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Tea consumption modulates hepatic drug metabolizing enzymes in Wistar rats

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Abstract

The antioxidant, antimutagenic and anticarcinogenic activities of green tea and its polyphenols have been reported. As bioactivation of the precarcinogens and detoxification of ultimate carcinogens are mainly carried out by hepatic metabolizing enzymes, we have investigated the modulation of these enzyme activities subsequent to tea consumption in rats. Female Wistar rats were divided into eight groups (n = 5). Six groups were given aqueous solutions (2 %, w/v) of six different teas (New Zealand green tea, Australian green tea, Java green tea, Dragon green tea, Gunpowder green tea or English Breakfast black tea) as the sole source of fluid. One group was given a standard green tea extract (0.5 %, w/v) while the control group had free access to water. At the end of four-weeks treatment, different cytochrome P450 (CYP) isoform and phase II enzyme activities were determined by incubation of the liver microsomes or cytosols with appropriate substrates. CYP 1A2 activity was markedly increased in all the tea treatment groups ($P < 0.05$). CYP 1A1 activity was increased significantly in most of the groups except for the Madura, Gunpowder, and Java green tea-treatment groups. Cytosolic glutathione-*S*-transferase activity was significantly increased ($P < 0.05$) in the New Zealand, Gunpowder, and Java green tea-treatment groups. The microsomal UDP-glucuronosyl transferase activity remained unchanged or was moderately increased in most of the groups. The balance between the phase I carcinogen-activating enzymes and the phase II detoxifying enzymes could be important in determining the risk of developing chemically-induced cancer.

Introduction

There is increasing evidence that nutrition plays an important causative role in the initiation, promotion and progression stages of several types of human cancer (Katiyar & Mukhtar 1996a). The diet may contain many chemicals that can antagonize the effects of chemical carcinogens. One of the mechanisms could be by modulation of the enzyme systems involved in the activation and deactivation of chemical carcinogens (Parke & Ioannides 1981; Guengerich 1984). A large number of the potentially genotoxic environmental chemicals and natural products, to which man is exposed, require metabolic activation to exhibit their mutagenic and carcinogenic effects (Eaton et al 1995). This bioactivation is mainly carried out by some of the phase I enzymes including cytochrome P450 (CYP), which give rise to reactive intermediates that attack DNA and other cellular macromolecules (Smith et al 1995). Inhibition of bioactivating enzymes and/or induction of detoxification enzymes by either naturally occurring substances or synthetic agents continues to be a promising chemo-preventive strategy.

Tea (*Camellia sinensis*) is one of the most popular beverages consumed worldwide.

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The antioxidative, antimutagenic and anticarcinogenic activities of tea and its polyphenols have been reported in various animal models using different carcinogens (Li et al 1999). Many studies have shown that tea polyphenol fractions significantly inhibit tumorigenesis in the skin, oral cavity, oesophagus, lung and duodenum in several rodent models (Yang & Wang 1993; Weisburger 1996, 1999a). Fujiki et al (1998) showed that tea polyphenols inhibited the growth of lung cancer in human cell lines. Japanese epidemiologists reported a significantly lower risk of gastric cancer among patients whose green tea consumption was more than 10 cups per day (Fujiki et al 1996, 1999).

Green tea contains 35–52% (measured in weight percent extract of solids) catechins and flavonols combined (Bushman 1998). The four major catechins are (−)-epicatechin, (−)-epicatechin-3-gallate, (−)-epigallocatechin and (−)-epigallocatechin gallate. Some studies have demonstrated that tea possesses the ability to prevent cancer and this protective property is related to its effects on CYP enzymes (Kohlmeier et al 1989). Tea polyphenols have been shown to inhibit rat liver microsomal monooxygenase activities, including those of aryl hydrocarbon hydroxylase, 7-ethoxyresorufin *O*-deethylase and 7-ethoxycoumarin *O*-deethylase (Yang & Wang 1993; Sohn et al 1994). It has been demonstrated that green tea polyphenols enhance the activities of antioxidant and phase II enzymes (Khan et al 1992; Yang & Wang 1993). It is also claimed that drinking green tea may prevent cardiovascular disease and disorders of the liver in man. These could be mediated by reducing total serum cholesterol and triacylglycerol levels as well as reducing levels of glutamic-oxaloacetic transaminase and glutamic-pyruvic transaminase (Han et al 1999). Those authors have reported that (−)-epigallocatechin gallate, (−)-epigallocatechin and (−)-epicatechin-3-gallate strongly inhibited lipid peroxidation in rat liver mitochondria and microsomes. Furthermore they suggested that tea might be an effective crude drug for the treatment of obesity and fatty liver caused by a high fat diet.

This study was aimed at investigating any modulation of xenobiotic metabolizing enzymes in rats by green teas cultivated in New Zealand and Australia, and to compare metabolic enzyme activity changes with those induced by other imported green teas and standard green tea extract. In addition, the effect of tea consumption on blood lipid profiles was monitored.

Materials and Methods

Chemicals and reagents

All chemicals and reagents used were of HPLC analytical grade. Sodium dodecyl sulfate, hexan-1-ol, sodium hydroxide, potassium dihydrogen orthophosphate, potassium chloride, HPLC-grade acetonitrile, *tert*-methylbutyl ether, diethyl ether and methanol were purchased from BDH Chemicals (Poole, UK). Tetrabutylammonium bromide, NADPH, sodium dithionite, *p*-nitrophenol, phenacetin, paracetamol, 2-aminophenol, glutathione, and 1-chloro-2,4-dinitrobenzene were purchased from Sigma Chemical Co (St Louis, MO). Quinine hydrochloride was purchased from Merck–Schuchardt (Schuchardt, Germany). 3-Hydroxyquinine was a gift from Dr P. Winstanley, University of Liverpool, UK. Debrisoquine and 4-hydroxydebrisoquine were kindly donated by Roche (Auckland, New Zealand). Guanoxan hemisulfate was supplied by Pfizer (Auckland, New Zealand).

Treatment of rats with tea

The study was approved by the University of Otago Animal Ethics Committee, Dunedin, New Zealand. Female Wistar rats, eight- to nine-weeks-old, were divided into eight groups ($n = 5$). They were housed in hanging wire cages in a temperature-controlled room with a 12-h light–dark cycle and free access to food (commercial rodent diet). The animals were allowed to acclimatize for five days before the study.

Six different varieties of tea were purchased from local supermarkets in Dunedin, New Zealand. These were New Zealand green tea (Tasman Extracts, Nelson, New Zealand), Madura green tea (Murwillumban, NSW, Australia), Gunpowder green tea (Twining and Company Ltd, London, UK), Dragon green tea (Import and Export corporation, Shanghai, China), English Breakfast tea (Twining and Company Ltd, London, UK) and Java green tea (Twining and Company Ltd, London, UK). In New Zealand green tea is grown in the Nelson region of the South Island. Madura green tea is 97% caffeine-free and is free of tannic acid. Java green tea is a blend of Indonesian green teas from the Island of Java. English Breakfast is a popular brand of black tea consumed in western as well as Asian countries. Tea solutions (2%, w/v) were prepared simulating the usual way of brewing tea. Boiled tap water was added to the required weight of tea powder with intermittent stirring for 10 min, then strained through filters to obtain a clear tea solution. The six different tea solutions were then poured into clean feeding bottles (protected from light)

and given to the appropriate rat group. The seventh rat group was given a 0.5% w/v aqueous solution of green tea extract (courtesy Dr Masami Suganuma, Saitama Cancer, Saitama –Ken 362, Japan). The eighth group (control) received tap water only. Rats were pretreated with tea solutions or appropriate solutions for four weeks. The rats were weighed at the start of the experiment and at the end of each week to monitor any influence of tea on growth rate.

After four weeks of treatment, rats were killed by CO_2 asphyxiation. Blood was collected by cardiac puncture; plasma was separated and kept at −20°C for analysis of blood lipids. Livers were removed and kept frozen at −80°C until required. The liver microsomes and cytosols were prepared by a differential centrifugation method (Robson et al 1987) and stored in phosphate buffer (pH 7.4) containing 20% w/v glycerol at −80°C until required. The microsomal protein content was determined by the method of Lowry et al (1951). The P450 content was determined by the method described by Omura & Sato (1964).

Determination of CYP 1A1/CYP 1A2 activity

The activity of CYP 1A1 and CYP 1A2 was determined using phenacetin as a specific substrate probe as described by Tassaneeyakul et al (1993). The activity of the high affinity component (CYP 1A2) of phenacetin-*O*-deethylase was determined by incubating 5 μM phenacetin with liver microsomes (0.5 mg mL^{-1}) for 30 min. The reaction was terminated by addition of 1 M NaOH. The formation of the metabolite, paracetamol, was measured by a specific HPLC method (Tassaneeyakul et al 1993). The activity of the low affinity isozyme CYP 1A1 was determined by using phenacetin at a concentration of 300 μM, approximately the K_m of CYP 1A1 reported in rat liver microsomes (Boobis et al 1981). The procedures for incubation and HPLC assay were the same for CYP 1A2.

Determination of CYP 3A activity

Quinine was used as the marker for determining CYP 3A activity. The reaction mixture (0.5 mL) containing 30 μM quinine, NADPH (1 mM), and rat liver microsomes (0.5 mg protein mL^{-1}) was incubated at 37°C for 12 min. The reaction was stopped by the addition of cold methanol (1 mL). Formation of the 3-hydroxyquinine metabolite was monitored by an HPLC method as described by Wanwimolruk et al (1996).

Determination of CYP 2E activity

This was determined by using an HPLC method, previously developed in our laboratory, using *p*-nitrophenol as substrate. In brief, the reaction mixture containing *p*-nitrophenol (140 μM), rat liver microsomes (0.8 mg mL^{-1}), ascorbic acid (1 mM) and NADPH (1 mM) in a total volume of 0.5 mL phosphate buffer (pH 7.4) was incubated at 37°C for 25 min. After terminating the reaction using perchloric acid (0.6 M), an internal standard (phenacetin 1.5 μg mL^{-1}) was added. The metabolite formed, *p*-nitrocatechol, and the internal standard were extracted into *tert*-methylbutyl ether after alkalinizing with ammonium sulfate. The residue obtained after the ether was evaporated off was analysed using an HPLC system. The system consisted of a stainless steel HPLC column (150 mm × 4.6 mm i.d.) packed with a reversed-phase C18 material (5 μm Nucleosil, Macherey–Nagel, Duren, Germany), an acetonitrile:water mobile phase (16:84 v/v, pH 3.0) and a UV detector (243 nm).

Determination of CYP 2D activity

Debrisoquine 4-hydroxylase (CYP 2D) activity was measured using a method described by Wanwimolruk et al (1995). Briefly, each reaction mixture (1 mL) consisted of debrisoquine (substrate, 60 μM), rat liver microsomal protein (1 mg mL^{-1}) and NADPH (1.2 mM). Guanoxan hemisulfate (98 μM) was used as the internal standard. Analysis of the metabolite formed (4-hydroxydebrisoquine) was performed using an HPLC system consisting of a CN column (Spherisorb-CN, 5 μ, 15 cm × 4.6 mm i.d), mobile phase (acetonitrile:phosphate buffer, 12:88 v/v, pH 5) and a UV detector (214 nm).

Determination of glucuronosyl transferase activity

Microsomal glucuronosyl transferase activity was determined using two different substrates, *p*-nitrophenol and 2-aminophenol. UDP-glucuronosyl transferase activity towards *p*-nitrophenol was determined using the spectrophotometric method described by Luquita et al (1994). *p*-Nitrophenol (800 μM) was used as a substrate for the assay. A membrane perturbant Triton–X 100 (0.05 mg/protein) and a β-glucuronidase inhibitor, D-saccharic acid lactone (0.5 mM) were systematically incorporated in the reaction medium. The UDP-glucuronosyl transferase activity towards 2-aminophenol was determined by the method described by Burchell (1974).

Table 1 Effect of a four-week pretreatment with tea solution (2% w/v) on liver weight and P450 content in rats.

Group	Liver weight (g)	P450 content (nmol (mg protein)$^{-1}$)
Control	12.2 ± 1.3	0.207 ± 0.04
Green tea extract (0.5%)	12.5 ± 0.9	0.281 ± 0.05
New Zealand green tea	13.1 ± 0.8	0.290 ± 0.04
Dragon green tea	12.8 ± 0.5	0.280 ± 0.10
Australian green tea	11.8 ± 1.1	0.307 ± 0.05
English Breakfast tea	12.6 ± 0.9	0.401 ± 0.03*
Gunpowder green tea	13.1 ± 1.2	0.403 ± 0.09*
Java green tea	12.4 ± 0.4	0.345 ± 0.07*

Results are given as mean ± s.d., n = 5 per group. *$P < 0.05$ compared with the control group.

Determination of glutathione-*S*-transferase activity

Hepatic cytosolic glutathione-*S*-transferase activity was determined using a spectrophotometric method (Habig et al 1974). This procedure was based on the enzyme catalysed condensation of glutathione with the model substrate 1-chloro-2,4-dinitrobenzene.

Blood lipid analysis

Plasma lipids were analysed for content of total cholesterol, triglycerides and high-density lipoproteins using a COBAS FARA machine (Roche Diagnostics, S-Hoffmann-La-Roche Ltd, Basle, Switzerland).

Statistical analysis

Results are expressed as mean ± s.d. Statistical analysis was performed using an SPSS (version 8.0) programme for Windows 95. The differences between groups were evaluated using one way analysis of variance, followed by Dunnette's test for pair-wise comparison and Tukey's family error rate. In all cases, $P < 0.05$ was considered as the minimum level of statistical significance.

Results

The growth rate of the rats was not affected by treatment with the different tea solutions (2% w/v). None of the animals showed any gross pathology of the internal organs. Liver weights of rats in the treatment groups were not significantly different from controls (Table 1). The hepatic P450 contents in all the treatment groups showed a trend to increase compared with the controls, but significant differences were observed in only the groups treated with English Breakfast tea, Gunpowder green tea or Java green tea (Table 1).

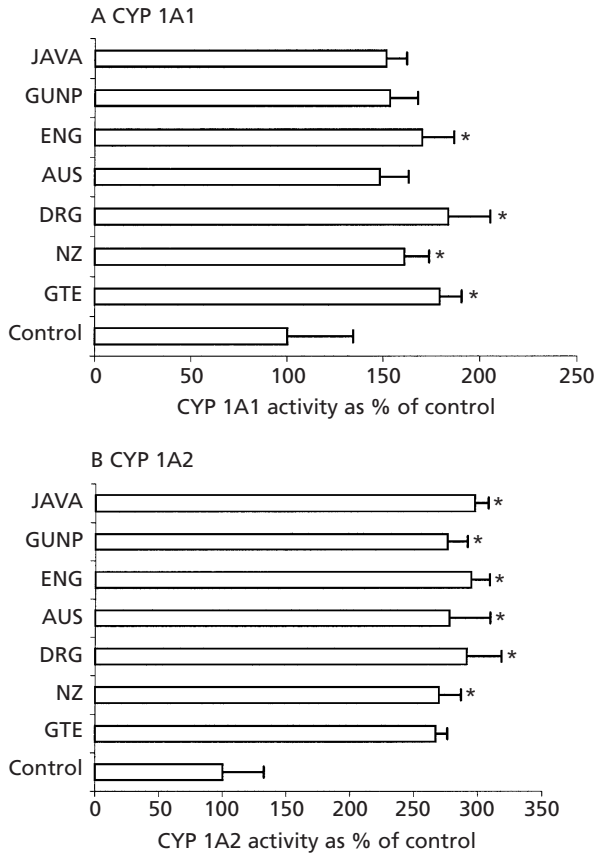

Figure 1 Effect of tea consumption on activities of hepatic CYP 1A1 (A) and CYP 1A2 (B) in rats. Rats were maintained on freshly prepared tea solutions (2% w/v) or green tea extract (0.5%, w/v) as their sole drinking fluid for four weeks. Results are presented as % of the control group's activity with an error bar. Mean ± s.d. CYP 1A1 and CYP 1A2 activity in the control group was 0.486 ± 0.17 and 0.084 ± 0.03 nmol mg^{-1} min^{-1}, respectively. GTE, Green tea extract (0.5%, w/v); NZ, New Zealand green tea; DRG, Dragon green tea; AUS, Australian green tea; ENG, English Breakfast tea; GUNP, Gunpowder green tea; JAVA, Java green tea. *$P < 0.05$ compared with control.

Hepatic CYP activity

An increase in activity of CYP 1A isoforms was detected in most of the groups treated with various tea solutions as well as green tea extract (0.5%). However, the in-

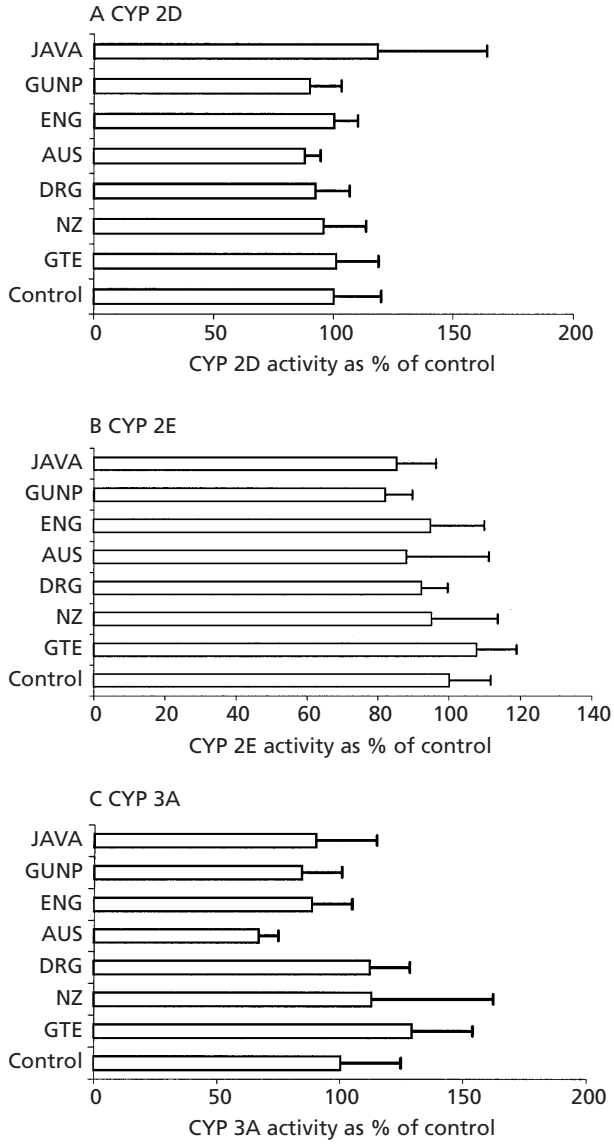

Figure 2 Activity of hepatic CYP 2D (A), CYP 2E (B) and CYP 3A (C) in rats treated with tea solutions (2%, w/v). Rats were maintained on freshly prepared tea solutions (2% w/v) or green tea extract (0.5%) as their sole drinking fluid for four weeks. Mean ± s.d. CYP 2D, CYP 2E and CYP 3A activity in the control group was 0.898 ± 0.18, 0.568 ± 0.7 and 0.121 ± 0.03 nmol mg^{-1} min^{-1}, respectively. GTE, Green tea extract (0.5%, w/v); NZ, New Zealand green tea; DRG, Dragon green tea; AUS, Australian green tea; ENG, English Breakfast tea; GUNP, Gunpowder green tea; JAVA, Java green tea.

crease was statistically significant only for the rats treated with New Zealand green tea, Dragon green tea, English Breakfast black tea or green tea extract 0.5% (Figure 1A). The maximum increase (183% of the control) in CYP 1A1 activity was seen in the group treated with Dragon (Chinese) green tea. Java green tea, Gunpowder green tea and Madura green tea produced moderate increases ranging from 120 to 160% of the control groups. Significant modulation of the hepatic CYP 1A2 isoform activities were quite evident in all the treatment groups (Figure 1B). The maximum increase (298% of the control) was observed with Java green tea. The effect on CYP 1A2 activity caused by treatment with any of the tea solutions was comparable with that seen in the group receiving green tea extract (0.5%).

Four-week pre-treatment with tea solutions caused a slight decrease in CYP 2E activity; however, these changes were not statistically significant (Figure 2B). CYP 2D and CYP 3A activity remained unaltered in almost all of the treatment groups (Figure 2A, C).

UDP-glucuronosyl transferase activity

Two planar phenol substrates, *p*-nitrophenol and 2-aminophenol, were selected to determine activity of the hepatic UDP-glucuronosyl transferase isozymes in rat liver microsomes. Pre-treatment with any of the tea solutions did not cause a significant difference in the activity of UDP-glucuronosyl transferase catalysing the conjugation of *p*-nitrophenol (Table 2). Java green tea produced a 25% increase in the UDP-glucuronosyl transferase activity compared with control, but this was not statistically significant. Similarly the catalysing activity of UDP-glucuronosyl transferase toward 2-aminophenol conjugation did not change significantly in any of the treatment groups (Table 2).

Glutathione-*S*-transferase activity

A significant increase in the activity of cytosolic glutathione-*S*-transferase was observed in the rats treated with New Zealand green tea, Gunpowder green tea and Java green tea (Table 2). There were no significant changes in the activity of glutathione-*S*-transferase in the other treatment groups.

Blood lipid profiles

After four-weeks pretreatment with tea solutions, the total plasma cholesterol content remained unaltered while the high-density lipoprotein fraction of plasma cholesterol showed an increasing trend (Table 3). There was a trend of decreasing triglyceride levels in rats treated with the various tea solutions as well as green tea

Table 2 Microsomal UDP-glucuronosyl transferase (UDPGT) and cytosolic glutathione-*S*-transferase activities in rats receiving different freshly-prepared tea solutions (2% w/v) or green tea extract (0.5% w/v) as their sole drinking fluid for four weeks.

Group	UDPGT activity towards *p*-nitrophenol (nmol min^{-1} mg^{-1})	UDPGT activity towards 2-aminophenol (nmol min^{-1} mg^{-1})	Glutathione-*S*-transferase activity (μmol min^{-1} mg^{-1})
Control	7.8 ± 3.1	0.51 ± 0.19	0.22 ± 0.05
Green tea extract (0.5%)	5.0 ± 1.1	0.53 ± 0.05	0.23 ± 0.03
New Zealand green tea	6.0 ± 1.9	0.58 ± 0.10	0.38 ± 0.03*
Dragon green tea	7.4 ± 3.1	0.63 ± 0.33	0.25 ± 0.03
Australian green tea	5.6 ± 1.7	0.40 ± 0.02	0.21 ± 0.01
English Breakfast tea	7.6 ± 1.6	0.54 ± 0.05	0.25 ± 0.05
Gunpowder green tea	6.7 ± 1.9	0.50 ± 0.06	0.30 ± 0.03*
Java green tea	10.4 ± 2.3	0.62 ± 0.09	0.36 ± 0.06*

Data are presented as mean ± s.d. of five individual rats. *Significantly different from the control group ($P < 0.05$).

Table 3 Plasma lipid content in rats receiving tea solutions (2% w/v) or green tea extract (0.5% w/v) as their sole drinking fluid for four weeks.

Group	Total cholesterol (mmol L^{-1})	High-density lipoproteins (mmol L^{-1})	Triglycerides (mmol L^{-1})
Control	1.82 ± 0.10	1.39 ± 0.22	1.57 ± 0.78
Green tea extract (0.5%)	2.30 ± 0.41	1.87 ± 0.29	1.77 ± 0.29
New Zealand green tea	1.83 ± 0.41	1.45 ± 0.29	1.33 ± 0.48
Dragon green tea	2.22 ± 0.36	1.85 ± 0.25	1.01 ± 0.16
Australian green tea	2.18 ± 0.24	1.83 ± 0.36	1.06 ± 0.18
English Breakfast tea	2.25 ± 0.34	1.97 ± 0.33	0.87 ± 0.24
Gunpowder green tea	1.88 ± 0.17	1.69 ± 0.18	1.06 ± 0.44
Java green tea	2.00 ± 0.45	1.65 ± 0.37	1.06 ± 0.28

Data are presented as mean ± s.d. of five individual rats.

extract (0.5%). However, none of these changes reached statistical significance (Table 3).

Discussion

Green tea is claimed to effectively antagonize the carcinogenicity of a number of dietary constituents including nitrosamines and polycyclic aromatic hydrocarbons (Bu-Abbas et al 1994b). The advantages of using green tea for cancer chemoprevention are that it is safe with no known harmful effects, it is cheap and it is easy to mass-produce. It has been shown to be effective in numerous experimental studies, and this effectiveness has been supported by epidemiological studies.

This study was part of an on-going research programme to investigate the anticarcinogenic properties of different teas and their constituents. Tea extracts and the major constituents of tea have been shown to possess anticarcinogenic potential. Therefore, it was important to establish whether this effect involved perturbation of the CYP isoform and phase II enzyme-mediated metabolism of chemical carcinogens in such a way so as to reduce bioactivation and/or favour their detoxification in-vivo in rats.

The results demonstrated that consumption of various fresh tea solutions as well as green tea extract 0.5% did not cause any apparent toxic response or growth retardation. Of the five major CYP isoforms studied, CYP 1A activity was shown to be increased in rats given tea

solutions, although some failed to produce statistically-significant differences. CYP 1A1 is primarily involved in the metabolism of polycyclic aromatic hydrocarbons, whereas CYP 1A2 preferentially metabolizes heterocyclic amines, arylamines and aflatoxin B1 (Gonzalez 1992; Eaton et al 1995; Williams 1995). These isoforms may play a very important role in activation of these environmental carcinogens. CYP 1A2 is normally expressed in liver. The level of CYP 1A1 expression in the liver is substantially lower than for CYP 1A2 and inducers of CYP 1A may have considerable potential for toxicity/carcinogenicity (Tassaneeyakul et al 1993).

The increase in rat hepatic CYP 1A activity suggested that some tea components induced activity of CYP 1A1/1A2 in rat liver. These results are consistent with some work reported by other investigators. Exposure to green tea resulted in an increase in *O*-demethylation of CYP 1A substrate methoxyresorufin and, to a lesser extent, deethylation of ethoxyresorufin and pentoxyresorufin (Bu-Abbas et al 1994a). Treatment of rats with green and black teas caused significant induction of CYP 1A2, 1A1, 2B, and 4A1 (Wang et al 1994; Chen et al 1996; Weisburger 1999b). Immunoblot analysis of solubilized microsomes using polyclonal antibodies against rat CYP 1A1/1A2 revealed an increase in the apoprotein level of CYP 1A2 but not CYP 1A1 following pretreatment with green tea (Bu-Abbas et al 1994b). Also microsomal preparations from rats pretreated with tea were much more effective than controls in converting the carcinogen, 2-amino 3-methyl imidazole quinoline to mutagenic intermediates in the Ames test (Bu-Abbas et al 1994a). Induction of phase I and phase II enzymes in livers of F344 rats receiving a 2% solution of green or black tea for six weeks was observed. Activity of CYP 1A1, CYP 1A2, CYP 2B1 and UDP-glucuronosyl transferase was increased significantly but glutathione transferase was not (Bu-Abbas et al 1994b; Sohn et al 1994; Katiyar & Mukhtar 1996b). Some in-vitro studies have shown contradictory results. For example, when green tea fractions were added to microsomes of Sprague-Dawley rats, inhibition of several enzymes such as ethoxyresorufin-*O*-deethlylase (CYP 1A), 7-ethoxycoumarin-*O*-deethylase (CYP 1A) and arylhydrocarbon hydroxylase (CYP 1A) was observed (Wang et al 1994). Decaffeinated green tea did not cause any induction of CYP enzymes (Chen et al 1996). Treatment with (−)-epigallocatechin gallate, the major and putative active component of green tea, inhibited CYP 1A and 2B1 (Sinha et al 1994).

In this study, green tea and black tea caused preferential induction of hepatic CYP 1A2 vs CYP 1A1, suggesting involvement of different mechanisms of induction. Pretreatment of rats with caffeine solution as the sole source of drinking fluid for 21 days caused a significant increase in the methoxyresorufin-*O*-deethylase (CYP 1A) activity similar to that seen with 2% tea solutions, while decaffeinated green tea did not (Chen et al 1996). Those authors suggested that previous reports of CYP 1A2 induction could be due to the effect of caffeine and not due to tea polyphenols. The Madura tea used in our study, confirmed as caffeine free, increased CYP 1A2 activity similar to that of green tea extract (0.5%) and other green teas. This suggested that the causative agent for increase in CYP 1A activity could be due to polyphenols.

Glutathione-*S*-transferase activity (GST) was significantly increased in rats receiving different green teas. Glutathione conjugation is probably the most effective means of detoxification of reactive intermediates (Wilkinson & Clapper 1997). It is noteworthy that pretreatment with the same teas did not cause any increase in activity of another carcinogen-detoxifying enzyme, glucuronosyl transferase. The possibility that other forms of glucuronosyl transferase, not monitored by the substrates used, have been modulated cannot be excluded. As the UDP-glucuronosyl transferases are a multi-gene family subject to differential regulation by inducers (Tephly & Burchell 1990), it is reasonable to infer that the effect of tea may reflect differential modulation of isoforms. This aspect of the results of this study is different from a few earlier reports where an increase in UDP-glucuronosyl transferase activity was observed but not GST (Bu-Abbas et al 1994b; Sohn et al 1994). However, there are previous reports of a significant increase in the activity of GST, glutathione peroxidase, catalase and quinone reductase after feeding mice with green tea or green tea polyphenols (Khan et al 1992; Katiyar et al 1993). It is possible that such differences in activity of phase II enzymes among those studies could be attributed to the variability in the content of the polyphenols and other flavonoids in the tea preparation from different geographical locations. It could also be attributed to the differences among species and strains of the experimental rodents in the susceptibility of their enzymes towards modulatory effect and capacity of tea constituents.

The results from rat plasma lipid analysis in this study were in agreement with previous reports showing the beneficial effects of tea on the plasma lipid profile. Tea polyphenols have been shown to have a hypocholesterolaemic effect in experimental animals by markedly lowering lymphatic cholesterol absorption and increasing faecal excretion of cholesterol and total lipids in cholesterol-fed rats (Muramatsu et al 1986). Tea

polyphenols are powerful antioxidants, and thus may play a role in the lowering of oxidation of low-density lipoprotein-cholesterol with a consequent decrease in risk of heart disease (Weisburger 1999b). However, further studies with controlled and high fat diet in a larger group of animals are required to investigate potential hypolipidaemic effects of tea and its constituents.

In conclusion, green tea may owe its anticarcinogenic potential to its ability to modulate the initiation stage of chemical carcinogenesis by affecting the enzyme systems that catalyse the activation and detoxification processes. It could be envisaged that the mutagenic and carcinogenic process, and the ultimate risk of developing a chemically-induced cancer, lie in the delicate balance between phase I carcinogen activating enzymes and phase II detoxifying enzymes. It is possible that teas grown in different geographical locations or harvested in different seasons may have a different pharmacological effect, which could probably be related to the polyphenol content of the leaves.

References

Boobis, A. R., Kahn, G. C., White, C., Hrodie, M. J., Davies, D. S. (1981) Biphasic *O*-deethylation of phenacetin and 7-ethoxycoumarin by human and rat liver microsome fractions. *Biochem. Pharmacol.* **30**: 2451–2456

Bu-Abbas, A., Clifford, M. N., Walker, R., Ioannides, C. (1994a) Selective induction of rat hepatic CYP1 and CYP4 proteins and of peroxisomal proliferation by green tea. *Carcinogenesis* **15**: 2575–2579

Bu-Abbas, A., Clifford, M. N., Walker, R., Ioannides, C. (1994b) Marked antimutagenic potential of aqueous green tea extracts. Mechanism of action. *Mutagenesis* **9**: 325–331

Burchell, B. (1974) Substrate specificity of UDP-glucuronyltransferase, purified to apparent homogeneity from phenobarbital treated rat liver. *Biochem. J.* **173**: 749–757

Bushman, J. L. (1998) Green tea and cancer in humans: a review of the literature. *Nutr. Cancer* **31**: 151–159

Chen, L., Bondoc, F., Lee, M., Hussin, A., Thomas, P., Yang, C. (1996) Caffeine induces cytochrome P4501A2: Induction of CYP1A2 by tea in rats. *Drug Metab. Dispos.* **24**: 529–533

Eaton, D. L., Gallagher, E. P., Bammler, T. K., Kunze, K. L. (1995) Role of cytochrome P4501A2 in chemical carcinogenesis: implications for human variability in expression and enzyme activity. *Pharmacogenetics* **5**: 259–274

Fujiki, H., Suganuma, M., Okabe, S., Komori, A., Sueoka, E., Sueoka, N., Kozu, T., Sakai, Y. (1996) Japanese green tea as a cancer preventive in humans. *Nutr. Rev.* **54**: S67–S70

Fujiki, H., Suganuma, M., Okabe, S., Sueoka, N., Komori, A., Sueoka, E., Kozu, T., Tada, Y., Suga, K., Imai, K., Nakachi, K. (1998) Cancer inhibition by green tea. *Mutat. Res.* **402**: 307–310

Fujiki, H., Suganuma, M., Okabe, S., Sueoka, E., Suga, K., Imai, K., Nakachi, K., Kimura, S. (1999) Mechanistic findings of green tea as cancer preventive for humans. *Proc. Soc. Exp. Biol. Med.* **220**: 225–228

Gonzalez, F. J. (1992) Human cytochromes P450: problems and prospects. *Trends Pharmacol. Sci. Rev.* **13**: 346–350

Guengerich, F. P. (1984) Effects of nutritive factors on metabolic processes involving bioactivation and detoxification of chemicals. *Annu. Rev. Nutr.* **4**: 207–231

Habig, W. H., Pabst, M. J., Jakoby, W. B. (1974) Glutathione-*S*-transferase, the first enzymatic step in mercapturic acid formation. *J. Biol. Chem.* **249**: 7130–7139

Han, L. K., Takaku, T., Li, J., Kimura, Y., Okuda, H. (1999) Anti-obesity action of oolong tea. *Int. J. Obes.* **23**: 98–105

Katiyar, S. K., Mukhtar, H. (1996a) Tea consumption and cancer. *World Rev. Nutr. Diet.* **79**: 154–184

Katiyar, S. K., Mukhtar, H. (1996b) Tea in chemoprevention of cancer. *Int. J. Oncol.* **8**: 221–238

Katiyar, S. K., Agarwal, R., Zaim, M. T., Mukhtar, H. (1993) Protection against N-nitrosodiethylamine and benzo(a)pyrene induced forestomach and lung tumorigenesis in A/J mice by green tea. *Carcinogenesis* **14**: 849–855

Khan, S. G, Katiyar, S. K, Agarwal, R., Mukhtar, H. (1992) Enhancement of antioxidant and phase II enzymes by oral feeding of green tea polyphenols in drinking water to SKH-1 hairless mice: possible role in cancer chemoprevention. *Cancer Res.* **52**: 4050–4052

Kohlmeier, L., Weterings, K., Steck, S., Boobis, A. (1989) The specificity of inhibition of debrisoquine 4-hydroxylase activity by quinidine and quinine in the rats is the inverse of that in man. *Biochem. Pharmacol.* **38**: 2795–2799

Li, N., Sun, Z., Han, C., Chen, J. (1999) The chemopreventive effects of tea on human oral precancerous mucosa lesions. *Proc. Soc. Exp. Biol. Med.* **220**: 218–224

Lowry, O., Rosebrough, N., Farr, A., Randall, R. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265–275

Luquita, M. G., Sanchez Pozzi, E. J., Cotania, V. A., Mottino, A. D. (1994) Analysis of p-nitrophenol glucuronidation in hepatic microsomes from lactating rats. *Biochem. Pharmacol.* **47**: 1179–1185

Muramatsu, K., Fukuyo, M., Hara, Y. (1986) Effect of green tea catechins on plasma cholesterol level in cholesterol-fed rats. *J. Nutr. Sci. Vitaminol.* **32**: 613–622

Omura, T., Sato, R., (1964) The carbon monoxide-binding pigment of liver microsomes. *J. Biol. Chem.* **239**: 2370–2379

Parke, D. V., Ioannides, C. (1981) The role of nutrition in toxicology. *Annu. Rev. Nutr.* **1**: 207–234

Robson, R. A., Matthews, A. P., Miners, J. O. (1987) Characterisation of theophylline metabolism by human liver microsomes. *Br. J. Clin. Pharmacol.* **24**: 293–300

Sinha, R., Rothman, N., Brown, E., Mark, S., Hoover, R., Caporaso, N., Levander, O., Knize, M., Lang, N., Kadlubar, F. (1994) Pan-fried meat containing high levels of heterocyclic aromatic amines but low levels of polycylic aromatic hydrocarbons induces cytochrome P4501A2 activity in humans. *Cancer Res.* **54**: 6154–6159

Smith, T., Hong, J., Wang, Z., Yang, C. (1995) How can carcinogenesis be inhibited? *Ann. N.Y. Acad. Sci.* **768**: 82–90

Sohn, O. S., Surace, A., Fiala, E. S., Richie, J. P., Colosimo, S., Zang, E., Weisburger, J. H. (1994) Effects of green and black tea on hepatic xenobiotic metabolising systems in the male F344 rat. *Xenobiotica* **24**: 119–127

Tassaneeyakul, W., Birkett, D. J., Veronese, M. E., Mcmanus, M. E., Tukey, R. H., Quattrochi, L. C., Gelboin, H. V., Miners, J. O.

(1993) Specificity of substrate and inhibitor probes for human cytochromes P4501A1 and 1A2. *J. Pharmacol. Exp. Ther.* **265**: 401–407

Tephly, T. R., Burchell, B. (1990) UDP-glucuronosyl transferases: a family of detoxifying enzymes. *Trends Pharmacol. Sci.* **11**: 276–279

Wang, Z. Y., Huang, M. T., Lou, Y. R., Xie, J. G., Rehul, K. R., Newmark, H. L., Ho, C. T., Yang, C. S., Coney, A. H. (1994) Inhibitory effects of black tea, green tea, decaffeinated tea and decaffeinated green tea on ultraviolet B induced skin carcinogenesis in 7,12 dimethyl benz(a) anthracenes-initiated SKH-1 mice. *Cancer Res.* **54**: 3428–3435

Wanwimolruk, S., Thou, M. R., Woods, D. J. (1995) Evidence for the polymorphic oxidation of debrisoquine and proguanil in a Khmer (Cambodian) population. *Br. J. Clin. Pharmacol.* **40**: 166–169

Wanwimolruk, S., Wong, S. M., Zhang, H., Coville, P. F. (1996) Simultaneous determination of quinine and a major metabolite 3-hydroxyquinine in biological fluid by HPLC without extraction. *J. Liq. Chromatogr.* **19**: 293–305

Weisburger, J. H. (1996) Tea antioxidants and health. In: Cadenas, E., Packer, L. (eds) *Handbook of Antioxidants*. Dekker, New York, pp 469–486

Weisburger, J. H. (1999a) Second international scientific symposium on tea and human health. *Proc. Soc. Exp. Biol. Med.* **220**: 193–194

Weisburger, J. H. (1999b) Tea and human health: the underlying mechanisms. *Proc. Soc. Exp. Biol. Med.* **220**: 271–276

Wilkinson, J., Clapper, M. L. (1997) Detoxification enzymes and chemoprevention. *Proc. Soc. Exp. Biol. Med.* **216**: 192–200

Williams, D. A. (1995) Drug Metabolism. In: Foye, W. O., Lemke, T. L., Williams, D. A. (eds) *Principles of Medicinal Chemistry*, 4th edn. Williams & Wilkins, Baltimore, pp 83–140

Yang, C. S, Wang, Z.-Y. (1993) Tea and cancer. *J. Natl. Cancer Inst.* **85**: 1038–1049