

# Effect of a 4-month tea intervention on oxidative DNA damage among heavy smokers: role of *XRCC1* genotypes

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**Abstract:** Reduced DNA repair capacity is believed to increase susceptibility to smoking-related cancers. Polymorphisms in several DNA repair genes have been reported but the role of these variants in generating DNA damage phenotypes in human populations has been less well studied. The aim of this study was to determine whether variation in DNA repair genes is related to smokers' increased susceptibility to DNA damage, and the impact of high tea drinking on this. We designed a phase II randomised controlled, 3-arm tea intervention trial to study the effect of high consumption (4 cups per day) of decaffeinated green or black tea or water on oxidative DNA damage, as measured by urinary 8-hydroxydeoxyguanosine (8-OHdG), among heavy smokers over a 4-month period and to evaluate the roles of *XRCC1* genotypes as effect modifiers. A total of 120 heavy smokers were included in the analysis. Multiple linear regression models were used to estimate the main effects and interaction effect of green and black tea consumption on creatinine-adjusted urinary 8-OHdG, with or without adjustment for potential confounders. Finally, we studied whether the effect of treatment varied by *XRCC1* status of the individual. In this randomised controlled trial among smokers, daily drinking of 4 cups of decaffeinated green tea was associated with statistically significant decrease in urinary excretion of 8-OHdG. We did find a greater effect of green tea consumption on urinary 8-OHdG levels among Arg399/Arg ( $p=0.02$ ) than among Arg399/Gln+Gln/Gln ( $p=0.079$ ) smokers. Decaffeinated black tea consumption had no effect on urinary 8-OHdG levels among heavy smokers. Our data show that consumption of 4 cups of tea per day is a feasible and safe approach and was associated with significant decrease in urinary 8-OHdG among green tea consumers after 4 months of use. Our results suggest that carriers of the polymorphic *XRCC1* Gln399 allele may not significantly benefit from a green tea, and hence an antioxidant, intervention.

**Keywords:** tea, smokers, DNA damage, *XRCC1*, clinical trial

## Introduction

Preventive strategies require identification of individuals who are cancer susceptible as a result of combinations of carcinogen exposure and lack of protective factors. Changes in dietary habits, with the intake of more cancer-chemopreventive agents, appear to be a practical approach for cancer prevention in persons with increased oxidative stress, as is the case for smokers. Tea is one of the most ancient beverages and, next to water, is the most widely consumed liquid in the world. Tea polyphenols are the major polyphenolic compounds of tea. They scavenge active oxygen radicals (Cheng et al 1989) and inhibit DNA biosynthesis of tumour cells (Katiyar et al 1992) and chemocarcinogen-induced carcinogenesis (Xu and Song 1991). Total antioxidant capacity of plasma was significantly increased after taking green tea in amounts of 300 and 450 mL. A positive increment according to green tea dosage was also observed (Sung et al 2000).

Oxidative damage to DNA is considered an important target for carcinogenesis. The most abundant of the purine and pyrimidine oxidation products is 8-hydroxy deoxyguanosine (8-OHdG), which is a promising biomarker for evaluation of oxidative DNA damage. It has been shown that hydroxyl radicals, singlet oxygen and direct photodynamic action produce 8-hydroxylation of the guanine base, then damaged DNA is repaired in vivo by endonucleases and free, water-soluble 8-OHdG is excreted into the urine without further metabolism (Shigenaga et al 1989). It is believed that the presence of 8-OHdG in urine represents the primary repair product of the oxidative DNA damage in vivo.

Inherited polymorphisms in genes controlling both carcinogen metabolism and repair of DNA damage have been

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suggested to underlie the variability in cancer susceptibility (Mohrenweiser and Jones 1998; Hecht 1999). Recently, three coding polymorphisms in x-ray cross-complementing group 1 (*XRCC1*) DNA repair gene have been identified, and it is possible that these polymorphisms may affect DNA repair capacity and thus modulate cancer susceptibility (Shen et al 1998). The *XRCC1* codon 399 polymorphism has been studied in relation to several cancers, with various results. In previous studies, the Arg/Gln genotype was classified in the same group with the Arg/Arg or Gln/Gln genotype according to the distribution of genotypes in controls and cases. Therefore, it has not been determined if individuals carrying one Gln allele have an increased risk for cancer. However, the Gln/Gln genotype has been consistently identified as the risk genotype for various smoking-related cancers (Sturgis et al 1999; Shen et al 2000; Divine et al 2001). The reported frequency of the Gln399 allele among Asians (0.22–0.26) is lower than among Caucasians (0.32–0.37) (Lunn et al 1999).

DNA repair plays a critical role in protecting the genome of the cell from insults of cancer-causing agents, such as those found in tobacco smoke. Reduced DNA repair capacity, therefore, can increase the susceptibility to smoking-related cancers. It is therefore important to take account of genotypes when assessing biomarkers of cancer risk in population studies. Since the *XRCC1* polymorphisms occur at reasonable frequencies, statistically meaningful conclusions can be drawn from studies of relatively small numbers of subjects. We evaluated the relationship between the codon 399 polymorphism in *XRCC1* gene, smoking and tea consumption in a randomised controlled intervention among heavy smokers.

## Materials and methods

### Study population

The study population consisted of 143 heavy smokers recruited in cohorts ( $n \approx 36$  per cohort) between October 1999 and April 2001 in Tucson, Arizona. A total of 133 smokers completed the study. Thirty men and 90 women with complete *XRCC1* genotype data were included in this analysis. All of the subjects were screened by questionnaire to exclude those who smoked fewer than 10 cigarettes per day for less than 1 year, pregnant women, persons with a history of schizophrenia or cancer, current drug or alcohol abusers, individuals with an abnormal liver function blood test, or those currently being treated with antidepressants. The study was approved by the Institutional Review Board

of the University of Arizona, and all of the subjects provided informed consent before enrolment.

### Study protocol

The detailed study protocol has been published elsewhere (Hakim et al 2003). In summary, the study was a 3-arm randomised and controlled tea intervention trial. Randomisation was carried out by use of a random permuted block design (block size = 6), with separate schedules for men and women. Each individual was assigned randomly to drink 4 cups per day of decaffeinated green tea, decaffeinated black tea or water. Study participants were asked to maintain the beverage consumption pattern (4 cups per day) for 4 months, returning to the clinic at monthly intervals to receive the monthly tea supply, return completed tea and smoking diaries, and provide blood and urine specimens. We used decaffeinated green and black tea to control for the potential independent effect of caffeine intake on oxidative damage. All the tea used in the trial was obtained from the same supplier (Unilever Bestfoods, NJ, USA) and tea analysis was performed for each cohort (Hakim et al 2003). Participants were telephoned during the week before each follow-up visit to confirm the date and time of the next appointment and to identify any problems or side effects associated with study participation. Blood and urine were collected monthly.

### Data collection

An in-person screening visit was conducted to ascertain eligibility and to obtain baseline data using a standardised, self-administered health and lifestyle questionnaire (Hakim et al 2003). The questionnaire also sought detailed information on smoking, physical exercise and alcohol drinking. The smoking questionnaire included the following variables: number of cigarettes smoked per day, total years of smoking, age at onset of smoking and pack-year (number of packs smoked per day multiplied by years of smoking).

Dietary information on the frequency of consumption of more than 150 foods and drinks, in a 12-month period prior to enrolment, was obtained by the self-administered Arizona food frequency questionnaire (AFFQ). All individual questionnaires were checked and coded by trained staff, scanned and then transformed into estimates of intake for a series of over 30 nutrients.

Body mass index (BMI) was computed as measured weight in kilograms divided by the square of the measured height in metres. Percentage of body fat was estimated as part of a body composition assessment that was done using

dual energy x-ray absorptiometry. This technology estimates lean body mass, percentage body fat and bone density. Blood (45 mL) and urine (100 mL) samples were collected at baseline and then monthly throughout the intervention.

### Urinary 8-OHdG

One merit of urinary 8-OHdG analysis is that the results are reproducible and are not increased by air oxidation. This may be due to the presence of a high concentration of an antioxidant, uric acid, and the low level of the precursor deoxyguanosine (dG) in urine (Kasai 2002). Urinary 8-OHdG was measured by an enzyme-linked immunosorbent assay (ELISA) kit, the validity and comparability of which to high-performance liquid chromatography with electrochemical detection (HPLC-ECD) had already been verified (Osawa et al 1995; Yoshida et al 2002). First-void urine samples were centrifuged at 300 g for 10 minutes to remove any particulate material. Baseline through 4-month urinary samples from the same individual were batched for 8-OHdG analysis, with the laboratory blinded to treatment status. All reagents and urine samples were brought to room temperature before use and all standards and samples were typically assayed in triplicate (Hakim et al 2003). The intra-assay coefficient of variation of this assay was 4.9%. Data were corrected by urinary creatinine concentration and expressed as nanograms 8-OHdG per milligram creatinine.

### Urinary creatinine

Levels were determined using a creatinine assay kit (Sigma Chemicals, Catalogue nr 555, St. Louis, MO, USA) developed based on the method reported by Heinegard and Tiderstrom (1973) with an intra-assay coefficient of variation of 3.6%. Urinary cotinine was measured in a commercially certified service laboratory for the cancer centre.

### Catechins

Total catechins in plasma were determined spectrophotometrically following complexation with 4-dimethylaminocinnamaldehyde (DMACA; Merck, Darmstadt, Germany) (Kivits et al 1997). The tea catechin levels in urine were determined by use of HPLC with an electrochemical array detection system (Lee et al 1995).

### XRCCI analyses: exon 10, codon 399

Polymorphisms were analysed in genomic DNA extracted from whole blood using the ABI Prism 7700 sequence detector (TaqMan; PE Biosystems, Foster City, CA, USA). Polymerase chain reaction primers and dual-labelled allele

discrimination probes were designed using the Primer Express software package (PE Biosystems). Oligonucleotide probes were labelled with two different fluorescent dyes to discriminate between the two alleles of the single nucleotide polymorphism (SNP). Two oligonucleotide probes each anneal perfectly with one allele at the site of the SNP. During the reaction, the Taq enzyme recognises the perfect match and cleaves the dye molecule off the probe, releasing it into solution. The mismatched probe remains quenched by the quencher dye attached to the other end of the probe. The increased level of fluorescent emission of each dye indicates the corresponding homozygous genotype. Heterozygous individuals have increased emission of both dyes. Reactions with no template and with DNA of known genotype were run on each assay plate to control for contamination and to aid in signal processing for genotype determination by the software.

Oligonucleotide sequences for the analyses were as follows. Forward primer: TGGACTGTACCGCATGC; reverse primer: TGCCCAGCACAGGATAAGG; probe 1: TGCCCTCCCGGAGGTAAGGCCT; probe 2: TGCCCTCCAGAGGTAAGGCCTCA. The assay was set up by using 2× volume of the Master Mix provided by PE Biosystems, which contains all four deoxynucleotides, Taq polymerase and TaqMan buffer, 2000 nm of forward and reverse primers and the double-labelled probe, and 1–5 ng of genomic DNA in 25-μL reactions. The thermal cycling conditions for the ABI Prism 7700 Sequence Detector was set up at initial settings of 50 °C for 2 minutes and 95 °C for 10 minutes, followed by 40 cycles each of 95 °C for 15 seconds and 60 °C for 1 minute. All laboratory personnel were blind to status of the samples.

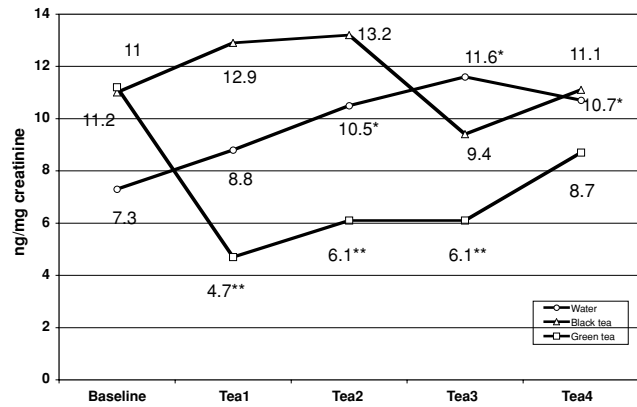
### Statistical methods

All statistical analyses were performed using Stata Statistical Software (Intercooled stata 7; College Station, TX, USA). The primary end-point was the change in the level of creatinine-adjusted urinary 8-OHdG from baseline to 4 months after commencement of intervention. Associations between baseline characteristics, urinary 8-OHdG and intervention group were assessed, using a t-test, chi-square test or Wilcoxon Rank Sum test. Tests for significance of the change (pre-intervention versus post-intervention values) in urinary 8-OHdG were performed. Multiple linear regression models were used to estimate the main effects of green and black tea intake on creatinine-adjusted urinary 8-OHdG, with or without adjustment for potential confounders.

Potential confounders considered were baseline levels of creatinine-adjusted urinary 8-OHdG, BMI, percentage body fat, cohort effect and physical activity. Finally, we studied whether the effect of treatment varied by *XRCC1* status of the individual. Analyses were performed on both raw and log-transformed data; however, because results did not differ substantially, only results based on original data are presented. Statistical tests were two-sided with a significance level of 0.05.

## Results

Of the 235 persons screened, 143 smokers were randomised and 120 smokers completed the 4-month intervention and *XRCC1* analyses. The main reason for non-enrolment was loss of interest, while the reasons for dropout ( $n = 10$ ) were moving out of Tucson and not having enough time. The study population consisted of 90 female and 30 male smokers. There were no statistically significant differences by gender, smoking variables or treatment group between those who completed the study and those who did not. Adherence to the study protocol was assessed through self-report and detection of catechins in plasma and urine. Ninety-five percent of participants reported consuming at least 4 cups of tea or water per day at each of the 4-month study points. There were no differences in smoking level between the three groups and throughout the 4-month intervention, and levels of creatinine-adjusted urinary cotinine did not change in any group during study participation (data not shown). Levels of dietary and plasma antioxidants did not change in any group during study participation (data not shown).



**Figure 1** Median levels of creatinine-adjusted urinary 8-OHdG by treatment group at the five time points. \*  $p < 0.01$  compared with baseline level in water group; \*\*  $p < 0.01$  compared with baseline level in green tea group.

The mean age of trial participants was 57 years (range 18–79); 75% were women, and 87% were non-Hispanic whites. The prevalence of the *XRCC1* Arg399/Arg, Arg399/Gln and Gln399/Gln genotypes was 47.9%, 40.5% and 11.6%, respectively. Baseline characteristics were similar across the three groups (Table 1). The distribution of *XRCC1* genotypes was not significantly different between the three groups. There were no significant differences in dietary intake or plasma levels of antioxidants between the three groups (data not shown).

Figure 1 presents the median levels of creatinine-adjusted urinary 8-OHdG by treatment group at the five time points. There was a significant increase in the median levels of urinary 8-OHdG in the water group in months 2, 3 and 4 compared with the baseline. In the green tea group, we found

**Table 1** Baseline characteristics (mean  $\pm$  SE or percentage) of participants by randomisation group ( $n = 120$ )

Characteristics	Water ( $n = 42$ )	Black tea ( $n = 43$ )	Green tea ( $n = 35$ )	<i>p</i>
Females (%)	74	72	77	0.88
Age $\geq 50$ years (%)	50	51	54	0.93
Non-Hispanic white (%)	17	12	11	0.73
Education $\geq 12$ years (%)	95	91	91	0.70
<i>XRCC1</i> (%)				
Arg/Arg	45	53	43	
Arg/Gln	41	35	48	
Gln/Gln	14	12	9	0.75
Cigarettes per day	20.7 $\pm$ 1.3	19.8 $\pm$ 1.3	20.6 $\pm$ 1.8	0.96
Packs per year	32.3 $\pm$ 3.4	33.8 $\pm$ 3.9	34.5 $\pm$ 4.8	0.97
Cotinine (ng/mg creatinine)	2324.9 $\pm$ 215.1	1896.6 $\pm$ 180.7	2367.2 $\pm$ 308.6	0.47
Calories (kcal/day)	1642.5 $\pm$ 88.7	1636.2 $\pm$ 90.4	1788.6 $\pm$ 89.8	0.16
Percentage body fat	36.6 $\pm$ 1.7	37.3 $\pm$ 1.6	36.7 $\pm$ 1.6	0.90
Body mass index	25.9 $\pm$ 1.0	26.5 $\pm$ 1.1	26.7 $\pm$ 0.8	0.48
Urinary 8-OHdG (ng/mg creatinine)	8.7 $\pm$ 1.3	10.8 $\pm$ 1.3	9.5 $\pm$ 2.1	0.08

SE, standard error.

**Table 2** Unadjusted means ( $\pm$ SE) of urinary 8-OHdG (ng/mg creatinine) by intervention group and XRCCI genotype

Population	Water	Black tea	Green tea	<i>p</i> <sup>a</sup>
Total population	42	43	35	
Baseline	8.7 $\pm$ 1.3	10.8 $\pm$ 1.3	9.5 $\pm$ 2.1	0.24
Month 4	11.1 $\pm$ 1.3	13.5 $\pm$ 1.8	7.9 $\pm$ 1.0	0.11
Arg/Arg	19	23	15	
Baseline	8.6 $\pm$ 1.4	10.4 $\pm$ 1.7	11.2 $\pm$ 3.9	0.63
Month 4	11.8 $\pm$ 1.9	14.3 $\pm$ 2.4	8.9 $\pm$ 1.6	0.33
Arg/Gln+Gln/Gln	23	20	20	
Baseline	9.2 $\pm$ 2.2	10.8 $\pm$ 1.8	7.5 $\pm$ 1.8	0.44
Month 4	10.5 $\pm$ 2.1	12.5 $\pm$ 2.8	7.0 $\pm$ 1.3	0.31

<sup>a</sup>Between-groups comparison. SE, standard error.

a significant decrease in the median levels of urinary 8-OHdG in months 1, 2 and 3 compared with baseline level. No effect was seen among smokers in the black tea group.

Table 2 presents the unadjusted mean ( $\pm$ standard error) of urinary 8-OHdG (ng/mg creatinine) by treatment group and XRCCI genotypes at baseline and month 4. At the end of the intervention, the decrease in urinary 8-OHdG (ng/mg creatinine) in the green tea group was 19.6% and 6.7%, respectively, in the XRCCI Arg399/Arg and the Arg399/Gln+Gln/Gln genotypes.

To account for the wide variation in baseline levels of urinary 8-OHdG and potential confounding of other characteristics, we performed multivariate regression analyses. The dependent variable was the change in creatinine-adjusted 8-OHdG from baseline to month 4. Table 3 shows the results of the final model for the two tea interventions, adjusted for baseline 8-OHdG levels, BMI, percentage body fat, amount of beverage consumed, cohort effect and physical activity. Our results showed a highly

**Table 3** Adjusted mean change<sup>a</sup> (95% CI) in urinary excretion of 8-OHdG (ng/mg creatinine) by tea group and XRCCI genotypes compared with water

	Urinary 8-OHdG (mean $\pm$ SE)					
	Black tea			Green tea		
	Mean change	95% CI	<i>p</i>	Mean change	95% CI	<i>p</i>
Total population	2.0	-4.1, 3.6	0.882	-1.8	-8.0, -2.0	0.001
Arg/Arg	3.3	-4.9, 5.2	0.964	-2.0	-12.1, -1.1	0.02
Arg/Gln + Gln/Gln	3.2	-6.4, 4.5	0.73	-2.2	-10.1, 0.56	0.079

<sup>a</sup> Linear regression analysis of urinary 8-OHdG as a function of treatment group, baseline measurement, body mass index, percentage body fat, amount of beverage consumed, physical activity and cohort effect. SE, standard error; CI, confidence interval.

significant decrease in urinary 8-OHdG after 4 months of drinking decaffeinated green tea ( $p=0.001$ ). The change from baseline was significant in the XRCCI Arg399/Arg green tea group ( $p=0.02$ ) but not in the Arg399/Gln+Gln/Gln green tea group ( $p=0.079$ ). Decaffeinated black tea consumption had no effect on urinary 8-OHdG levels among heavy smokers.

## Discussion

DNA damage is generally considered a necessary step in cancer initiation and is being used extensively as an outcome in intervention studies (Santella 1997). 8-OHdG is known to represent one of the major forms of oxidative DNA damage, and the ratio of urinary 8-OHdG to urinary creatinine (8-OHdG/creatinine) is considered a good biological indicator of DNA oxidation (Erhola et al 1997). In a trial of 40 male smokers in China and 27 men and women (smokers and nonsmokers) in the USA, urinary 8-OHdG was reported to be reduced after consuming approximately 6 cups a day of green tea for 7 days; however, levels of urinary 8-OHdG were not reported (Klaunig et al 1999).

In this randomised controlled trial among smokers, daily drinking of 4 cups of decaffeinated green tea was associated with a statistically significant decrease in urinary excretion of 8-OHdG. Smoking behaviour and levels of dietary and plasma antioxidants did not change in any group during study participation. This suggests that changes in smoking behaviour and/or diet were not responsible for the observed decrease in DNA damage. In contrast to the findings for green tea, we showed no change in 8-OHdG levels with black tea consumption.

Polymorphisms in several DNA repair genes have been reported (Park et al 2002), but the role of these variants in generating DNA damage phenotypes in human populations has been less well studied. Common polymorphisms in DNA repair genes may alter protein function and an individual's capacity to repair damaged DNA; deficits in repair capacity may lead to genetic instability and carcinogenesis. Individuals carrying the Gln allele are thought to have impaired ability to repair DNA damage and therefore are at increased cancer risk. Although several epidemiological studies have found the Gln/Gln genotype to be associated with increased risk for the development of lung and other tobacco-related cancers, the findings in relation to the Arg/Gln genotype are conflicting, and this association remains controversial (Sturgis et al 1999; Shen et al 2000; Divine et al 2001). In a recent study (Park et al 2002), the presence of at least one Gln allele was associated with a significant

increased risk for squamous cell carcinoma of the lung. The risk for the disease increased as the number of Gln alleles increased (Arg/Gln genotype: adjusted OR (odds ratio) = 1.45, 95% CI (confidence interval): 0.84–2.5; Gln/Gln genotype: adjusted OR = 3.26, 95% CI: 1.17–9.15).

In our study, smokers carrying the polymorphic *XRCC1* Gln399 allele did not have elevated baseline urinary 8-OHdG when compared with their Arg399/Arg counterparts. We did find a greater effect of green tea consumption on urinary 8-OHdG levels among Arg399/Arg than among Arg399/Gln+Gln/Gln smokers; that is, the change from baseline was significant in the Arg399/Arg green tea group but not in the Arg399/Gln+Gln/Gln green tea group.

Some limitations of this present study should be noted. Concerns have been raised about the validity of methods used to measure 8-OHdG (Halliwell 1996). Nevertheless, even with the variation in methods, the creatinine-standardised concentrations of 8-OHdG seem broadly similar among different laboratories (Halliwell 2000). Furthermore, the 4.9% intra-assay coefficient of variation we found in this study suggests satisfactory repeatability of the ELISA assay. Another potential limitation is the lack of a study blind. It was necessary to use the commercially available products because we were mainly interested in studying the effect of regular consumption of black and green tea in the forms in which they are commonly consumed. However, all the tea used in the trial was obtained from the same supplier, and tea content analyses were performed for each cohort to assure standardisation of product. Data from the self-reported diaries and recalls suggest high adherence to all of the interventions with no use of other tea products to supplement the intervention. This high adherence to all regimens suggests that any bias based on prior beliefs of the intervention is reduced.

In conclusion, in this randomised controlled trial, drinking 4 cups of decaffeinated green tea daily for 4 months was associated with a statistically significant decrease in urinary 8-OHdG among heavy smokers. We also demonstrated that regular use of these products was safe and feasible.

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