linköping, Sweden

M. Josefsson

Correspondence: I. Persson, Department of Medicine and Care, Division of Pharmacology, Faculty of Health Sciences, Linköping University, SE-581 85 Linköping, Sweden. E-mail: ingpe@imv.liu.se

Acknowledgement: We thank Tebladet, Linköping for the kind gift of the tea.

Introduction

A diversity of pharmacological effects of *Camellia sinensis* (Theaceae) on the cardiovascular system has been reported: antioxidative (Locher et al 2002; Kurihara et al 2004), antiproliferative and anti-angiogenic activity (Locher et al 2002; Oak et al 2005), and nitric oxide synthase activation (Nakagawa & Yokozawa 2002; Anter et al 2004; Lorenz et al 2004). Epidemiological studies have shown reduced cardiovascular morbidity among tea drinkers (Mukamal et al 2002; Hakim et al 2003).

Rooibos tea, produced from the leaves and stems of *Aspalathus linearis* (Leguminosae), is a caffeine-free alternative. Reported pharmacological effects attributed to Rooibos tea have been antioxidative activity (Standley et al 2001; Lee & Lang 2004) and immunostimulatory effects (Lambert & Yang 2003).

The diversity of the pharmacological effects of *C. sinensis* and *A. linearis* have been attributed to polyphenols. The unfermented leafs of *C. sinensis*, green tea, contain 30–40% polyphenols (Yang et al 2000; Lambert & Yang 2003) such as flavanols, flavadiols, flavonols and phenolic acids. The dominating group of polyphenols in green and black tea is the tea flavanols i.e. catechins. The four major catechins in tea are (–)-epicatechin, (–)-epicatechingallate, (–)-epigallocatechin and (–)-epigallocatechingallate (Balentine et al 1997; Nakagawa & Yokozawa 2002; Del Rio et al 2004).

The content of polyphenols in black tea is approximately 3–10% (Lambert & Yang 2003). In green tea, 77% of the polyphenols are catechins (Del Rio et al 2004). In black tea, 3–4% of the polyphenols are catechins (Del Rio et al 2004). Catechin content is

thus reduced by approximately 85% in black tea compared with green tea (Balentine et al 1997). Purine alkaloids such as caffeine, theobromine and theophylline are also present in *C. sinensis*.

The main polyphenol compounds in *A. linearis*, Rooibos tea, are dihydrochalcones, flavones and flavanols e.g. aspalathin, nothofagin, isoorientin, orientin, isovitexin, vitexin and rutin (Bramati et al 2002, 2003).

The renin–angiotensin system (RAS) is one of the most important mechanisms in the body concerning the regulation of blood pressure, fluid and electrolyte balance. The angiotensin-converting enzyme (ACE), a carboxypeptidase, is involved in the RAS, where it converts angiotensin I to the octapeptide angiotensin II. The two active sites of ACE contain a Zn^{2+} ion and ACE inhibitors are designed on the basis of the ability to bind to the Zn^{2+} ion (Sturrock et al 2004). ACE inhibitors are the first line treatment of patients with hypertension and heart failure.

ACE proteolytically cleaves several peptides including bradykinin; ACE inhibitors increase bradykinin levels. Bradykinin causes smooth muscle relaxation by binding to bradykinin receptors on the endothelial cell surface, thereby activating eNOS and increasing nitric oxide (NO) production. NO from endothelial cells relaxes vascular smooth muscle and inhibits platelet aggregation. Reduced bioavailability of NO is believed to be an initial step in the development of cardiovascular disease. Antioxidants may increase NO. It has been proposed that part of the positive effect seen in tea drinkers regarding cardiovascular disease is due to the tea's antioxidative effects (Tijburg et al 1997; Miura et al 2001; Locher et al 2002).

There are several interactions known between the RAS and NO (Yan et al 2003). It has been shown that exogenous and endogenous NO can inhibit ACE in pigs and man in-vitro (Persson & Andersson 1999; Persson et al 2000). This NO-mediated ACE inhibition is additive with traditional ACE inhibitors such as captopril, and of functional importance concerning angiotensin II-induced vasoconstriction and platelet aggregation.

We have investigated the effects of green tea, black tea and Rooibos tea on ACE activity and NO production in cultured human endothelial cells from umbilical veins. The effects of the main flavanols and purine alkaloids in green tea and black tea were examined.

For this study, teas were selected from the different major tea-producing continents. Japanese Sencha is a green tea from Asia, Assam B.O.P. is a black tea from India, and Rooibos tea comes from Africa. The extracts were prepared in the same way that tea is usually prepared as an everyday beverage. This was done to enable us to investigate if our ordinary tea-drinking behaviour might have protective abilities on the cardiovascular system.

Materials and Methods

The study on cultured endothelial cells from human umbilical veins (HUVEC) was approved by the regional ethics committee at the Faculty of Health Sciences, Linköping, Sweden (Dnr 03-602).

C. sinensis and *A. linearis* extraction

Japanese Sencha was used for preparation of green tea extract. Indian Assam B.O.P. (Broken Orange Pekoe) was used for preparation of black tea extract. Rooibos tea was used for preparation of *A. linearis* extract. Infusions were made with 1 g tea leaves in 20 mL fresh boiled sterile phosphate-buffered saline (PBS) for 5 min (the green tea and the black tea) or 10 min (the Rooibos tea). The infusion was filtered twice, first through a standard filter and then through a sterile filter 0.2 μm (Millipore). The obtained filtrate was considered as 1:20 and samples were frozen at -20°C.

Cultured endothelial cells from HUVEC

Human umbilical cords were obtained after normal vaginal delivery (after informed consent from the mothers), and kept in sterile bottles containing PBS, penicillin, streptomycin and gentamicin. Endothelial cells were then isolated by treatment with 0.5 mg mL^{-1} collagenase for 20 min at 37°C as described by Nyhlén et al (2000). In short, the collagenase + cell perfusate was washed twice, then resuspended in culture medium (Dulbecco's modified Eagle's medium, DMEM, with normal glucose) supplemented with nonessential amino acids (NEAA, 1:100), oxalacetic acid (1.2 mM), insulin (0.24 IE mL^{-1}), penicillin (5 U mL^{-1}), streptomycin (0.5 μg mL^{-1}), HEPES (10 mM), endothelial cell growth factor (ECGF, 30 μg mL^{-1}) and 17% inactivated fetal calf serum (FCS). Resuspended HUVEC were seeded in 25 cm^2 tissue culture flasks coated with 0.2% gelatin, and kept in an incubation chamber. Medium was replaced every 48–72 h. At confluence, cells were harvested with trypsin-EDTA for 5–10 min, and then reseeded 1:2. Second passage was seeded in a 96-well microtitre plate, and allowed to reach confluence. Medium was then removed and replaced with medium without FCS to avoid discrepancies in the results due to ACE or NO present in the serum. Cells were treated with green tea, black tea, Rooibos tea, (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechingallate (ECG), (−)-epigallocatechingallate (EGCG), caffeine, theobromine or theophylline. Tea extracts were diluted in PBS, all other drugs in dimethylsulfoxide (DMSO). Corresponding volumes of PBS or DMSO were used as controls. After 10-min incubation with drugs, ACE activity and NO production were analysed as described below. NO production was also analysed after 24-h incubation with drugs.

The used concentrations of tea extracts, flavanols and purine alkaloids corresponded to the amount expected in tea taken as a beverage (Balentine et al 1997; Del Rio et al 2004).

ACE activity

After stimulation of the cells with drugs, ACE activity was analysed with a commercial radioenzymatic assay

(ACE-direct REA, Bühlmann Laboratories, Allschwil, Switzerland) with the following modifications. Blank or standard serum was added to wells with corresponding volumes of medium without FCS. The synthetic substrate [^{3}H]hippuryl-glycyl-glycine was then added directly to all the wells of the microtitre plate. Cells were incubated with substrate for 2 h at 37°C to allow for the ACE present to cleave the substrate to [^{3}H]hippuric acid. After this time, 150 μl medium + substrate was transferred from each well into scintillation vials containing 50 μL 1 M HCl (to stop the enzymatic reaction). Scintillation fluid was added and the samples were counted in a scintillation counter.

NO determination

After incubation with the different substances, medium was removed and stored at −20°C until nitrite analysis. Nitrite was analysed as a marker of NO production as proposed by Lauer et al (2001). Nitrite concentration in the medium was analysed with a commercially available nitrite/nitrate assay: nitric oxide (NO_2^-/NO_3^-) assay kit obtained from R&D Systems, UK. The principle for the colorimetric detection of nitrite is the Griess reaction. In short, diazonium ions are produced when acidified nitrite reacts with sulfanilic acid. These diazonium ions form chromophore agents when reacting with N-(1-naphthyl) ethylenediamine. Optical density was determined with a Spectramax reader at 540 nm.

Liquid chromatography–mass spectrometry–mass spectrometry (LC-MS-MS)

For quantifications of EC, EGC, ECG and EGCG an ESI-LC-MS-MS system for gradient chromatography was used. The instrumentation consisted of a Perkin Elmer 200 chromatographic system equipped with two micro pumps, a solvent degasser and an autosampler (Norwalk, CT, USA). Mass detection was performed on a Sciex API 2000 triple quadrupole instrument equipped with a turbo ion-spray interface (PE Sciex, Ontario, Canada) operating in positive ion mode. The interface probe was set at 350°C and the ion-spray needle was operated at −4500 V. Nitrogen was used as nebulizer-, auxiliary-, curtain- and CAD-gas and was set at 25, 50, 30 psi and a value of 5, respectively. The HPLC was carried out on a Hypersil Polar-RP 150 × 3.0 mm (Phenomenex, Torrance, CA, USA) equipped with an Opti-Solv 2 μm column inlet filter (Optimize, Portland, Oregon, USA). The mobile phases consisted of acetonitrile, methanol and 20 mM ammonium formiate buffer, pH 3, in mixtures of 5:5:90 (v/v/v) for phase A and 40:40:20 (v/v/v) for phase B. A linear gradient chromatography from 0 to 100% B-phase over 7 min was run. Total run-time including wash and reconditioning was 10 min. A flow-rate of 0.25 mL min^{-1} at ambient temperature was used. The detection and quantification of the selected compounds were performed in the Multiple Reaction Monitoring mode (MRM). MS-MS product ion spectra were produced by collision activated dissociation (CAD) of the de-protonated molecule ion $(M+1)^-$. The most intense transitions; 289/245 (EC), 305/179 (EGC) 441/169 (ECG), 457/169 (EGCG) were used for quantification. Linear calibration curves were performed ranging from 0.5 to 20 μg mL^{-1}. Tea extracts were diluted in methanol and buffer 1:10 and 1:100, respectively, before analysis. Instrument control, integration and calculation were performed with the PC based PE Sciex software, Analyst 1.4.

Chemicals

All chemicals for culturing cells were obtained from Life Technologies (Scotland, UK), with the exception of the ECGF that was bought from Boehringer-Mannheim (Germany) and heparin (Heparin LEO) from LEO Pharma AB (Malmö, Sweden). The catechins were bought from Sigma Chemical Co. (St Louis, MO, USA). Japanese Sencha (imported by Charabang, Stockholm, Sweden), Assam B.O.P. (imported by Norrköpings Kolonial, Sweden) and Rooibos tea (imported by Norrköpings Kolonial, Sweden) were a kind gift from Tebladet, Linköping, Sweden.

Viability

A viability test was performed by incubating HUVEC with 1:200 green tea, black tea or Rooibos tea for 24 h. Trypan blue 0.05% was then added for 5 min at room temperature before counting the cells.

Calculations

Results are presented as mean ± s.e.m. (standard error of the mean). One unit (U) of ACE activity was defined as the amount of the enzyme required to release 1 μmol hippuric acid min^{-1} and L^{-1}. Graph Pad Prism 3.0 was used for the statistical calculations. One way analysis of variance was performed followed by Dunnett's post test. Statistical significance is denoted as $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$.

Results

ACE activity

After incubation with the green tea, black tea or Rooibos tea for 10 min, a significant and dose-dependent inhibition of ACE activity in HUVEC was seen with the green tea and the black tea (Figure 1). No significant effect on ACE was seen with the Rooibos tea (Figure 1).

After 10-min incubation with (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechingallate and (−)-epigallocatechingallate, a dose-dependent and significant inhibition of the ACE activity in HUVEC was seen for the four tea catechins (Figure 2).

Compared with the DMSO control, the purine alkaloids caffeine, theobromine and theophylline did not show

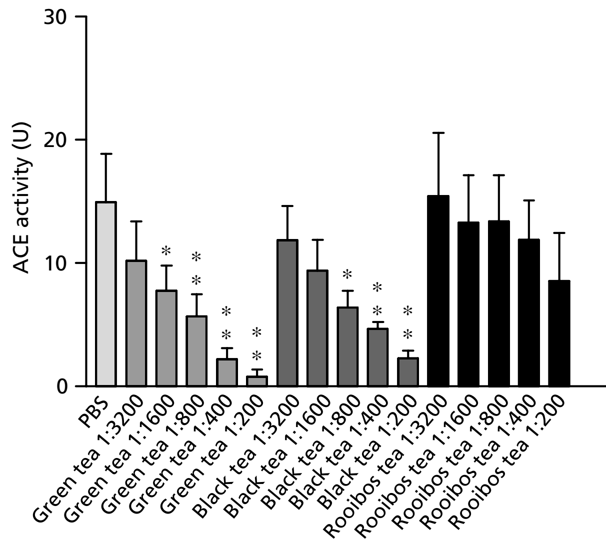

Figure 1 ACE activity in HUVEC after 10-min incubation with green tea, black tea or Rooibos tea. $*P < 0.05$ and $**P < 0.01$, $n = 5$.

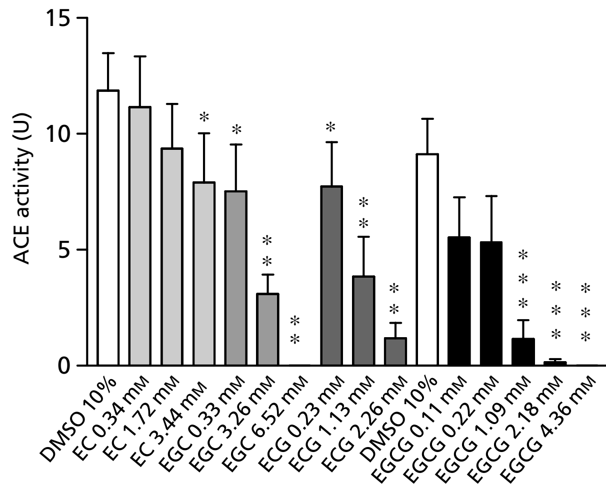

Figure 2 ACE activity in HUVEC after 10-min incubation with: (−)-epicatechin (EC), $n = 6$, (−)-epigallocatechin (EGC), $n = 6$; (−)-epicatechingallate (ECG), $n = 6$; or (−)-epigallocatechingallate (EGCG), $n = 8$. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$.

any significant effect on the ACE activity after 10-min incubation (data not shown).

NO production

After 10-min incubation with the green tea, black tea or Rooibos tea, an increase in NO production was seen (Figure 3).

After 24-h incubation, an increased dosage-dependent and significant effect on the NO production in HUVEC was seen for the green tea, the black tea and the Rooibos tea (Figure 3).

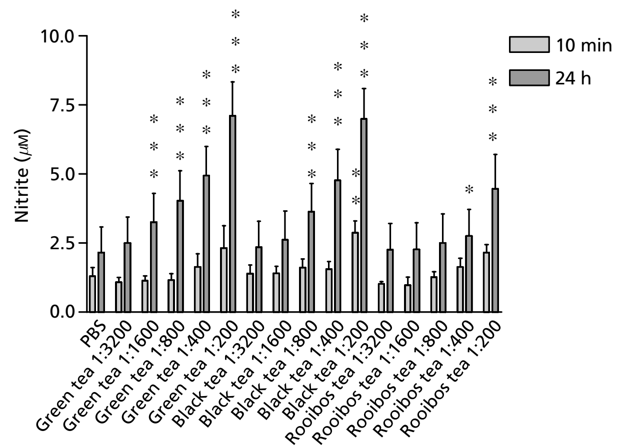

Figure 3 NO production in HUVEC after 10-min ($n = 5$) or 24-h ($n = 9$) incubation with green tea, black tea, or Rooibos tea. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ compared with PBS controls.

After 24-h incubation with (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechingallate and (−)-epigallocatechingallate, a dose-dependent and significant increase in NO production was seen (Figure 4).

After 24-h incubation with caffeine, theobromine and theophylline, NO production was significantly increased ($P < 0.05$) for caffeine 5.15 μM, while no significance was shown for theobromine or theophylline (data not shown).

LC-MS-MS

The green tea contained a higher amount of the four catechins (epicatechin, epigallocatechin, epicatechingallate and epigallocatechingallate) compared with the

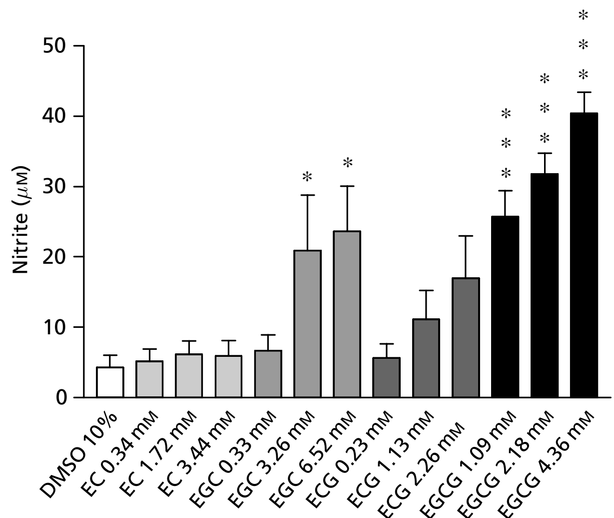

Figure 4 NO production in HUVEC after 24-h incubation with (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechingallate (ECG), or (−)-epigallocatechingallate (EGCG). $*P < 0.05$ and $***P < 0.001$, $n = 6$.

Table 1 The quantity of four catechins (epicatechin (EC), epigallocatechin (EGC), epicatechingallate (ECG) and epigallocatechingallate (EGCG)) in Japanese Sencha (green tea), Assam B.O.P. (black tea) and Rooibos tea

Tea extract 1:20	EC ($\mu g\,mL^{-1}$)	EGC ($\mu g\,mL^{-1}$)	ECG ($\mu g\,mL^{-1}$)	EGCG ($\mu g\,mL^{-1}$)
Green tea Japanese Sencha	140	410	220	730
Black tea Indian Assam B.O.P.	35	10	140	100
Rooibos tea	0	0	0	0

black tea, while no catechins where traced in Rooibos tea. Table 1 shows the amount of the four catechins found in Japanese Sencha (green tea), Assam B.O.P. (black tea) and Rooibos tea.

Viability

The cell viability after exposure to the different tea extracts was green tea 99.9% ± 0, black tea 99.6% ± 0.4, Rooibos tea 100% ± 0. PBS control was 99.7% ± 0.3, n = 3.

Discussion

ACE inhibition decreases the amount of angiotensin II production by interuption of the conversion of angiotensin I to angiotensin II. Reduced levels of angiotensin II decrease blood pressure, and inhibit smooth muscle cell proliferation and superoxide anion production. ACE inhibitors decrease cardiovascular morbidity and mortality. An inhibitory effect of *C. sinensis* on ACE activity may be one explanation for the beneficial effects seen of this plant on cardiovascular diseases.

The pharmacological mechanism of the allopathic ACE inhibitors is their ability to bind to the Zn^{2+} at the active site of ACE. Catechins are known to have chelate-binding capacity to metal ions like Zn^{2+} and Fe^{2+}. A diversity of metal ions are essential elements for plants, and Fe^{2+} and Zn^{2+} are so called micronutrients. Zinc is known as an activator or component of many plant enzymes. The dihydroxy or the trihydroxy structure of the catechins probably contributes to the ion metal-chelating properties.

The results of this study showed that extracts of green tea and black tea inhibited ACE activity and increased NO production in cultured human endothelial cells. The inhibitory effect on ACE activity was associated with the flavanols i.e. epicatechin, epigallocatechin, epicatechingallate and in particular epigallocatechingallate. An increased number of hydroxy groups and addition of a double-bound oxygen seemed to increase the inhibitory effect on ACE activity.

The ability of catechins to form insoluble compounds with proteins and to chelate metal ions like Zn^{2+} may explain the action of green and black tea as ACE inhibitors. This could be one explanation of the pharmacological effect of *C. sinensis* on the cardiovascular system.

This study showed significantly increased NO production from HUVEC. The effect on NO was seen after 10-min incubation but was more pronounced after 24-h treatment with the tea extracts.

Epigallocatechingallate is considered to have antioxidative properties (Miura et al 2001; Ahmed et al 2002). Since antioxidants are known to increase the bioavailability of NO, the results of our study were not surprising. Studies have described that the antioxidative ability can be explained by the chelate binding to Fe^{2+} (Balentine et al 1997; Ishizaka et al 2002; Saito et al 2004).

NO inhibits platelet aggregation. The result of an increased NO production should be inhibition of platelet aggregation. Chang & Hsu (1991) showed that epigallocatechingallate inhibited platelet aggregation. The most significant results on NO production in our study was achived by epigallocatechingallate. Thus, epigallocatechingallate acted as an ACE inhibitor and as an NO producer. There are several interactions known between the RAS and NO (Yan et al 2003); ACE inhibitors increase bradykinin levels and bradykinin activates eNOS and increases NO production. Tea polyphenols have been shown to activate eNOS (Anter et al 2004).

Compared with green tea, black tea had less effect on ACE activity and NO production. This could be explained by the fermentation process, which is known to reduce the content of tea flavanols (Balentine et al 1997). According to the results of this study, the effects of tea on ACE and NO may be explained by the catechins e.g. epigallocatechingallate. In the fermentation process, polyphenol oxidases come into contact with polyphenols in the resins, thereby degrading the content of flavanols. The amount of polyphenolic constituents in tea plants varies with climate, season, age of the leafs and species of the tea (Lin et al 2003). According to this, the effect of tea on ACE and NO may vary.

Roobois tea contained no catechin compounds, which may explain why Rooibos tea did not show any significant effects on ACE activity. However, Rooibos tea (1:200 dosage) had a significant effect on NO production. As Rooibos tea is considered to have antioxidative effects (Bramati et al 2002, Lee & Lang 2004) and antioxidants may increase NO, this could be the possible pharmacological mechanism of Rooibos tea. Rooibos tea does contain several other polyphenols and so Rooibos tea may have other possibilities.

Conclusions

Tea extracts and the flavanols of green tea and black tea inhibited ACE activity and increased NO production in human endothelial cells in-vitro. *Camellia sinensis* as a beverage may have the potential to prevent and protect against cardiovascular disease.

References

Ahmed, S., Rahman, A., Hasnain, A., Lalonde, M., Goldberg, V. M., Haqqi, T. M. (2002) Green tea polyphenol epigallocatechin-3-gallate inhibits the IL-1beta-induced activity and expression of cyclooxygenase-2 and nitric oxide synthase-2 in human chondrocytes. *Free Radic. Biol. Med.* **33**: 1097–1105

Anter, E., Thomas, S. R., Schulz, E., Shapira, O. M., Vita, J. A., Keaney, J. F. Jr (2004) Activation of endothelial nitric-oxide synthase by the p38 MAPK in response to black tea polyphenols. *J. Biol. Chem.* **279**: 46637–46643

Balentine, D. A., Wiseman, S. A., Bouwens, L. C. M. (1997) The chemistry of tea flavonoids. *Crit. Rev. Food Sci. Nutr.* **37**: 693–704

Bramati, L., Minoggio, M., Gardana, C., Simonetti, P., Mauri, P., Pietta, P. (2002) Quantitative characterization of flavonoid compounds in Rooibos tea (*Aspalathus linearis*) by LC-UV/DAD. *J. Agr. Food Chem.* **50**: 5513–5519

Bramati, L., Aquiland, F., Pietta, P. (2003) Unfermented Rooibos tea. Quantitative characterization of flavonoids by HPLC-UV and determination of the total antioxidant activity. *J. Agr. Food Chem.* **51**: 7472–7474

Chang, W. C., Hsu, F. L. (1991) Inhibition of platelet activation and endothelial cell injury by flavan-3-ol and saikosaponin compounds. *Prostaglandins Leukot. Essent. Fatty Acids.* **44**: 51–56

Del Rio, D., Stewart, A. J., Mullen, W., Burns, J., Lean, M. E. J., Brighenti, F., Crozier A. (2004) HPLC-MS analysis of phenolic compounds and purine alkaloids in green and black tea. *J. Agr. Food Chem.* **52**: 2807–2815

Hakim, I. A., Alsaif, M. A., Alduwaihy, M., Al-Rubeaan, K., Al-Nuaim, A. R., Al-Attas, O. S. (2003) Tea comsumption and the prevalence of coronary heart disease in Saudi adults: results from a Saudi national study. *Prev. Med.* **36**: 64–70

Ishizaka, N., Saito, K., Mitani, H., Yamazaki, I., Sata, M., Urui, S.-I., Mori, I., Ohno, M., Nagai, R. (2002) Iron overload augments angiotensin II-induced cardiac fibrosis and promotes neointima formation. *Circulation* **106**: 1840–1846

Kurihara, H., Fukami, H., Asami, S., Toyoda, Y., Makai, M., Shibata, H., Yao. X.-S. (2004) Effects of Oolong tea on plasma antioxidative capacity in mice loaded with restraint stress assessed using the oxygen radical absorbance capacity (ORAC) assay. *Biol. Pharm. Bull.* **27**: 1093–1098

Lambert, J. D., Yang, C. S. (2003) Mechanisms of cancer prevention by tea constituents. *Am. Soc. Nutr. Sci.* **133**: 3262S–3267S

Lauer, T., Preik, M., Rassaf, T., Strauer, B.E., Deussen, A., Feelisch, M., Kelm, M. (2001) Plasma nitrite rather than nitrate reflects regional endothelial nitric oxide synthase activity but lacks intrinsic vasodilator action. *Proc. Natl. Acad. Sci. USA* **98**: 12814–12819

Lee, E. J., Lang, H. D. (2004) Antioxidant activity and protective effects on DNA strand scission of Rooibos tea (*Aspalathus linearis*). *Biofactors* **21**: 285–292

Lin, Y.-S., Tsal, Y.-J., Tsay, J.-S., Lin, J.-K. (2003) Factors affecting the levels of tea polyphenols and caffeine in tea leaves. *J. Agr. Food Chem.* **51**: 1864–1873

Locher, R., Emmanuele, L., Suter, P. M., Vetter, W., Barton, M. (2002) Green tea polyphenols inhibit human vascular smooth muscle cell proliferation stimulated by native low-density lipoprotein. *Eur. J. Pharmacol.* **434**: 1–7

Lorenz, M., Wessler, S., Follmann, E., Michaelis, W., Düsterhöft, T., Baumann, G., Stangl, K., Stangl, V. (2004) A constituent of green tea, epigallocatechin-3-gallate, activates endothelial nitric oxide synthase by a phosphatidylinositol-3-OH-kinase-, cAMP-dependent protein kinase-, and Akt-dependent pathway and leads to endothelial-dependent vasorelaxation. *J. Biol. Chem.* **279**: 6190–6195

Miura, Y., Chiba, T., Tomita, I., Koizumi, H., Miura, S., Umegaki, K., Hara, Y., Ikeda, M., Tomita, T. (2001) Tea catechins prevent the development of atherosclerosis in apoprotein e-deficient mice. *J. Nutr.* **131**: 27–32

Mukamal, K. J., Maclure, M., Muller, J. E., Sherwood, J. B., Mittleman, M. A. (2002) Tea consumption and mortality after acute myocardial infarction. *Circulation* **105**: 2476–2481

Nakagawa, T., Yokozawa, T. (2002) Direct scavenging of nitric oxide and superoxide by green tea. *Food Chem. Toxicol.* **40**: 1745–1750

Nyhlén, K., Linden, M., Andersson, R. G. G., Uppugunduri, S. (2000) Corticosteroids and interferons inhibit cytokine induced production of IL-8 by human endothelial cells. *Cytokine* **12**: 355–360

Oak, M.-H., El Bedoui, J., Schini-Kerth, V. B. (2005) Antiangiogenic properties of natural polyphenols from red wine and green tea. *J. Nutr. Biochem.* **16**: 1–8

Persson, K., Andersson, R. G. G. (1999) Nitric oxide modulates captopril-mediated angiotensin-converting enzyme inhibition in porcine iliac arteries. *Eur. J. Pharmacol.* **385**: 21–27

Persson, K., Whiss, P. W., Nyhlén, K., Jacobsson-Strier, M., Glindell, M., Andersson, R. G. G. (2000) Nitric oxide donors and angiotensin-converting enzyme inhibitors act in concert to inhibit human angiotensin-converting enzyme activity and platelet aggregation in vitro. *Eur. J. Pharmacol.* **406**: 15–23

Saito, K., Ishizaka, N., Aizawa, T., Sata, M., Iso-o, N., Noiro, E., Mori, I., Ohno, M., Nagai, R. (2004) Iron chelation and a free radical scavenger suppress angiotensin II-induced upregulation of TGF-β1 in the heart. *Am. J. Physiol. Heart Circ. Physiol.* **288**: H1836–H1843

Standley, L., Wintertom, P., Marnewick, J. L., Gelderblom, W. C. A., Joubert, E., Britz, T. J. (2001) Influence of processing stages on antimutagenic and antioxidant potentials of Rooibos tea. *J. Agr. Food Chem.* **49**: 114–117

Sturrock, E. D., Natesh, R., van Rooyen, J. M., Acharya, K. R. (2004) Structure of angiotensin I-converting enzyme. *Cell. Mol. Life Sci.* **61**: 2677–2686

Tijburg, L. B. M., Wiseman, S. A., Meijer, G. W., Weststrate, J. A. (1997) Effects of green tea, black tea and dietary lipophilic antioxidants on LDL oxidizability and atherosclerosis in hypercholesterolic rabbits. *Atherosclerosis* **135**: 37–47

Yan, C., Kim, D., Aizawa, T., Berk, B. (2003) Functional interplay between angiotensin II and nitric oxide: cyclic GMP as a key mediator. *Arterioscler. Thromb. Vasc. Biol.* **23**: 26–36

Yang, C. S., Chung, J. Y., Yang, G.-Y., Chhabra, S. K., Lee, M.-J. (2000) Tea and tea polyphenols in cancer prevention. *J. Nutr.* **130**: 472S–478S