Anti-Cancer Drugs 1996, **7**, pp. 461–468

Inhibitory effect of six green tea catechins and caffeine on the growth of four selected human tumor cell lines

Susanne Valcic, Barbara N Timmermann, David S Alberts,[1] Gerald A Wächter, Mary Krutzsch,[1] Julie Wymer[1] and José M Guillén[1]

Department of Pharmacology and Toxicology, College of Pharmacy, The University of Arizona, Tucson, AZ 85721, USA. Tel: (+1) 520 626-2481; Fax: (+1) 520 626-4063. [1]Section Hematology and Oncology, Department of Medicine and the Arizona Cancer Center, College of Medicine, The University of Arizona, Tucson, AZ 85721, USA.

Green tea is an aqueous infusion of dried unfermented leaves of *Camellia sinensis* (family Theaceae) from which numerous biological activities have been reported including antimutagenic, antibacterial, hypocholesterolemic, antioxidant, antitumor and cancer preventive activities. From the aqueous-alcoholic extract of green tea leaves, six compounds (+)-gallocatechin (GC), (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin gallate (EGCG) and caffeine, were isolated and purified. Together with (+)-catechin, these compounds were tested against each of four human tumor cell lines (MCF-7 breast carcinoma, HT-29 colon carcinoma, A-427 lung carcinoma and UACC-375 melanoma). The three most potent green tea components against all four tumor cell lines were EGCG, GC and EGC. EGCG was the most potent of the seven green tea components against three out of the four cell lines (i.e. MCF-7 breast cancer, HT-29 colon cancer and UACC-375 melanoma). On the basis of these extensive *in vitro* studies, it would be of considerable interest to evaluate all three of these components in comparative preclinical *in vivo* animal tumor model systems before final decisions are made concerning which of these potential chemopreventive drugs should be taken into broad clinical trials.

Key words: *Camellia sinensis*, catechins, green tea components, growth inhibitory activity, human tumor cell lines, isolation.

Introduction

Green tea is an aqueous infusion of dried unfermented leaves of *Camellia sinensis* (family Theaceae) a plant native to Southeastern China and parts of India, Burma, Thailand, Laos and Vietnam. Its popularity as a beverage, particularly in the Far Eastern countries and in a few countries of North Africa and the Middle East, together with its reputation for health benefits prompted a large number of investigations of its chemical constituents and their biological activities. The tea leaves are distinguished by their content of methylxanthines, and polyphenols, especially flavanols of the catechin type. Although its chemical composition varies with growing conditions, season, age of the leaves and variety cultivated, the major green tea phenols (GTP) are (−)-epigallocatechin gallate (EGCG), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), (−)-epicatechin (EC), (+)-gallocatechin (GC) and (+)-catechin (Figure 1), which, altogether, may constitute up to 30% of the dry leaf weight.[1]

Numerous biological activities have been reported for green tea. The aqueous infusion itself and its polyphenolic constituents are known for their antimutagenic,[2–8] antibacterial,[9–12] hypocholesterolemic,[13] antioxidant,[14–17] antitumor[18–21] and, above all, cancer preventive activities.[22–30]

While the results of epidemiologic studies regarding the association between different types of cancer and tea consumption are very complex and sometimes even contradictory,[31] the cancer preventive effect of green tea or its components has been shown by *in vitro*[23,25,27] and *in vivo* experiments.[22,24,26–30] The majority of these studies report the effects of crude green tea extracts, the green tea phenolic fraction (GTP) or its major constituent EGCG. There are some studies, however, that report on various activities of EGCG as well as other catechin derivatives isolated from the GTP fraction.[2,6,10,16,19,21,32–35] In most of these studies EGCG was found solely responsible for biological activity or the most active compound among those catechins tested. In addition to these observations, other studies suggest that the activity of the cate-

This project was supported by grants CA-27502 and CA-23074 from the National Institutes of Health, Bethesda, MD 20892.

Correspondence to BN Timmermann

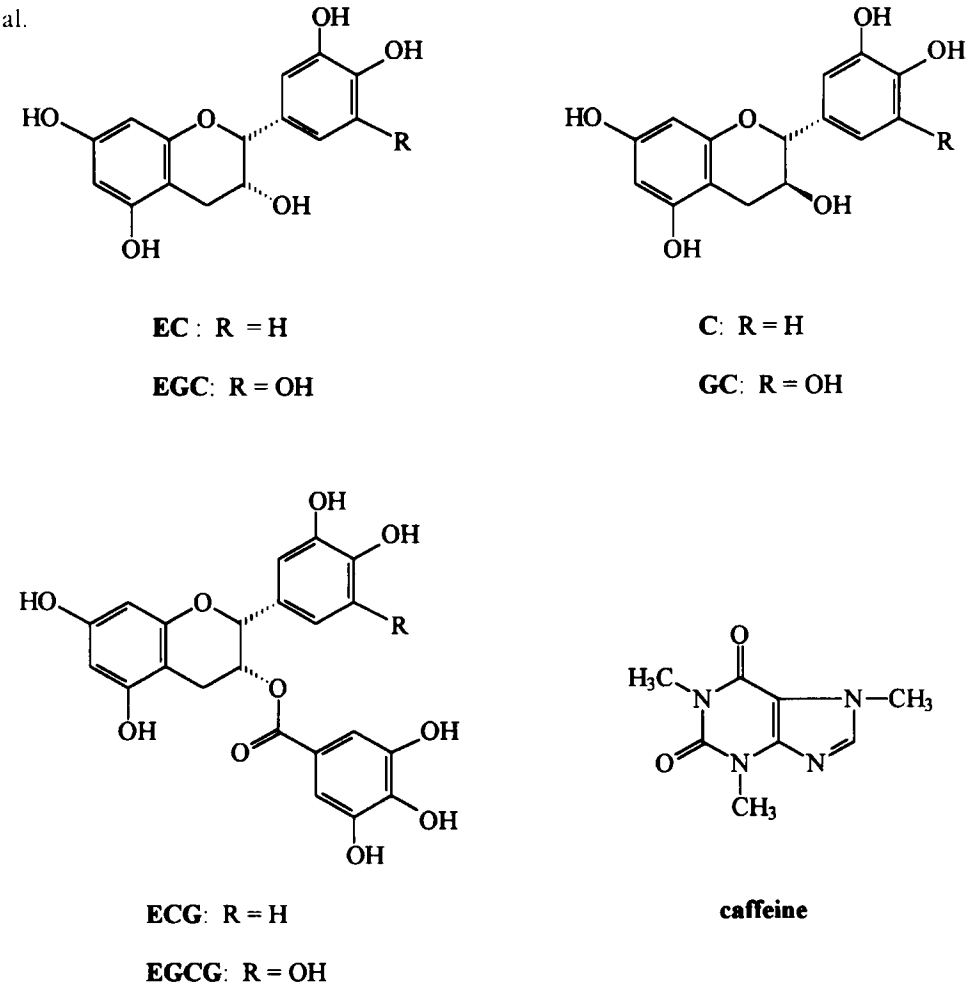

Figure 1. Chemical structures of the seven green tea components, catechin, EC, GC, EGC, ECG, EGCG and caffeine.

chins depends on the nature of the biological targets. For example, a triphenolic B-ring of polyphenols from green tea seems essential for the growth inhibition of E1A-3Y1 cells, rat 3Y1 diploid fibroblasts transformed by the E1A gene of human adenovirus type 12.[21] In another study, it was reported that the presence of a galloyl group in the 3-*O*-position in flavan-3-ols increased their potency in inhibiting lipid peroxidation.[35] Based on these results, we think that it is important to further evaluate the biological activity not only of pure EGCG, but the activities of all the major constituents present in green tea.

In this study, we isolated and purified EGCG, EGC, ECG, EC, GC and caffeine from green tea leaves. These compounds together with (+)-catechin were tested and their individual activities were compared for the first time for their relative growth inhibitory effects in an *in vitro* screening assay using four different human cancer cell lines as part of a program to search for chemopreventive agents from natural dietary sources.

Materials and methods

Plant material

The plant material was provided by the Royal Estates Tea Company, Division of Thomas J Lipton (Englewood Cliffs, NJ). The green tea blend was labeled 'Green Research Standard'.

Extraction and isolation

Dried, green tea leaves (1 kg) were ground to a fine powder and macerated four times with ethanol (95%):water 1:1. The extracts were filtered, combined and dried at room temperature. After redis-

solution in a small amount of water, the crude extract was freeze-dried yielding 307 g of a green powder. This crude extract was dissolved in water and the aqueous phase was exhaustively extracted with ethyl acetate. After evaporation *in vacuo* 141 g crude green tea polyphenols (GTP) remained. A portion of GTP (65 g) was chromatographed on a Sephadex LH 20 (Pharmacia, Uppsala, Sweden) column (78 × 10 cm) with ethanol (95%) yielding five different semipurified catechin derivatives and caffeine.

Purification of EC, EGC, GC, ECG and EGCG

EC and EGC were purified separately by column chromatography (75 × 5.5 cm) using silica gel (silica gel 60, 0.05–0.2 mm; Macherey-Nagel, Düren, Germany) and dichloromethane:ethyl acetate:acetic acid (40:60:1). ECG and GC were purified separately by column chromatography using silica gel and dichloromethane:acetone:acetic acid (60:40:1). The obtained EC, GC, EGC and ECG were dissolved in a small amount of ethyl acetate, precipitated with dichloromethane and filtered through a fritted funnel. Semipurified EGCG was stirred with water overnight at room temperature and filtered through a fritted funnel. The powder was washed repeatedly with water to obtain pure EGCG.

Catechin

(+)-Catechin was purchased as (+)-catechin hydrate 98% from Aldrich (Milwaukee, WI).

Detection and identification

The catechin derivatives were detected by TLC on silica gel plates (Polygram Sil G/UV_{254}; Macherey-Nagel with dichloromethane:acetone:acetic acid (60:40:1) using UV light (254 nm) and vanillin/sulfuric acid spray for visualization. All compounds were monitored for purity ($\geq$99%) by HPLC (Alltech Model 425 HPLC pump, model 450 UV detector) using a VYDAC 218TP54 (C-18) (250 × 4.6 mm, 5 μ) column and acetonitrile:ethyl acetate:0.05% phosphoric acid (12:2:86). The structural identities of all compounds were determined by spectral data including $^{1}H/^{13}C$-NMR (300 MHz) and MS.

Cell culture technique

The human lung carcinoma cell line A-427,[36] the human colon carcinoma cell line HT-29[37] and the human breast carcinoma cell line MCF-7[38] were obtained from ATCC (Rockville, MD; ATCC numbers HTB 53, HTB 38 and HTB 22 respectively) and previously have been characterized. The human melanoma cell line UACC-375 was obtained from Dr A Leibovitz (Arizona Cancer Center). Cells were maintained in monolayer culture in RPMI 1640 media supplemented with 5% fetal bovine serum (Gemini Bioproducts, Calabasas, CA), 2 mM L-glutamine, 50 IU/ml penicillin and 50 μg/ml streptomycin in a humidified atmosphere of 95% air and 5% CO_2 at 37°C. Cell lines were monitored for the absence of mycoplasmas using the Mycoplasma Detection Kit (Boehringer Mannheim, Indianapolis, IN). Subculturing was done at subconfluent densities. The cells were dispersed with a phosphate buffered saline solution of 0.05% trypsin and 0.02% ethylenediamine tetraacetic acid.

Measurement of cell proliferation

Cell growth was determined using the sulforhodamine B (SRB) colorimetric protein stain assay. Optimum cell number per well was determined for each cell line. Cells were plated in non-perimeter wells of 96-well microtiter plates and incubated for 24 h prior to addition of drugs. All drugs were solubilized in 100% dimethyl sulfoxide (DMSO). The drugs were then diluted with media so that the maximum amount of DMSO did not exceed 0.5% within the wells. Each drug was tested over a concentration range of six 10-fold dilutions. Smaller serial dilutions were tested subsequently to further clarify a drug concentration which achieved a growth inhibition of 50% (IC_{50}). The media controls, the DMSO controls and each of the drug concentrations were tested in a minimum of six wells per experiment. After an additional 6 days of culture, cells were fixed by addition of cold trichloroacetic acid at a final concentration of 10%. The plates were kept at 4°C for 1 h, the supernatant was then aspirated and the plates washed with deionized water. SRB (Aldrich) solution was prepared to 0.4% w/v in 1% acetic acid. SRB (50 μl) was added to each well and the cells were stained for 10 min at room temperature. Unbound SRB was removed by washing with 1% acetic acid followed by air drying. Bound stain was solubilized with 50 mM unbuffered tris and optical

density (OD) measured by an automated spectrophotometer at a single wavelength of 540 nm.

Determination of IC_{50} values

For each drug, a minimum of three experiments measuring the growth inhibition of all the cell lines was conducted. For these experiments, the mean ± SD of OD data from the replicated wells was calculated for each drug concentration tested. The inhibitory effect of the drug at each concentration was expressed as a percentage [(mean OD of treated cells/mean OD of control cells) × 100]. The IC_{50}, the drug concentration causing a 50% reduction in the mean OD value relative to the control, was then estimated by interpolation from the inhibitory effects measured at each concentration tested.

Statistical methods

Several methods were used to fit the tumor cell growth inhibition–drug concentration data for each of the individual experiments. The majority of the time, a logistic model was the best fit selected (Curve fitting 1.1 for Windows, Central, SC). After the coefficients of the fitting function were obtained, the concentration causing a 50% reduction in the absorbance relative to the control wells was estimated using the equation: percent inhibition = (absorbance of treated cells/absorbance of control cells) × 100.

The resulting IC_{50}s from each experiment were then compared in an analysis of variance for differences among the seven green tea components for their growth inhibitory activities against each of the four human tumor cell lines. The statistical significance between all pairs of components was assessed using Tukey's multiple comparisons test.[39]

Results

From the aqueous-alcoholic extract of green tea leaves, five polyphenolic compounds (i.e. GC, EC, EGC, ECG and EGCG) and caffeine were isolated and purified. For this study, (+)-catechin, which also occurs in green tea, was purchased. The seven green tea components were used in 106 individual experiments against each of the four human tumor cell lines (MCF-7 breast carcinoma, HT-29 colon carcinoma, A-427 lung carcinoma and UACC-375 melanoma). The number of experiments performed with each green tea component against each of the four human tumor cell lines is shown in Table 1. Shown in Table 2 and in Figure 2 are the mean IC_{50}s and corresponding standard deviations for the seven green tea components against the human

Table 1. Number of experiments by green tea components against human breast, colon, lung and melanoma cell lines

Component	Breast	Colon	Lung	Melanoma
Caffeine	4	4	4	4
Catechin	4	4	5	4
EC	4	4	4	4
ECG	4	4	2	4
EGC	4	4	3	4
EGCG	4	4	4	4
GC	3	3	3	3

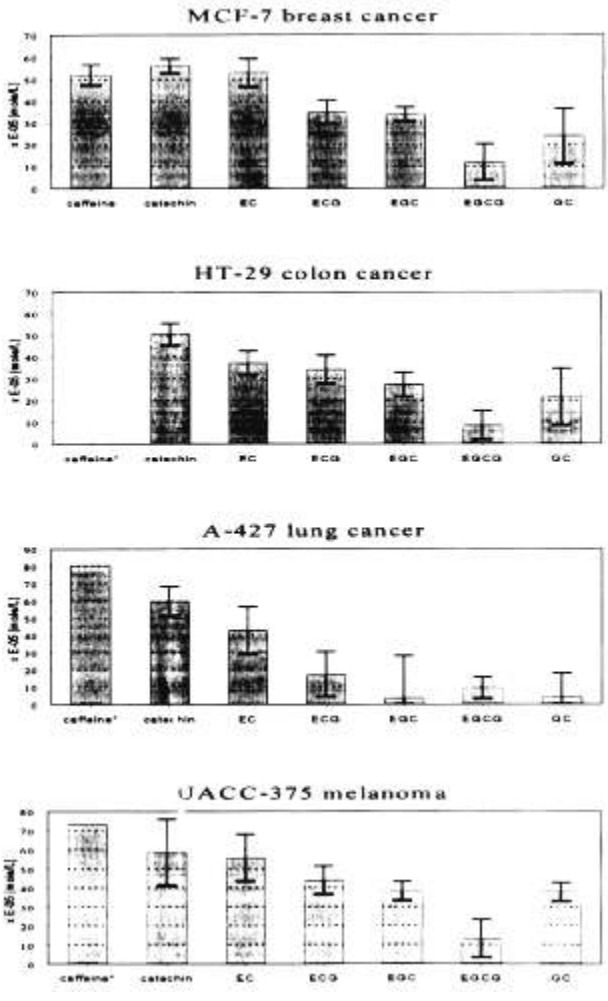

Figure 2. Mean IC_{50} concentrations and SD for seven green tea components against four human tumor cell lines (MCF-7 breast, HT-29 colon, A-427 lung and UACC-375 melanoma). *IC_{50} value and/or SD not available.

Table 2. Mean IC_{50}s and SD for the seven green tea components against human breast, colon, lung and melanoma cell lines

	Breast			Colon			Lung			Melanoma		
Component	*N*	mean	SD	*N*	mean	SD	*N*	mean	SD	*N*	mean	SD
Caffeine	3	5.2E − 04	4.70E − 05		–[a]	–[a]	1	8.04E − 04	–[a]	1	7.34E − 04	–[a]
Catechin	3	5.6E − 04	3.30E − 05	3	5.04E − 04	5.20E − 05	4	5.98E − 04	8.60E − 05	3	5.85E − 04	1.75E − 04
EC	3	5.3E − 04	6.30E − 05	3	3.72E − 04	5.40E − 05	3	4.28E − 04	1.39E − 04	3	5.57E − 04	1.24E − 04
ECG	4	3.5E − 04	5.60E − 05	4	3.41E − 04	6.60E − 05	2	1.72E − 04	1.32E − 04	4	4.38E − 04	7.60E − 05
EGC	4	3.4E − 04	3.40E − 05	4	2.73E − 04	5.50E − 05	3	3.41E − 05	2.48E − 04	4	3.81E − 04	4.90E − 05
EGCG	4	1.2E − 04	8.40E − 05	4	8.64E − 05	6.70E − 05	4	9.47E − 05	6.50E − 05	4	1.33E − 04	1.00E − 04
GC	3	2.4E − 04	1.27E − 04	3	2.15E − 04	1.28E − 04	3	3.83E − 05	1.41E − 04	3	3.78E − 04	4.80E − 05

[a] IC_{50} value and/or SD not available.

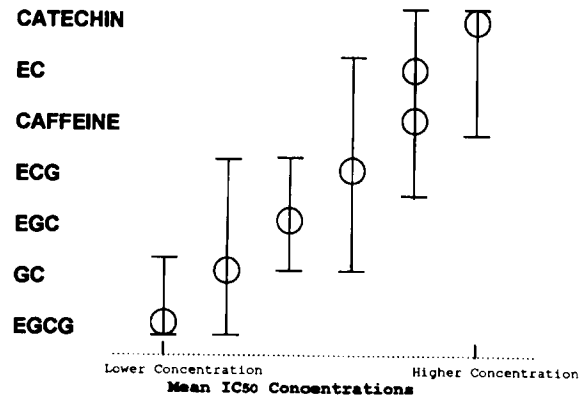

Figure 3. Relative potency of seven green tea components in the inhibition of MCF-7 human breast cancer cells *in vitro* using Tukey's multiple comparisons test. Vertical lines, groups of green tea components which are not significantly different from the circled component on the basis of their relative IC_{50} values.

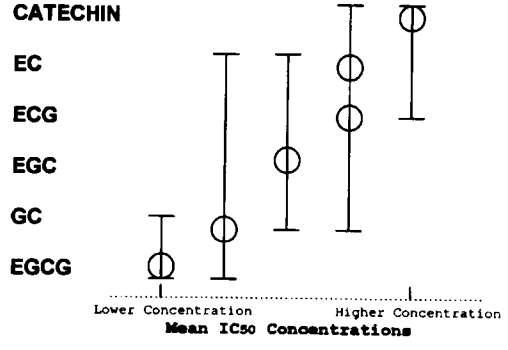

Figure 4. Relative potency of seven green tea components in the inhibition of HT-29 human colon cancer cells *in vitro* using Tukey's multiple comparisons test. Vertical lines, groups of green tea components which are not significantly different from the circled component on the basis of their relative IC_{50} values.

breast, colon, lung and melanoma cell lines. The IC_{50}s were determined as described in Materials and methods. The lower the IC_{50} value, the more potent is the green tea component as an inhibitor of tumor cell growth.

Shown in Figures 3–6 are the relative potencies of each of the seven green tea components, with respect to inhibition of cell growth of the MCF-7 breast cancer, HT-29 colon cancer, A-427 lung cancer and UACC-375 melanoma cell lines, respectively. Against all four tumor cell lines, the three most potent green tea components were EGCG, GC and EGC. EGCG was the most potent of the seven green tea components against three out of the four cell lines (i.e. MCF-7 breast cancer, HT-29 colon cancer and UACC-375 melanoma).

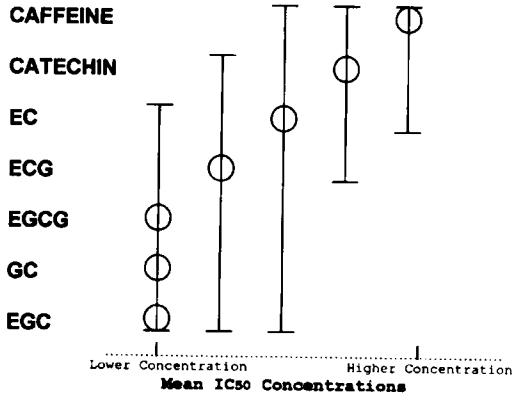

Figure 5. Relative potency of seven green tea components in the inhibition of A-427 lung cancer cells *in vitro* using Tukey's multiple comparisons test. Vertical lines, groups of green tea components which are not significantly different from the circled component on the basis of their relative IC_{50} values.

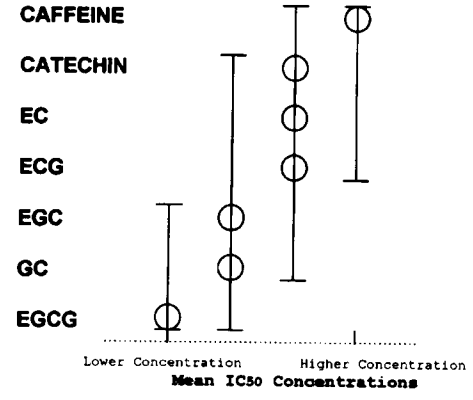

Figure 6. Relative potency of seven green tea components in the inhibition of UACC-375 melanoma cells *in vitro* using Tukey's multiple comparisons test. Vertical lines, groups of green tea components which are not significantly different from the circled component on the basis of their relative IC_{50} values.

Discussion

Whole green tea extract and its polyphenolic components have attracted considerable attention during the 1990s for their potential cancer preventive activities.[22–30] Because of the increasing interest to develop at least one of its polyphenolic components as a chemopreventive agent, we performed an initial comparison of the potential growth inhibitory activities of pure green tea components against human tumor cells. Although EGCG is the most prevalent of these polyphenolic compounds, we believed that others may be more potent and/or pharmaceutically attractive for drug development.

The data from the present *in vitro* studies of the growth inhibitory activities of seven pure green tea components against four different human tumor cell lines suggest that three of the verified polyphenolic compounds are considerably more potent than the others, i.e. EGCG, GC and EGC. On the basis of these extensive *in vitro* studies, it would be of considerable interest to evaluate all three of these components in comparative preclinical *in vivo* animal tumor model systems, before final decisions are made concerning which of these potential chemopreventive drugs should be taken into broad clinical trials.

Acknowledgments

We thank Mr JH Wertheim, Tea Importers, Inc., Westport, CT, and the Royal Estates Tea Company, Division of Thomas J Lipton, Co., Englewood Cliffs, NJ, for providing biomass of green tea leaves, and Dr Yong-Long Liu, Mr Ming Zhao and Mr Mark Shafer for assistance in the isolation of pure catechins.

References

1. Graham HN. Green tea composition, consumption, and polyphenol chemistry. *Prev Med* 1992; **21**: 334–50.
2. Kada T, Kaneko K, Matsuzaki S, Matsuzaki T, Hara Y. Detection and chemical identification of natural bio-antimutagens. A case of the green tea factor. *Mutat Res* 1985; **150**: 127–32.
3. Mukhtar H, Wang ZY, Katiyar SK, Agarwal R. Tea components: antimutagenic and anticarcinogenic effects. *Prev Med* 1992; **21**: 351–60.
4. Hayatsu H, Inada N, Kakutani T, Arimoto S, Negishi T, Mori K, Okuda T, Sakata I. Suppression of genotoxicity of carcinogens by (−)-epigallocatechin gallate. *Prev Med* 1992; **21**: 370–6.
5. Bu-Abbas A, Clifford MN, Walker R, Ioannides C. Marked antimutagenic potential of aqueous green tea extracts: mechanism of action. *Mutagenesis* 1994; **9**: 325–31.
6. Horikawa K, Mohri T, Tanaka Y, Tokiwa H. Moderate inhibition of mutagenicity and carcinogenicity of benzo[*a*]pyrene, 1,6-dinitropyrene and 3,9-dinitrofluoranthene by Chinese medicinal herbs. *Mutagenesis* 1994; **9**: 523–6.
7. Yamada J, Tomita Y. Antimutagenic activity of water extracts of black tea and oolong tea. *Biosci Biotech Biochem* 1994; **58**: 2197–200.
8. Apostolides Z, Weisburger JH. Screening of tea clones for inhibition of PhIP mutagenicity. *Mutat Res* 1995; **326**: 219–25.
9. Horiba N, Maekawa Y, Ito M, Matsumoto T, Nakamura H. A pilot study of Japanese green tea as a medicament: antibacterial and bactericidal effects. *J Endodont* 1991; **17**: 122–4.
10. Ikigai H, Nakae T, Hara Y, Shimamura T. Bactericidal catechins damage the lipid bilayer. *Biochim Biophys Acta* 1993; **1147**: 132–6.
11. Shetty M, Subbannayya K, Shivananda PG. Antibacterial activity of tea (*Camellia sinensis*) and coffee (*Coffea arabica*) with special reference to *Salmonella typhimurium*. *J Commun Dis* 1994; **26**: 147–50.
12. Kono K, Tatara I, Takeda S, Arakawa K, Hara Y. Antibacterial activity of epigallocatechin gallate against methicillin-resistant *Staphylococcus aureus*. *Kansenshogaku Zasshi* 1994; **68**: 1518–22.
13. Muramatsu K, Fukuyo M, Hara Y. Effect of green tea catechins on plasma cholesterol level in cholesterol-fed rats. *J Nutr Sci Vitaminol* 1986; **32**: 613–22.
14. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis* 1989; **10**: 1003–8.
15. Xie B, Shi H, Chen Q, Ho CT. Antioxidant properties of fractions and polyphenol constituents from green, oolong and black teas. *Proc Natl Sci Council (Repub China) Part B Life Sci* 1993; **17**: 77–84.
16. Katiyar SK, Agarwal R, Mukhtar H. Inhibition of spontaneous and photo-enhanced lipid peroxidation in mouse epidermal microsomes by epicatechin derivatives from green tea. *Cancer Lett* 1994; **79**: 61–6.
17. Yen GC, Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J Agric Food Chem.* 1995; **43**: 27–32.
18. Zhen Y, Cao S, Xue Y, Wu S. Green tea extract inhibits nucleoside transport and potentiates the antitumor effect of antimetabolites. *Chin Med Sci J* 1991; **6**: 1–5.
19. Kashiwada Y, Nonaka GI, Nishioka I, Chang JJ, Lee KH. Antitumor agents, 129. Tannins and related compounds as selective cytotoxic agents. *J Nat Prod* 1992; **55**: 1033–43.
20. Wang ZY, Huang MT, Ho CT, *et al.* Inhibitory effect of green tea on the growth of established skin papillomas in mice. *Cancer Res.* 1992; **52**: 6657–65.
21. Mitsui T, Yamada K, Yamashita K, *et al.* E1A-3Y1 cell-specific toxicity of tea polyphenols and their killing mechanism. *Int J Oncol* 1995; **6**: 377–83.
22. Xu Y, Ho CT, Amin SG, Han C, Chung FL. Inhibition of tobacco-specific nitrosamine-induced lung tumorigenesis in A/J mice by green tea and its major polyphenol as antioxidants. *Cancer Res* 1992; **52**: 3875–9.
23. Klaunig JE. Chemopreventive effects of green tea components on hepatic carcinogenesis. *Prev Med* 1992; **21**: 510–9.
24. Narisawa T, Fukaura Y. A very low dose of green tea polyphenols in drinking water prevents *N*-methyl-*N*-nitrosourea-induced colon carcinogenesis in F344 rats. *Jpn J Cancer Res* 1993; **84**: 1007–9.
25. Fujiki H, Suganuma M, Komori A, *et al.* A new tumor promotion pathway and its inhibitors. *Cancer Detect Prev* 1994; **18**: 1–7.
26. Mukhtar H, Katiyar SK, Agarwal R. Green tea and skin—anticarcinogenic effects. *J Invest Dermatol* 1994; **102**: 3–7.
27. Nishida H, Omori M, Fukutomi Y, *et al.* Inhibitory effects of (−)-epigallocatechin gallate on spontaneous hepatoma in C3H/HeNCrj mice and human hepatoma-derived PLC/PRF/5 cells. *Jpn J Cancer Res* 1994; **85**: 221–5.
28. Wang ZY, Huang MT, Lou YR, *et al.* Inhibitory effects of

black tea, green tea, decaffeinated black tea, and decaffeinated green tea on ultraviolet B light-induced skin carcinogenesis in 7,12-dimethylbenz[*a*]anthracene-initiated SKH-1 mice. *Cancer Res.* 1994; **54**: 3428–35.
29. Hirose M, Akagi K, Hasegawa R, *et al.* Chemoprevention of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)-induced mammary gland carcinogenesis by antioxidants in F344 female rats. *Carcinogenesis* 1995; **16**: 217–21.
30. Yamane T, Takahashi T, Kuwata K, *et al.* Inhibition of *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine-induced carcinogenesis by (−)-epigallocatechin gallate in the rat glandular stomach. *Cancer Res* 1995; **55**: 2081–4.
31. Yang CS, Wang ZY. Tea and cancer. *J Natl Cancer Inst* 1993; **85**: 1038–49.
32. Agarwal R, Katiyar SK, Zaidi SI, Mukhtar H. Inhibition of skin tumor promoter-caused induction of epidermal ornithine decarboxylase in SENCAR mice by polyphenolic fraction isolated from green tea and its individual epicatechin derivatives. *Cancer Res* 1992; **52**: 3582–8.
33. Makimura M, Hirasawa M, Kobayashi K, *et al.* Inhibitory effect of tea catechins on collagenase activity. *J Periodontol* 1993; **64**: 630–6.
34. Huang MT, Ho CT, Wang ZY, *et al.* Inhibitory effect of topical application of a green tea polyphenol fraction on tumor initiation and promotion in mouse skin. *Carcinogenesis* 1992; **13**: 947–54.
35. Hong CY, Wang CP, Lo YC, Hsu FL. Effect of flavan-3-ol tannins purified from *Camellia sinensis* on lipid peroxidation of rat heart mitochondria. *Am J Chin Med* 1994; **22**: 285–92.
36. Giard DJ, Aaronson SA, Todaro GJ, *et al. In vitro* cultivation of human tumors: establishment of cell lines derived from a series of solid tumors. *J Natl Cancer Inst* 1973; **51**: 1417–23.
37. Fogh J, Trempe G. New human tumor cell lines. In: Fogh J, ed. *Human tumor cells in vitro.* New York: Plenum 1975: 115–9.
38. Soule HD, Vazquez J, Long A, Albert S, Brennan M. A human cell line from a pleural effusion derived from a breast carcinoma. *J Natl Cancer Inst* 1973; **51**: 1409–16.
39. Morrison DF. *Applied linear statistical methods.* Englewood Cliffs, NJ: Prentice-Hall 1983: 312–4.

(Received 27 February 1996; accepted 12 March 1996)