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Effects of single dose and regular intake of green tea (*Camellia sinensis*) on DNA damage, DNA repair, and heme oxygenase-1 expression in a randomized controlled human supplementation study

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Regular intake of green tea (*Camellia sinensis*) lowers DNA damage in humans, but molecular mechanisms of genoprotection are not clear. Protection could be via direct antioxidant effects of tea catechins, but, paradoxically, catechins have pro-oxidant activity in vitro, and it is hypothesized that mechanisms relate to redox-sensitive cytoprotective adaptations. We investigated this hypothesis, focusing particularly on effects on the DNA repair enzyme human oxoguanine glycosylase 1 (hOGG1), and heme oxygenase-1, a protein that has antioxidant and anti-inflammatory effects. A randomized, placebo-controlled, human supplementation study of crossover design was performed. Subjects ($n = 16$) took a single dose (200 mL of 1.5%, w/v) and 7-days of (2×200 mL 1%, w/v per day) green tea (with water as control treatment). Lymphocytic DNA damage was ~30% ($p < 0.001$) lower at 60 and 120 min after the single dose and in fasting samples collected after 7-day tea supplementation. Lymphocytic hOGG1 activity was higher ($p < 0.0001$) at 60 and 120 min after tea ingestion. Significant increases ($p < 0.0005$) were seen in hOGG1 activity and heme oxygenase-1 after 7 days. Results indicate that molecular triggering of redox-sensitive cytoprotective adaptations and posttranslational changes affecting hOGG1 occur in vivo in response to both a single dose and regular intake of green tea, and contribute to the observed genoprotective effects of green tea.

Keywords:
DNA damage and repair / Green tea / Heme oxygenase-1 / hOGG1 / Redox tone

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Regular consumption of green tea is reported to have various health benefits and decrease oxidative stress [1–3]. Significant genoprotection was reported in a placebo-controlled study of healthy subjects after 4-wk green tea supplementation [4], but molecular mechanisms remain elusive. Decreased oxidation-induced DNA damage could be mediated by enhanced antioxidant defense and/or to more efficient repair of DNA lesions. Higher intake of dietary-derived antioxidants could enhance defense, and green tea is very rich in catechins, which have powerful antioxidant properties [4, 5]. Though catechin bioavailability is low, there is a significant increase in plasma antioxidant capacity and catechin concentrations within 1 h of drinking green tea [5, 6]. Paradoxically, green tea has pro-oxidant activity in vitro [7]. If this is shown to occur in vivo, green tea could shift intracellular redox balance. Interestingly, in vitro studies have shown that a mild pro-oxidant shift in cellular redox tone activates redox-sensitive gene promoter regions called the antioxidant response element (ARE; also known as the electrophile response element) [8, 9]. ARE activation leads to upregulation of genes that produce an array of cytoprotective factors, including human oxoguanine glycosylase human oxoguanine glycosylase 1 (hOGG1), which catalyzes the first step in base excision repair (BER) of oxidation-induced lesions in DNA, and heme oxygenase-1 (HMOX-1), which is regarded as a "therapeutic funnel" because of its

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Abbreviations: ARE, antioxidant response element; **Fpg**, formamidopyrimidine DNA glycosylase; **HMOX-1**, heme oxygenase-1; **hOGG1**, human oxoguanine glycosylase 1

Table 1. DNA damage, assessed using the comet assay, in lymphocytes collected before and after green tea or water (as control) treatment

		Day 1 fasting (baseline)	Day 1 (acute study) 60 min postingestion	Day 1 (acute study) 120 min postingestion	Day 8 fasting (after 7-days supplementation)
Water	Fpg-treated cells	15.7 (2.26)	15.6 (2.49)	15.0 (2.60)	15.7 (3.34)
	Buffer-treated cells	4.4 (1.14)	4.6 (0.99)	4.4 (0.72)	4.6 (0.95)
Green tea	Fpg-treated cells	16.8 (3.09)	11.6 (2.45)*	11.7 (2.28)*	11.6 (1.82)*
	Buffer-treated cells	4.5 (1.05)	4.4 (0.83)	4.2 (1.15)	4.3 (0.71)

Data are mean (SD) %DNA in comet tail, $n = 16$. Data on buffer treated cells represent preexisting strand breaks; data on Fpg-treated cells represent the strand breaks created at oxidation-induced lesions in DNA plus the preexisting strand breaks; the difference between the Fpg- and buffer-treated cells represents oxidation-induced lesions.
*$p < 0.001$ compared to corresponding change from baseline and to time-matched samples after water treatment.

anti-inflammatory, immunomodulatory, and antioxidant effects [9–11]. There is evidence from animal and cell culture studies that tea catechins activate the ARE and cause increased expression of HMOX-1, indicating a pro-oxidant shift in intracellular redox balance, but data from human studies are lacking, and in vitro study showed no acute effect of exposure to green tea on expression of several ARE-associated genes [12, 13].

As noted, bioavailability of tea catechins is very low, and there is extensive and rapid conjugation and metabolism of catechins in humans—factors that restrict catechin concentrations in human plasma to nanomolar levels [5, 14]. Still, it is possible that catechins, or their metabolites, accumulate in tissues with regular intake of tea, enhancing direct antioxidant protection against oxidation-induced DNA damage and mutation. Alternatively, or in addition, there could be acute, subtle pro-oxidant effects triggered by catechins (and perhaps other phytochemicals). Regular, small, postingestion "pro-oxidant waves" could induce cytoprotective ARE-triggered adaptations, such as enhanced DNA repair and increased HMOX-1 [8–13]. To date, the hypothesized effects of pro-oxidant phytochemicals on redox tone and cellular adaptive response have not been demonstrated to occur in humans. Here, we present the results of a randomized, placebo-controlled, single-blinded human intervention trial of crossover design. The study was performed in two parts, an acute response study and a 7-day supplementation trial. Sixteen apparently healthy, nonsmokers (4 men, 12 women, all Chinese, aged 35–50 years) were recruited in the study, which was approved by the Human Subjects Ethics Subcommittee of The Hong Kong Polytechnic University and complied with the Declaration of Helsinki, as revised in 2008. The acute effects (at 60 and 120 min postingestion) of a single dose of green tea and 7 days of regular intake of green tea were investigated (the profile of the tea catechins is presented in Supporting Information Table 1). Hot water was used as the control treatment. Eight volunteers took tea as the first treatment and eight took water as the first treatment. There was a 4-wk washout period between treatments. The outcomes of interest were oxidation-induced lesions in DNA and hOGG1 activity (measured using two versions of the comet assay performed on lymphocytes harvested from venous blood [4,13,15,16]) and the expression in lymphocytes of several ARE-controlled genes (*HMOX1*, *NRF2*, *KEAP1*, *BACH1*, *NQO1*, *GST*α, *XRCC5*, and *hOGG1*), measured by real-time PCR. HMOX-1 protein in cells (by Western blotting) and in plasma (by commercial immunoassay) was also measured. Finally, relationships between the observed effects on these variables were explored. Detailed information on methods is available in Supporting Information. In brief, lymphocytes were harvested from venous blood by density centrifugation, and DNA damage and hOGG1 activity were measured using, respectively, the formamidopyrimidine DNA glycosylase (Fpg)-assisted comet assay and a cell extract version of the comet assay performed with oxidatively damaged "substrate" cells [4, 16]. The comet assay measures strand breaks, and Fpg (a microbial analogue of hOGG1) creates breaks at sites of oxidation-induced lesions in DNA. The extent of damage was measured as %DNA in the comet tail of examined cells. Cells treated with buffer in place of Fpg revealed the amount of preexisting DNA damage (as strand breaks) in lymphocytes, and cells treated with buffered Fpg solution revealed the preexisting strand breaks plus those created by the action of the enzyme on oxidation-induced lesions. In the cell extract version of the comet assay, hOGG1 in the cell extract takes the place of Fpg. Substrate cells that had been exposed to controlled photooxidation were embedded in agarose on microscope slides, lysed, and incubated with the hOGG1-containing cell extracts. Under controlled conditions, the amount of damage revealed in the substrate cells reflects hOGG1 activity in the cell extract, i.e. the greater the damage score in the extract-treated substrate cells, the higher the hOGG1 activity in the subject's lymphocyte extract [16]. In both versions of the comet assay, electrophoresis was run for 30 min at a constant 25 V and the current was set to 300 mA by adjusting the buffer volume. Immediately after staining with ethidium bromide, DNA damage (as %DNA in comet tail) was scored in 50 cells (nucleoids) in each of two gels (i.e. 100 nucleoids in total) for each sample for each treatment using Komet 5.5 image analysis system (Kinetic Imaging Limited, Liverpool, UK) and a fluorescent microscope (Nikon Eclipse E600, Nikon, Tokyo, Japan).

Results showed a significant decrease (~30%; $p < 0.001$) in lymphocytic DNA damage with green tea in both the acute

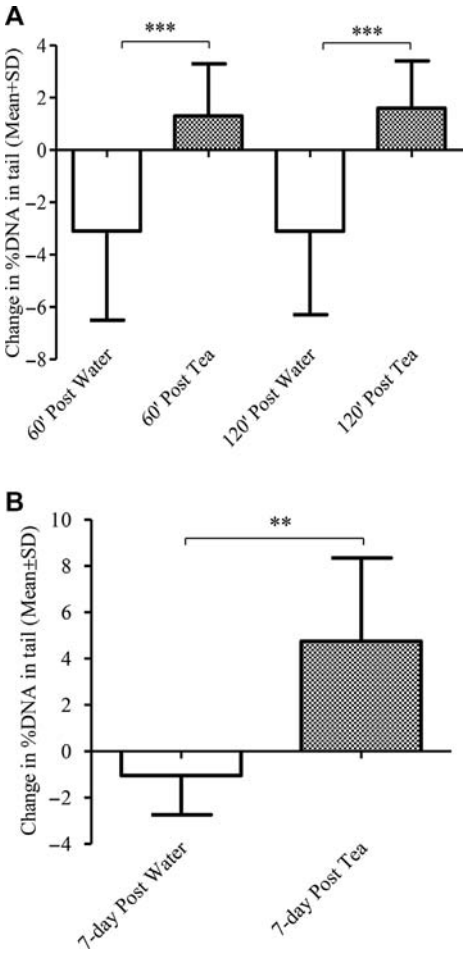

Figure 1. The change in hOGG1 activity in lymphocyte extracts (A) at 60 and 120 min postingestion of green tea (shaded bars) or water (control; open bars); (B) in fasting samples collected from 16 subjects pre and post 7 days twice daily ingestion of green tea (shaded bars) or water (control; open bars). Results are mean ± SD %DNA in comet tail of 100 nucleoids from extract-treated, oxidatively damaged substrate cells scored per sample per treatment and time point. The magnitude of damage measured in the substrate cells is determined by the hOGG1 activity in the lymphocyte extracts of the study subjects, i.e. the greater the damage score in the extract-treated substrate cells, the greater the hOGG1 activity in the lymphocyte extract [16]); $^{**}p < 0.0005$ and $^{***}p < 0.0001$ compared to change after water (for detailed information on methods, please see Supporting Information).

and 7-day supplementation parts of the study compared to both the day 1 fasting results and the time-matched responses to water (Table 1). The decrease was only in the oxidation-induced (Fpg-sensitive lesions) damage, as no change was seen in strand breaks in cells without Fpg treatment (Table 1). As shown in Fig. 1, a slight increase in hOGG1 activity (as evidenced by a greater amount of hOGG1-revealed damage in the substrate cells) was observed at 60 and 120 min after green tea intake, and the activity was significantly ($p < 0.0001$) different compared to the activity at the same time points after water intake. A significant ($p < 0.0001$) increase in hOGG1 activity was also observed after 7-day green tea supplementation, and the change in hOGG1 activity after 7 days of regular tea intake was significantly ($p < 0.0005$) greater than that after the single dose (Fig. 1).

No significant changes were observed in the genes of interest after a single dose of green tea, or after 7-day green tea supplementation (data are shown in Supporting Information Table 4 online). HMOX-1 protein expression in lymphocytes showed a small, nonsignificant increase after tea in the acute study, however after 7-day tea treatment, a significant ($p < 0.0001$) increase in HMOX-1 expression in lymphocytes was seen from (mean (SD)) of 0.97(0.42) on day 1 to 1.80(0.90) on day 8; after water the corresponding figures were, respectively, 0.94(0.46) and 1.10(0.79). Figure 2 shows representative images from Western blots run on samples (run in parallel) of two subjects, one of whom took tea as the first treatment, while the other took water as the first treatment. In relation to plasma HMOX-1 concentrations, no significant change was seen: mean (SD) pre and post 7-day tea supplementation were 1.34(0.46) and 1.31(0.49) ng/mL, respectively. No significant correlation was found between the tea-associated decreases in DNA damage and the increases in hOGG1 activity between days 1 and 8. A significant correlation was seen between decreases in DNA damage and increases in HMOX-1 protein after 7-day regular intake of green tea (Spearman's $r = -0.625$; $p < 0.02$). No significant correlation was seen between increases in hOGG1 activity and increases in lymphocyte HMOX-1 protein.

The genoprotective effect of regular intake of green tea was demonstrated in our previous study of middle-aged adults who took tea for 4 wk [4], but this current study is the first human trial to show a significant acute genoprotective effect of green tea, and is the first study to show that hOGG1 activity is enhanced by green tea. This finding of enhanced DNA repair by a dietary factor has important implications for our understanding of the function and molecular effects of phytochemicals and their role in cancer prevention. To our knowledge, there is only one other study that has shown increased hOGG1 activity in a human supplementation trial, and which was with kiwi fruit taken for 3 wk or longer [16]. Of particular interest in this current study with green tea is the finding that hOGG1 activity had increased significantly (and DNA damage had decreased significantly) as early as 1 h postingestion. Interestingly, we saw no tea-induced increase in *hOGG1* expression (or in any of the genes studied) at any time point. It has been reported that *hOGG1* has a rather consistent gene expression, and a poor correlation between hOGG1 mRNA and hOGG1 activity has been demonstrated [17, 18]. Therefore, we suggest that tea-induced posttranslational changes in hOGG1 that resulted in an increased half-life or increased activity of the enzyme could have occurred. We were unable to measure the hOGG1 protein content due to insufficient sample volume, but in a rat model of breast cancer induced by estrogen, co-administration of vitamin C (which is also known to have a pro-oxidant effect in

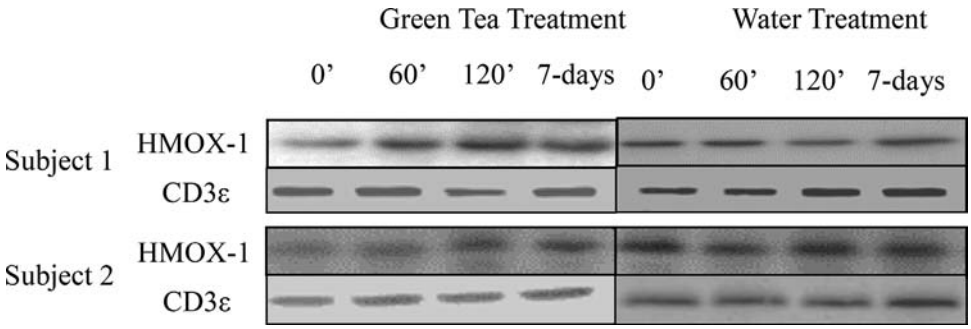

Figure 2. Representative Western blot images on samples from two subjects, showing protein expression of HMOX-1 before and after green tea and water ingestion in the acute and 7-day studies, with CD3ε as the reference protein. All samples were run in parallel. One subject took tea as the first treatment, the other took water as the first treatment.

vitro) was shown to prevent the estrogen-mediated decrease in OGG1 protein in mammary tissues, thereby offering protection against oxidation-induced DNA damage and estrogen-mediated mammary carcinogenesis [19]. Additionally, the activity of hOGG1 is reported to be altered by posttranslational changes, the most common of which are phosphorylation and acetylation [18,20,21]. Interestingly, it has been demonstrated that hOGG1 acetylation was increased by oxidant challenge to cells without changes in expression of the protein [20]. However, it has also been reported that short exposure of human cells to cadmium reversibly inhibited hOGG1 activity via redox modifications of cysteine residues at positions 253 and 255 [21]. Therefore, we speculate that tea-induced effects trigger posttranslational changes to hOGG1 that increase its activity. Increased HMOX-1 is recognized as a sign of oxidative stress [11], and the finding of increased intracellular HMOX-1 following green tea intake supports the hypothesis that green tea induces a pro-oxidant shift in redox balance in vivo. However, no changes in expression of *HMOX-1* or other redox-sensitive genes were seen. This could be due to timing. We may have missed the window of gene activation, but increased HMOX-1 protein after 7-day regular intake without increased *HMOX-1* expression could be due to an extended half-life of the protein because of (as suggested for hOGG1) posttranslational changes. This is noted to be speculative, and further work is required in this area. Yet, the finding of significant decreases in oxidation-induced lesions in DNA at 60 and 120 min after a single dose of green tea suggests that green tea catechins or their metabolites play a role in preventing the formation or accumulation of intracellular ROS. The Fpg-assisted comet assay results showed a sustained but similar level of effect after 7-day green tea supplementation when compared to the acute effect of a single dose of green tea. However, hOGG1 activity was significantly higher after 1 wk when compared to after a single dose of green tea. This suggests that regular intake of green tea has additional benefits in the prevention and/or repair of DNA damage.

In conclusion, results show that drinking green tea leads to a significant acute decrease in oxidation-induced lesions in DNA and increased activity of hOGG1 in lymphocytes, and these changes are maintained, and are more marked for hOGG1 activity, in fasting samples collected after 7 days of regular intake of green tea. The doses of tea used in this study were not large, and could be incorporated easily into the daily diet. The novel findings presented have important implications for health promotion through regular intake of green tea, and offer insight into the molecular mechanism of its action.

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The authors have declared no conflict of interest.

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