Original Article

The effects of green tea ingestion over four weeks on atherosclerotic markers

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Abstract

Background The objective of this study was to evaluate the effects of green tea ingestion over four weeks on atherosclerotic biological markers.

Methods After a one-week baseline period, 12 healthy male volunteers aged 28–42 years drank 600 mL of green tea daily for four weeks. Lipid profile, oxidized low-density lipoprotein (ox-LDL), total antioxidant capacity (TAC), C-reactive protein (CRP) and soluble cell adhesion molecules were measured at baseline and after two and four weeks ingestion of green tea.

Results There was no significant change in the concentrations of lipid profile, TAC, CRP, soluble intercellular adhesion molecule-1 (sICAM-1), or soluble E-selectin after ingestion of green tea. The levels of ox-LDL and soluble vascular cell adhesion molecule-1 (sVCAM-1) were significantly decreased after four weeks of green tea ingestion (Wilcoxon signed rank test, $P = 0.006$).

Conclusions The results of this study suggest an *in vivo* anti-oxidative effect for green tea and an influence of green tea on atherosclerotic biological markers. The effect of green tea seen on ox-LDL and sVCAM-1 provides a potential mechanism for the cardiovascular benefits of regular ingestion of green tea.

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Introduction

Tea is the most widely consumed drink in the world other than water.[1] Tea is made of *Camellia sinensis* leaves and classified as green tea, black tea or oolong tea according to the degree of fermentation. The tea leaf contains polyphenolic flavonoids (over 30% of the dry weight), most of which in green tea are flavanols, commonly known as catechins.[2]

A few epidemiological studies have reported that the incidence of coronary heart disease and cancer decreased with intake of green tea.[3,4] Tea or flavonoids derived from tea have *in vitro* antioxidant capacity[5–7] and green tea has been shown to suppress the oxidation of low-density lipoprotein (LDL) *in vitro*.[5] Previous studies have demonstrated that oxidation of human LDL is one of the risk factors in the development of atherosclerosis[8] and that dietary antioxidants lower the incidence of coronary heart diseases.[9]

We have previously reported that the total antioxidant capacity (TAC) of plasma was significantly increased after ingestion of green tea in amounts of 300 and 450 mL, and the increment was dose related.[10] There are some other reports that the TAC increased after tea-drinking.[7,11,12] However, the relationship between long-term ingestion of green tea and *in vivo* oxidation of LDL has seldom been investigated.[13]

Vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) mediate the adherence of leucocytes to the vascular endothelium and are therefore crucial for the initiation and progression of atherosclerosis.[14] Indeed, plasma levels of their soluble forms predict the cardiovascular risk in healthy individuals[15,16] and in patients with coronary artery disease.[17]

In this study, we measured the concentrations of atherosclerotic biological markers before and two and

four weeks after ingestion of green tea to ascertain the *in vivo* effect of green tea ingestion.

Materials and methods

Human subjects

The study was conducted in 12 healthy male volunteers aged 28–42 years. Potential volunteers were excluded after initial screening if they reported the use of any medication or dietary supplements. Further exclusion criteria consisted of a history of major illness, including heart disease, diabetes mellitus, liver disease and renal disease, a body mass index (BMI) $> 30\,kg/m^2$, alcohol intake averaging > 40 g/day, or regular tea or coffee intake averaging > 1 cup/day. Before the study, subjects were instructed to avoid drinking tea or other antioxidant-containing beverages for one week; otherwise they kept to their usual diet and lifestyle. This study had the approval of the Ethical Committee of the Asan Medical Center. Written informed consent was obtained from every volunteer.

Experimental design

Each subject took 150 mL of green tea four times a day (09:00, 11:00, 13:00 and 15:00) for four weeks. They were asked to abstain from wine, other types of tea and special dietary additives, but to otherwise continue their usual daily diet throughout the trial period. Compliance was checked by one of our investigators (HS) from Monday to Saturday. Compliance on Sundays was confirmed by direct questioning. Blood specimens were taken just before the start of the study (baseline) and two and four weeks into the study. All the samples were taken after a 12-h fast to rule out the acute effect of green tea intake. Heparinized blood for antioxidant capacity and soluble cellular adhesion molecule (sCAM) measurement, EDTA plasma for oxidized LDL (ox-LDL) measurement and serum for lipid profile and high-sensitivity C-reactive protein (hs-CRP) measurement were collected in a sodium heparin tube, a K_2EDTA tube and an SST tube, respectively (all from Becton Dickinson Vacutainer Systems, NJ, USA).

Preparation of tea

Tea infusions were prepared with commercially available tea bags (JinHyang, Amore Pacific Corporation, Seoul, Korea). One batch equalled 25 tea bags. *In vitro*, a tea bag was dipped into 150 mL boiled tap water (temperature 60–70°C) and was allowed to stand for 2 min. The antioxidant concentration of 30 tea preparations was measured. The mean (±standard deviation) total antioxidant concentration of 1.3 g of green tea in 150 mL water was 8.89 ± 0.41 mmol/L. When the antioxidant capacity of randomly selected tea bags from each batch were within mean ± 2SD, the tea bags of that batch were used. Every cup of tea was prepared by one of our investigators (HS) from Monday to Saturday. A tea bag containing 1.3 g of tea leaves was dipped into 150 mL of boiled tap water (temperature 60–70°C). On Sunday, the participants prepared the tea themselves. We asked them to pour 150 mL of boiled tap water into a scaled paper cup, wait for 3 min and dip one tea bag into the water. The mixture was allowed to stand for 2 min. Tea infusions were consumed hot and with no milk or sugar added.

CRP in serum

We measured hs-CRP using an immunoturbidimetric method (CRPLX, Roche Diagnostics, Indianapolis, IN, USA) on a COBAS INTEGRA 700 analyser (Roche Diagnostics). The lower detection limit of the hs-CRP was 0.064 mg/L. Internal quality control procedures are carried out 3–4 times daily. The target CV value is $< 2\%$ and was maintained as such. Our laboratory has been participating in the College of American Pathologists (CAP) survey and inspection.

Oxidized LDL in plasma

Plasma ox-LDL concentrations were measured by sandwich ELISA (intra-assay CV was 3.4% and inter-assay CV was 6.7%; Mercodia AB, Uppsala, Sweden), utilizing the same specific murine monoclonal antibody, mAB-4E6, as the assay described by Holvoet *et al.*[18,19] In order to avoid systematic differences in the current study, two internal controls were repeatedly included on all plates.

TAC in plasma

The Total Antioxidant Status kit (Randox Laboratories Ltd, Crumlin, UK) was applied to a Cobas Mira chemistry analyser (Roche Diagnostics). The assay principle is as follows: 2,2′-azino-di-2-ethyl-benzthiazoline sulphonate (ABTS) is incubated with a peroxidase (metmyoglobin) and H_2O_2 to produce the radical cation $ABTS^+$. This has a relatively stable blue–green colour which is measured at 600 nm. Antioxidants in the added sample cause suppression of this colour production to a degree proportional to their concentration. The assay was calibrated against an α-tocopherol analogue (Trolox) and the results were expressed as mmol/L of Trolox activity.

To measure the accuracy and reproducibility of the kit, the Randox Total Antioxidant Control (Randox Laboratories Ltd, UK) was used; the intra-assay CV was 1.1% and inter-assay CV 2.3%.

Soluble cellular adhesion molecules in plasma

The concentrations of soluble VCAM-1 (sVCAM-1), soluble ICAM-1 (sICAM-1) and soluble E-selectin (sE-selectin) were measured by human sVCAM-1 immunoassay, human sICAM-1 immunoassay and human sE-selectin immunoassay (R&D Systems Inc., Minneapolis, MN, USA), respectively. These assays employ the quantitative sandwich enzyme immunoassay technique using monoclonal antibodies specific for each sCAM.

Statistics

All the statistical analyses were done with SPSS 11.5 software (SPSS Inc., Chicago, IL, USA). We compared the parameters after two and four weeks of green tea ingestion with the baseline parameters. As the data points were not normally distributed and the sample size was relatively small ($n < 20$), we employed non-parametric statistics. For statistical analysis, hs-CRP concentrations below the detection limit were assigned a value of 0.064 mg/L. Results are expressed as median (interquartile range); significance was set at $P < 0.05$. The differences in biological markers according to duration of green tea ingestion were analysed statistically using the Wilcoxon signed rank test.

Results

The concentrations of ox-LDL after two weeks of green tea consumption decreased from 69.5 U/L (55.4–96.1) to 64.5 U/L (49.9–73.4; $P = 0.388$) and there was a statistically significant decrease after four weeks ingestion (54.8 U/L [40.9–60.6] $P = 0.006$; Table 1, Figure 1a).

The concentrations of sVCAM-1 showed a similar pattern. There was a non-significant decrease after green tea consumption from 323.8 ng/mL (269.4–447.6) at baseline to 300.5 ng/mL (248.5–364.5) after two weeks ($P = 0.774$), and a significant decrease after

Table 1 *Changes of atherosclerotic markers according to the duration of green tea intake**

	Baseline	After two weeks	After four weeks
Total cholesterol (mmol/L)	5.04 (3.90–5.77)	5.22 (3.98–5.64) *P*=0.774	4.84 (4.16–5.48) *P*=0.388
Triglyceride (mmol/L)	1.75 (1.03–2.44)	1.55 (0.88–2.11) *P*=0.388	1.69 (0.95–2.62) *P*=1.000
High-density lipoprotein cholesterol (mmol/L)	1.27 (1.03–1.37)	1.19 (1.14–1.42) *P*=0.774	1.34 (1.14–1.47) *P*=0.146
Low-density lipoprotein cholesterol (mmol/L)	2.87 (2.17–3.49)	3.28 (2.22–3.70) *P*=0.774	2.84 (2.43–3.21) *P*=0.388
Oxidized low-density lipoprotein (U/L)	69.5 (55.4–96.1)	64.5 (49.9–73.4) *P*=0.388	54.8 (40.9–60.6)† *P*=0.006
Total antioxidant capacity (mmol/L)	1.03 (0.94–1.12)	1.04 (0.98–1.10) *P*=1.000	1.05 (0.94–1.11) *P*=1.000
C-reactive protein (mg/L)	0.33 (0.06–0.82)	0.06 (0.06–0.07) *P*=0.453	0.06 (0.06–0.06) *P*=0.687
Soluble vascular cell adhesion molecule-1 (ng/mL)	323.8 (269.4–447.6)	300.5 (248.5–364.5) *P*=0.774	239.5 (173.5–317.0)† *P*=0.006
Soluble intercellular adhesion molecule-1 (ng/mL)	217.2 (177.7–257.5)	232.0 (171.2–267.2) *P*=0.388	209.2 (181.0–252.3) *P*=1.000
Soluble E-selectin (ng/mL)	43.4 (29.8–53.0)	45.6 (33.2–58.1) *P*=0.146	46.3 (37.1–53.2) *P*=0.388

*Data are represented by median (interquartile range) and *P* value compared with those at baseline by Wilcoxon signed rank test. Statistically significant differences compared with those at baseline (Wilcoxon signed rank test, †$P < 0.01$).

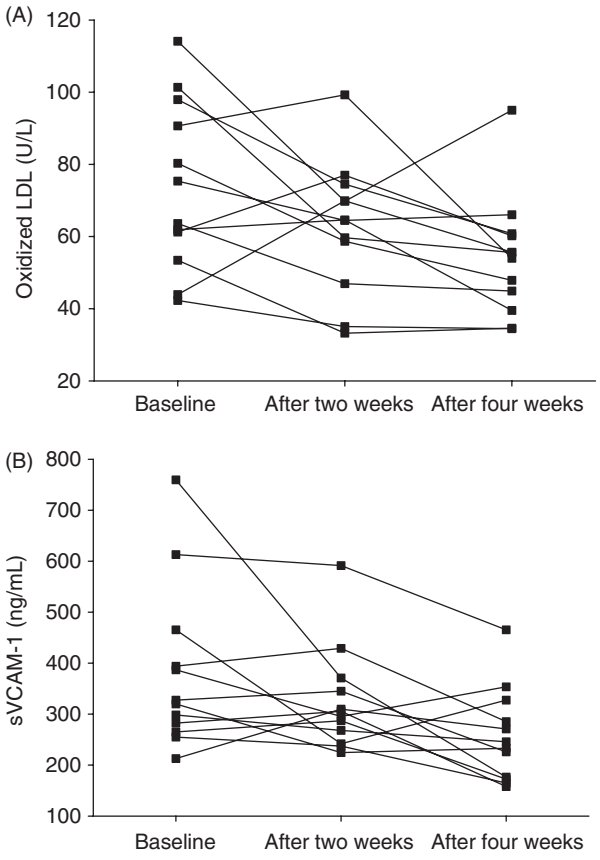

Figure 1 *Changes of individual oxidized LDL (A) and soluble vascular cell adhesion molecules (B) according to the duration of green tea intake.*

four weeks (239.5 ng/mL [173.5–317.0]; $P = 0.006$; Table 1, Figure 1b). The concentrations of sICAM-1 and sE-selectin showed no significant change after two and four weeks of green tea consumption compared with those at baseline.

The levels of TAC and CRP did not show significant change after two or four weeks of green tea consumption compared with those at baseline.

Discussion

Our study shows a significant decrease in ox-LDL concentration after four weeks daily green tea consumption of 600 mL. While this is the first study of the effects of subacute tea consumption using circulating ox-LDL as the marker for lipid oxidation, there have been several reports utilizing other markers.[6,13,20,21] Ishikawa *et al.*[20] reported that the lag time before LDL oxidation was significantly prolonged from 54 to 62 min in 14 healthy volunteers after consuming 750 mL of black tea per day for four weeks. Although there were differences between our study and that of Ishikawa *et al.* in the tea used (green versus black tea), the amount of daily consumption and the measuring method for LDL oxidation, both studies demonstrated that four weeks of tea consumption had protective effects on LDL oxidation *in vivo*.

Several studies have shown that ingestion of tea cannot inhibit LDL oxidation *ex vivo*.[6,13,21] McAnlis *et al.*[6] reported a four-week crossover study in which coffee was used as a control against black tea, showing no significant difference in the TAC or susceptibility of LDL to oxidation between the tea and coffee groups. The tea group had a daily dose of 1500 mL of black tea (equivalent to 126.8 $\pm$13.5 mg flavonoids). van het Hof *et al.*[13] reported that daily consumption of 900 mL (six cups) of green or black tea did not affect serum lipid concentrations, resistance of LDL to oxidation or markers of oxidative damage to lipids *in vivo*, although consumption of green tea slightly increased the TAC of plasma. In addition, Princen *et al.*[21] showed that daily consumption of 900 mL green or black tea for four weeks had no effect on resistance of LDL to oxidation *ex vivo*.

When Hodgson *et al.*[22] examined the acute effects of black tea and green tea on lipoprotein oxidation *ex vivo* without prior isolation of lipoproteins from serum, they found that there was a greater lag time for black tea than water control and a similar trend for green tea. They suggested that the lack of effects of tea on LDL oxidation *ex vivo* in previous controlled interventions[6,13,21] might be related to the method used to assess LDL oxidizability. Isolated LDL from serum was used for the LDL oxidation assay in these studies. As the oxidation of lipoprotein occurs in the presence of the aqueous phase of serum, LDL should not be isolated from serum for measurement. van het Hof *et al.*[23] observed the distribution of catechins in the body after drinking eight cups of green tea a day for three days. They reported that 60% were in a protein-rich fraction, 23% in high-density lipoprotein (HDL) and less than 10% in LDL, and that the concentration of catechins in LDL was not sufficient to enhance the resistance of LDL to oxidation *ex vivo*. These findings suggest that measurement of lipid oxidation using LDL isolated from serum may not represent the *in vivo* effects of green tea.

We used plasma concentration of ox-LDL as a marker for *in vivo* oxidation. Ox-LDL is known to be a clinically useful marker of oxidative stress[24,25] and is reported to be a biochemical risk marker for coronary heart disease.[18,19,26,27] Ox-LDL was measured by a competitive ELISA method using monoclonal antibody mAb-4E6, although we recognize that the routine measurement of circulating ox-LDL in clinical laboratory has limitations, for example lack of standardization and little clinical data.[18,19,26,27]

No previous study has examined the effects of green tea on CAMs. In our study, the concentration of sVCAM-1 decreased significantly after four weeks of green tea ingestion, but there was no change in

sICAM-1 or sE-selectin concentrations. Cominacini *et al.*[28] reported that the introduction of ox-LDL into human umbilical vein endothelial cells showed increased induction of VCAM-1 and ICAM-1 compared with the introduction of ox-LDL, which had been pretreated with vitamin E and probucol before oxidation. The decrease in sVCAM-1 observed in our study could be related to the antioxidant capacity of green tea. Peter *et al.*[29] suggest that serum concentration of sVCAM-1 had higher correlation with the degree of atherosclerosis than other CAMs, and may be of help in the risk assessment for development of atherosclerosis.[15,16]

TAC showed no significant change after two and four weeks of green tea consumption compared with basal concentration. Benzie *et al.*[11] reported that there was 4% increase in ferric/antioxidant power 40 min after taking 400 mL of green tea, which returned to basal value after 2 h. Serafini *et al.*[7] reported that plasma TAC reached a peak at 50 min after drinking 300 mL of green tea and showed subsequent decrease. In studies of long-term tea consumption, increases in total antioxidant activity were small (3–10%) and generally not significant.[6,13]

The results of this study demonstrate that the concentrations of ox-LDL and sVCAM-1 were significantly decreased after four weeks' ingestion of green tea, and these suggest the anti-oxidant effect of green tea and its influence on early inflammatory reactions. The effect of green tea on ox-LDL and sVCAM-1 provides a potential mechanism for cardiovascular benefits of regular ingestion of tea.

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