Biochemical and Biophysical Research Communications **288,** 101–105 (2001)
doi:10.1006/bbrc.2001.5736, available online at http://www.idealibrary.com on IDEAL®

Green Tea Constituent (−)-Epigallocatechin-3-gallate Inhibits Topoisomerase I Activity in Human Colon Carcinoma Cells

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Received September 10, 2001

DNA topoisomerases I and II are essential for cell survival and play critical roles in DNA metabolism and structure. Inhibitors of topoisomerase constitute a novel family of antitumor agents with demonstrated clinical activity in human malignancies. The clinical use of these agents is limited due to severe toxic effects on normal cells. Therefore, there is a need to develop novel, nontoxic topoisomerase inhibitors that have the ability to spare normal cells. Recent studies have shown that green tea and its major polyphenolic constituent, epigallocatechin-3-gallate (EGCG), impart growth inhibitory responses to cancer cells but not to normal cells. Based on the knowledge that EGCG induces DNA damage, cell cycle arrest, and apoptosis, we considered the possibility of the involvement of topoisomerase in the antiproliferative response of EGCG. Here, for the first time, we show that EGCG inhibits topoisomerase I, but not topoisomerase II in several human colon carcinoma cell lines. Based on this study it is tempting to suggest that combination of EGCG with other conventional topoisomerase inhibitors could be an improved strategy for treatment of colon cancer. The possible role of EGCG as a chemotherapeutic agent needs to be investigated. © 2001 Academic Press

Key Words: **topoisomerase; green tea; epigallocatechin-3-gallate; apoptosis; colon cancer.**

DNA topoisomerases (topo) I and II are ubiquitous and essential enzymes involved in multiple transactions involving DNA including DNA replication, transcription, chromosome condensation, and probably DNA recombination (1, 2). Topoisomerases are of two main types, causing transient breaks in one (type I) or both (type II) strands of DNA (1, 2). The importance of topo-mediated DNA cleavage in tumor cell death has been recognized as an effective molecular target for many antitumor drugs (3, 4). Both topoisomerases are important targets for cancer chemotherapeutic drugs. A large number of topo-directed agents are known which are currently in clinical use, however their utility is limited due to the fact that they induce severe toxic side effects such as myelosuppression, nausea, hair loss, congestive heart failure, and, in some instances, increases in the risk of secondary malignancies (5, 6). Therefore, novel nontoxic topo inhibitors that have the ability to spare normal cells from their cytotoxic effects are required. Such agents could be used in combination with other topo inhibitors.

Green tea, especially its major polyphenolic constituent, epigallocatechin-3-gallate (EGCG), has received much attention over the past few years as a potential cancer chemopreventive agent (7, 8). Recent data amassed from several laboratories is suggesting that EGCG may also possess cancer therapeutic effects (9–16). Studies from our laboratory have shown that EGCG, at physiologically attainable concentrations, exerts growth inhibitory effects on several human carcinoma cell lines, without affecting normal cell lines, resulting in a dose-dependent inhibition of cell growth, G0/G1 phase arrest of the cell cycle, and DNA damage leading to induction of apoptosis (17, 18). Subsequent studies from several other laboratories have reported similar findings in many other cancer cell types (19, 20). Further, studies from this and other laboratories have also shown that both topo I and II activities are higher in colon tumors and in various colon carcinoma cell lines compared to normal tissues (21). Based on these observations and many clinical studies, inhibitors of these enzymes are effective agents against colon cancer. Since EGCG has been shown to cause DNA

Abbreviations used: topo, topoisomerase; EGCG, epigallocatechin-3-gallate; TPT, topotecan; VEGF, vascular endothelial growth factor; NF-κB, nuclear factor-kappa B; STAT-1, signal transducers and activators of transcription-1; NOS, nitric oxide synthase; K DNA: Kinetoplast DNA.

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damage and apoptosis in cancer cells, we considered the possibility that EGCG may target topoisomerase enzymes. In the present study, we show that EGCG inhibits topo I but not topo II in several human colon carcinoma cell lines, suggesting the possible role of green tea, especially EGCG, in therapy of colon cancer.

MATERIALS AND METHODS

Materials. A purified preparation of EGCG (>98% pure) was kindly provided by Dr. Yukihiko Hara of Mitsui Norin Co., Ltd. (Shizuoka, Japan). Topotecan (TPT) was generously supplied by the Drug Synthesis and Chemistry Branch of the National Cancer Institute (Bethesda, MD). The human colon carcinoma cell lines HCT 116 and SW 480 were purchased from American Type Culture Collection (Manassas, VA). The VACO 241 cell line was provided by Dr. James K. V. Willson, Case Western Reserve University.

Cell culture and treatment. The human colon carcinoma cells HCT 116 and VACO 241 were grown in MEM (Gibco-BRL, Gaithersburg, MD) supplemented with 0.1 mM nonessential amino acids, 2.2 g/L sodium bicarbonate, 50 μg/ml gentamicin, 2-mM L-glutamine, 25 mM Hepes buffer and 8% heat-inactivated bovine calf serum (Hyclone). The SW 480 cells were grown in low glucose D-MEM (GibcoBRL) supplemented with sodium pyruvate, 3.7 g/L sodium bicarbonate, 25 mM Hepes buffer and 10% fetal bovine serum as described previously (22), in presence or absence of EGCG and/or TPT. EGCG and TPT were dissolved at 10 mM concentration in PBS and dimethyl sulfoxide (DMSO), respectively. The cells (30–40% confluent) were treated with desired concentrations of EGCG or TPT for 24 h in complete cell medium, whereas cells treated only with PBS or DMSO served as control.

Colony formation sensitivity assay. The sensitivity of human colon carcinoma HCT 116, SW 480, and VACO 241 cells was measured by colony formation sensitivity assay. Briefly, exponentially growing cells were dissociated into single-cell suspension using trypsin-EDTA (Gibco-BRL), counted, and plated into 60-mm tissue culture dish, placed in a 37°C and 5% CO_2 incubator for 16 h. The plates were then treated with desired concentration of EGCG or TPT for 24 h. At the end of this treatment, the plates were rinsed and replenished with fresh medium, and returned to the incubator. After 8–10 days the colonies were fixed and stained with formaldehyde-methylene blue solution. Colonies containing 50 cells or more were scored as positive and the surviving fraction for each drug concentration was calculated as the number of colonies for each concentration divided by the mean number of colonies for the control. For each drug concentration the IC_{50} and the IC_{10} values were calculated as the concentration of drug required to reduce the number of colonies to 50 or 10% of that of control, respectively.

Preparation of cell lysate. Cell extracts were prepared from 5×10^6 to 1×10^7 cells in log phase growth that were removed from suspension or attached cultures in tissue culture medium and centrifuged at 250*g* for 10 min at 4°C. The medium was removed and the pellets were washed once with PBS and were immediately homogenized at 4°C in 50 to 100 μl of extraction buffer containing 150 mM NaCl, 1 mM KH_2PO_4, 5 mM $MgCl_2$, 1 mM EGTA, 10% glycerol, 0.5 mM DTT, 1 mM phenylmethylsulfonyl fluoride, 2 μg/ml aprotinin at pH 6.4 followed by addition of 2 M NaCl to bring the final concentration of NaCl to 0.5 M. The mixture was incubated on ice for at least 30 min with frequent mixing and centrifuged at 10,000*g* at 4°C for 15 min. The supernatant was transferred to another microfuge tube and glycerol was added to a final concentration of 20%. Extracts were frozen at −80°C in small aliquots for topoisomerase assay. Protein was determined as previously described (21).

Topoisomerase assay. Topoisomerase I activity was determined as ATP-independent relaxation of supercoiled ϕX174RF DNA (Gibco BRL) with slight modification (23). Briefly, the reaction was carried out in 20 μl of reaction mixture at 37°C for 30 min. The final concentrations of the components in the reaction mixture were 10 mM Tris HCl pH 7.4, 0.2 mM EDTA, 0.5 mM DTT, 0.2 M NaCl, 6 μg/ml BSA 0.6 μg ϕX174RF DNA and various concentrations of cell extracts, usually containing 0.1 to 1.0 μg protein. The reaction was stopped by the addition of 10% SDS containing bromophenol blue, 0.42% xylene cyanole and 50% glycerol. The entire volume was quantitatively transferred and the DNA products analyzed on 1% agarose horizontal slab gels run in 45 mM Tris–borate buffer, pH 7.9, 1 mM EDTA for 18 h at 1 V/cm at room temperature. The gels were stained with ethidium bromide and photographed under short-wave UV light. Densitometric profiles of the negatives were quantitated using a SCI scan 5000densitometer (USB Chemicals, Cleveland, OH). One unit of topoisomerase I is defined as the amount of enzyme necessary to relax 50% of the 0.6 μg input supercoiled DNA in 30 min at 37°C.

Topoisomerase II activity was assayed as ATP-dependent decatenation of supercoiled kinetoplast DNA (K-DNA) (TopoGEN, Columbus, OH) in 20 μl reaction mixture containing 50 mM Tris–HCl pH 8.0, 85 mM KCl, 25 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM DTT, 30 μg/ml BSA, 1 mM ATP, 0.4 mg k-DNA with varying concentration of cell lysate usually containing 0.1 to 1.0 μg protein. The reactions were conducted at 30°C for 30 min and stopped as indicated above for topoisomerase I assay. Agarose gel electrophoresis and densitometric profiles were carried out as in topoisomerase I assay. One unit of topoisomerase II activity is defined as the amount of enzyme necessary to decatenate 50% of the 0.4 μg input K-DNA substrate in 30 min at 37°C.

TABLE 1

Activity of Topoisomerase I and II in Several Human Colon Carcinoma Cells

Cell line	Topoisomerase I (10^3 U/mg protein)	Topoisomerase II (10^3 U/mg protein)
HCT 116	4.6 ± 0.34	1.5 ± 0.08
VACO 241	4.7 ± 0.38	3.6 ± 0.24
SW 480	3.5 ± 0.26	2.0 ± 0.16

Note. Activity is expressed in 10^3 U/mg protein. Each value is the average of at least three independent experiments, each done in duplicate ± SE.

RESULTS AND DISCUSSION

In recent years, several lines of evidence on the anticancer activities of green tea polyphenols have emerged from cell culture systems and animal models (7, 8). Human epidemiological studies, though inconclusive, suggest that green tea consumption is associated with reduction in cancer risk in some but not all body sites (24). Many of these biological effects of green tea are mediated by its major polyphenolic constituent, EGCG (17, 18). Recent studies have shown that EGCG is a potent inducer of apoptosis (17) and inhibitor of NF-κB activation (18). EGCG has been shown to inhibit urokinase (10), gelatinase (11) and inhibits angiogenesis through blocking the induction of VEGF (12, 13), which are indispensable for tumor invasion and metastasis (12, 13). EGCG has also been shown to

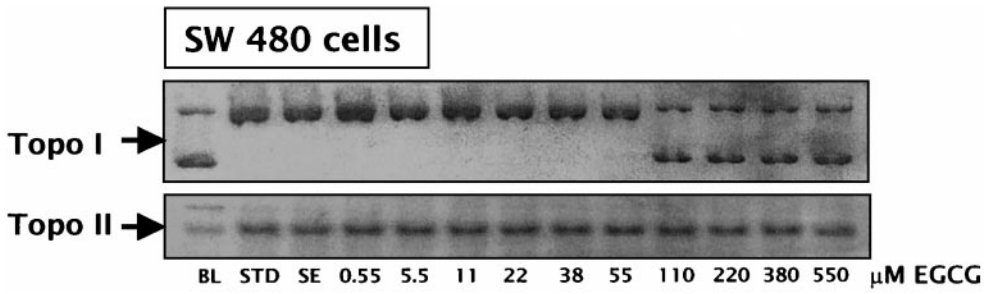

FIG. 1. Effect of EGCG on topoisomerase activity in SW480 human colon carcinoma cells. A significant inhibition of topo I activity was observed with EGCG treatment at concentrations between 110 and 550 μM. EGCG had no effect on topo II activity. BL, blank; STD, standard enzyme (complete reaction); SE, sample enzyme (complete reaction).

inhibit matrix metalloproteases that are essential for tumor cells for the degradation of extracellular matrix components (14). As a mechanism of action, EGCG has been shown to block the induction of nitric oxide synthase (NOS) II expression in lipopolysaccharide (LPS)-activated mouse macrophage (15) and STAT1 expression in interferon-γ activated human carcinoma cells (16). Recently, EGCG has been shown to inhibit the chymotrypsin-like activity of the proteasome, a cancer-related molecular target (25). Further, the presence of non toxic concentrations of EGCG to U937 monoblastoid leukemia cells and HT29 colon adenocarcinoma cells, results in life span limitations accompanied with telomere shortening and chromosomal abnormalities, leading to expression of senescence in these cell lines (26). Studies from our laboratory and elsewhere have shown that EGCG exerts growth inhibitory effects in several human carcinoma cells that result in DNA damage, cell cycle arrest and apoptosis (17–20). Since topoisomerase is known to be involved in the repair of DNA damage, we considered the possible involvement of topoisomerase in the ECGC induced events.

We examined the effect of EGCG on the activities of topo I and II in several established human colon carcinoma cells. As shown in Table 1, the catalytic activity of topo I in HCT 116, VACO 241, and SW 480 colon carcinoma cells is shown as 4.6, 4.7, and 3.5 $\times$ 10^3 U/mg protein while the topo II activity in these cell lines was found to be 1.5, 3.6 and 2.0 $\times$ 10^3 U/mg protein, respectively. These cell lines were used for further studies.

In the next series of experiments, we determined the effect of EGCG on SW 480 cells. As shown in Fig. 1, EGCG treatment of this cell line resulted in a significant inhibition of topo I activity at 110 μM to 550 μM concentrations. In sharp contrast, no inhibition in topo II activity was observed with EGCG treatment to SW 480 cells (Fig. 1). To further assess the growth inhibitory effect of EGCG, several human colon carcinoma cells *viz.* SW 480, HCT 116 and VACO 241, were used for the study. The growth inhibitory effect of EGCG toward different human colon carcinoma cells was compared (Fig. 2). EGCG treatment to HCT 116 cells was 9-fold more sensitive than SW 480 cells as indicated by the IC_{50} values of these cells. The IC_{50} value for HCT 116 was found to be 10 μM, while that of VACO 241 was 18 μM and SW 480 was 88 μM, respectively. The growth inhibitory effects of EGCG were further compared with topotecan (TPT), a well-known topo I inhibitor in these cell lines. As shown by the data in Table 2, the growth inhibitory concentration of EGCG was found to be much higher than TPT for all the cell lines studied. The ratio of the IC_{50} values of EGCG and TPT for HCT 116, VACO 241 and SW 480 cells was found to be 833, 2250 and 3667, respectively. SW 480 cells proved to be the most resistant to either drug.

Presently, all clinically useful topo I inhibitors act at the same step in the catalytic cycle of the enzyme, where they prevent religation of covalent DNA-topo reaction intermediates, thereby increasing the cellular

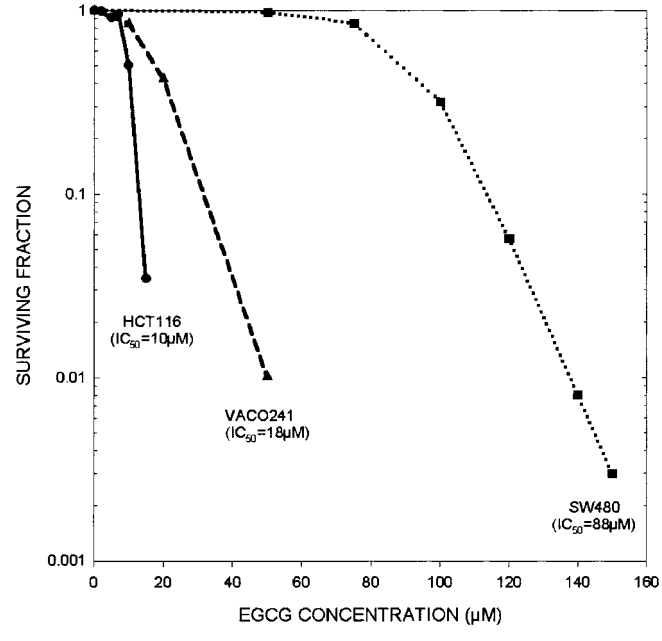

FIG. 2. Effect of EGCG on the surviving fraction in HCT 116, VACO 241, and SW 480 colon carcinoma cells. The cells were treated with various concentrations of EGCG for 24 h to obtain the survival curve.

TABLE 2

Inhibition of Growth Pattern of Several Human Colon Carcinoma Cells in Presence of EGCG and Topotecan (TPT)

Cells	Topoisomerase I (10^3 U/mg protein)	EGCG[a]		TPT[a]		EGCG/TPT
		IC_{50}	IC_{10}	IC_{50}	IC_{10}	IC_{50}
HCT 116	4.55 ± 0.34	10.0 ± 1.2	12.9 ± 1.4	0.012 ± 0.0001	0.026 ± 0.0001	833
VACO 241	4.65 ± 0.38	18.0 ± 2.0	31.4 ± 3.4	0.008 ± 0.0001	0.017 ± 0.0001	2250
SW 480	3.51 ± 0.30	88 ± 7.8	113.6 ± 10.5	0.024 ± 0.0002	0.046 ± 0.0003	3667

[a] Concentration (μM) inhibiting the growth of 50 and 10% EGCG and TPT treated cells compared with untreated controls. Each value is the average of at least three independent experiments, each done in duplicate ± SE.

levels of these complexes. Therefore, in the next series of experiments we compared the inhibition of topo I activity by the use of several known topo I inhibitors and compared them with the effects of EGCG (Table 3). Treatment of cells including HCT 116, VACO 241 and SW 480 with TPT, SN-38 and other topo I inhibitors caused a significant inhibition of topo I activity. TPT and CPT inhibited topo I activity in the concentration range of 3.8–9.6 μM while SN-38 inhibited topo I activity in the concentration range of 2.8–3.2 μM. EGCG was found to be effective in inhibiting topo I activity in concentrations ranging from 8.6 to 16.6 μM.

Inhibitors of topo I and II have been used as a class of anticancer drugs in various types of human malignancies (27, 28). Numerous topo inhibitors are currently being evaluated or used for the treatment of various human malignancies (27–29). Camptothecin and its derivatives, TPT and CPT-11 are inhibitors of topo I which are in current use for treatment of colon cancer (29). TPT has demonstrated significant anticancer activity in various tumors such as colorectal, ovarian, breast and other types of human cancers, however the clinical use of topo-inhibitors is limited due to severe side effects (3, 22, 30). Clinically used topo II inhibitors are VP-16, Adriamycin, doxorubicin, and m-AMSA, etc. (4, 31). Most of these commonly used inhibitors of topo I and II cause severe side effects such as myelosuppression, nausea, hair loss, congestive heart failure and even increase the risk of secondary malignancies leading to early death (27–31). Recent studies combining tea components with the topo II inhibitor, doxorubicin have shown a reduction in cytotoxicity and an increase in chemotherapeutic index in doxorubicin-resistant P388 leukemia cell bearing mice (32). These results lead to the suggestion that natural non-toxic topo inhibitors could be developed as chemotherapeutic agents. Our results demonstrate the possible use of EGCG as a chemotherapeutic agent in inhibiting topo I activity and suggest that it should be studied in combination with anticancer drugs toward eventual use in patients with colon cancer. The ability of EGCG to inhibit cancer cell growth, induce DNA damage and apoptosis both *in vitro* and *in vivo* suggests that EGCG, in combination with other drugs, may have possible chemotherapeutic potential for the treatment of colon cancer.

TABLE 3

Inhibition of Topoisomerase I Activity in Several Human Colon Carcinoma Cells by Various Topo Inhibitors

Compound	Topoisomerase I inhibition[a]		
	HCT 116	VACO 241	SW 480
9AC	4.25 ± 0.34	4.75 ± 0.38	3.25 ± 0.22
CPT	4.75 ± 0.36	4.95 ± 0.32	3.75 ± 0.24
CPT-11	675 ± 58.8	715 ± 62.5	645 ± 52.4
SN-38	3.25 ± 0.30	3.15 ± 0.24	2.85 ± 0.14
TPT	5.55 ± 0.44	5.25 ± 0.44	9.65 ± 0.78
EGCG	8.55 ± 0.28	10.75 ± 0.34	16.55 ± 0.44

[a] Inhibition is expressed as the concentration of compounds (μM) necessary to inhibit 50% of the topoisomerase activity. Each value is the average of at least three independent experiments, each done in duplicate ± SE.

ACKNOWLEDGMENTS

This research was supported by U.S. Public Health Service Grants P30 CA 43703 (H.M.), RO1 CA 78809 (H.M.), and K01 CA 79087 (C.A.B.) from the National Cancer Institute, DHHS.

REFERENCES

1. Li, T. K., and Liu, L. F. (2001) Tumor cell death induced by topoisomerase-targeting drugs. *Annu. Rev. Pharmacol. Toxicol.* **41,** 53–77.
2. Wang, J. C. (1996) DNA topoisomerases. *Annu. Rev. Biochem.* **65,** 635–692.
3. Takimoto, C. H., Wright, J., and Arbuck, S. G. (1998) Clinical applications of the camptothecins. *Biochim. Biophys. Acta* **1400,** 107–119.
4. Hande, K. R. (1998) Clinical applications of anticancer drugs targeted to topoisomerase II. *Biochim. Biophys. Acta* **1400,** 173–184.
5. Chen, A. Y., and Liu, L. F. (1994) DNA topoisomerases: Essential enzymes and lethal targets. *Annu. Rev. Pharmacol. Toxicol.* **34,** 191–218.
6. Anderson, R. D., and Berger, N. A. (1994) International Com-

mission for Protection against Environmental Mutagens and Carcinogens. Mutagenicity and carcinogenicity of topoisomerase-interactive agents. *Mutat. Res.* **309,** 109–142.

7. Mukhtar, H., and Ahmad, N. (1999) Green tea in chemoprevention of cancer. *Toxicol. Sci.* **52,** 111–117.
8. Isemura, M., Saeki, K., Kimura, T., Hayakawa, S., Minami, T., and Sazuka, M. (2000) Tea catechins and related polyphenols as anti-cancer agents. *Biofactors* **13,** 81–85.
9. Lin, J. K., and Liang, Y. C. (2000) Cancer chemoprevention by tea polyphenols. *Proc. Natl. Sci. Counc. Repub. China B* **24,** 1–13.
10. Jankun, J., Selman, S. H., Swiercz, R., and Skrzypczak-Jankun E. (1997) Why drinking green tea could prevent cancer. *Nature* **387,** 561.
11. Garbisa, S., Sartor, L., Biggin, S., Salvato, B., Benelli, R., and Albini, A. Tumor gelatinases and invasion inhibited by the green tea flavanol epigallocatechin-3-gallate. *Cancer* **91,** 822–832.
12. Cao, Y., and Cao, R. (1999) Angiogenesis inhibited by drinking tea. *Nature* **398,** 381.
13. Jung, Y. D., Kim, M. S., Shin, B. A., Chay, K. O., Ahn, B. W., Liu, W., Bucana, C. D., Gallick, G. E., and Ellis, L. M. (2001) EGCG, a major component of green tea, inhibits tumour growth by inhibiting VEGF induction in human colon carcinoma cells. *Br. J. Cancer* **84,** 844–850.
14. Demeule, M., Brossard,M., Page, M., Gingras, D., and Beliveau, R. (2000) Matrix metalloproteinase inhibition by green tea catechins. *Biochim. Biophys. Acta* **1478,** 51–60.
15. Lin, Y. L., and Lin, J. K. (1997) (−)-Epigallocatechin-3-gallate blocks the induction of nitric oxide synthase by down-regulating lipopolysaccharide-induced activity of transcription factor nuclear factor-kappa B. *Mol. Pharmacol.* **52,** 465–472.
16. Menegazzi, M., Tedeschi, E., Dussin, D., Carcereri, De Prati A., Cavalieri, E., Mariotto, S., and Suzuki, H. (2001) Anti-interferon-γ action of epigallocatechin-3-gallate mediated by specific inhibition of STAT1 activation. *FASEB J.* **15,** 1309–1311.
17. Ahmad, N., Feyes, D. K., Nieminen, A. L., Agarwal, R., and Mukhtar, H. (1997) Green tea constituent epigallocatechin-3-gallate and induction of apoptosis and cell cycle arrest in human carcinoma cells. *J. Natl. Cancer Inst.* **89,** 1881–1886.
18. Ahmad, N., Gupta, S., and Mukhtar, H. (2000) Green tea polyphenol epigallocatechin-3-gallate differentially modulates nuclear factor kappa B in cancer cells versus normal cells. *Arch. Biochem. Biophys.* **376,** 338–346.
19. Yang, G. Y., Liao, J., Kim, K., Yurkow, E. J., and Yang, C. S. (1998) Inhibition of growth and induction of apoptosis in human cancer cell lines by tea polyphenols. *Carcinogenesis* **19,** 611–616.
20. Hibasami, H., Komiya, T., Achiwa, Y., Ohnishi, K., Kojima, T., Nakanishi, K., Akashi, K., and Hara, Y. (1998) Induction of apoptosis in human stomach cancer cells by green tea catechins. *Oncol. Rep.* **5,** 527–529.
21. Berger, S. J., Tsai, M-L., Chatterjee, S., Markowitz, S. D., Willson, J. K. V., and Berger, N. A. (1993) Transcriptional down regulation of topoisomerase II relative to topoisomerase I in normal and malignant human colon tissues, implications for therapy of colon cancer. *Proc. Am. Assoc. Cancer Res.* **34,** 328.
22. Belfi, C. A., Chatterjee, S., Gosky, D. M., Berger, S. J., and Berger, N. A. (1999) Increased sensitivity of human colon cancer cells to DNA cross-linking agents after GRP78 up-regulation. *Biochem. Biophys. Res. Commun.* **257,** 361–368.
23. Duguet, M., Lavenot, C., Harper, F., Mirambeau, F., and De Recondo, A. M. (1983) DNA topoisomerases from rat liver: Physiological variation. *Nucleic Acids Res.* **11,** 1059–1075.
24. Yang, C. S. (1999) Tea and health. *Nutrition* **15,** 946–949.
25. Nam, S., Smith, D. M., and Dou, Q. P. (2001) Ester bond-containing tea polyphenols potently inhibit proteasome activity *in vitro* and *in vivo*. *J. Biol. Chem.* **276,** 13322–13330.
26. Naasani, I., Seimiya, H., and Tsuruo, T. (1998) Telomerase inhibition, telomere shortening, and senescence of cancer cells by tea catechins. *Biochem. Biophys. Res. Commun.* **249,** 391–396.
27. Li, T. K., and Liu, L. F. (2001) Tumor cell death induced by topoisomerase-targeting drugs. *Annu. Rev Pharmacol. Toxicol.* **41,** 53–77.
28. Sikic, B. I. (1999) New approaches in cancer treatment. *Ann. Oncol.* **10,** 149–153.
29. Saijo, N. (2000) Preclinical and clinical trials of topoisomerase inhibitors. *Ann. NY Acad. Sci.* **922,** 92–99.
30. Bailly, C. (2000) Topoisomerase I poisons and suppressors as anticancer drugs. *Curr. Med. Chem.* **7,** 39–58.
31. Capranico, G., Giaccone, G., and D'Incalci, M. (1999) DNA topoisomerase II poisons and inhibitors. *Cancer Chemother. Biol. Response Modif.* **18,** 125–143.
32. Sadzuka, Y., Sugiyama, T., and Sonobe, T. (2000) Efficacies of tea components on doxorubicin induced antitumor activity and reversal of multidrug resistance. *Toxicol. Lett.* **114,** 155–162.