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RESEARCH NOTE

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# Effects of Tea Constituents on Intracellular Level of the Major Tea Catechin, (-)-Epigallocatechin-3-gallate

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**Abstract** (-)-Epigallocatechin-3-gallate (EGCG), a major tea catechin, has been shown to have many interesting biological activities. In the present study, we studied the effects of green tea catechins, EGCG metabolites, and black tea theaflavins on accumulation of EGCG in HT-29 human colon cells. Intracellular levels of [$^3$H]-EGCG were not changed significantly in the presence of other tea catechins including (-)-epicatechin, (-)-epigallocatechin, and (-)-epicatechin-3-gallate. EGCG methyl metabolites and EGCG 4"-glucuronide did not affect cellular levels of [$^3$H]-EGCG. Black tea theaflavins and theasinensin A (TsA), an EGCG oxidative dimer, however, significantly decreased cellular accumulation of EGCG in HT-29 cells by 31-56%. This decrease was more pronounced when cells were incubated in the presence of theaflavin-3',3"-digallate (TFdiG) or TsA. When EGCG was added separately from TFdiG or TsA, the accumulation of EGCG in HT-29 cells was also significantly decreased regardless of when TFdiG or TsA was added during the uptake study ($p$<0.01). The results suggest that theaflavins and TsA may interrupt EGCG absorption through the gastrointestinal epithelium.

**Keywords:** green tea, epigallocatechin-3-gallate, theaflavin, theasinensin A, cellular uptake

## Introduction

Consumption of tea (*Camellia sinensis*) has been suggested to have a number of beneficial health effects (1). Tea consists of many potential health effective compounds including catechins, non-catechin polyphenols, caffeine, amino acids, and vitamins (2). Among these constituents, catechins are believed to account for the most effective health beneficial effects of tea, including its anti-carcinogenic effects (3). Epigallocatechin-3-gallate (EGCG) is the most abundant catechin found in green tea and is believed to be the most biologically active compound among tea constituents (4).

Studies with cell lines have demonstrated many anti-carcinogenic mechanisms of action for EGCG, including inhibition of AP-1 activation, signals of epidermal growth factor and insulin-like growth factor, inhibition of topoisomerase, telomerase, and proteasome activity, and modulation of arachidonic acid metabolism (5-11). Since studies in mice, rats, and humans have shown that the plasma concentration of EGCG is low (12, 13), it is not clear whether these effects of EGCG observed in cells using concentrations far exceed those in body are still achievable *in vivo*.

The role of several factors affecting cellular levels of EGCG and EGCG bioavailability has been investigated. Intracellular EGCG is transported out of cells by the action of the multidrug resistance-associated proteins (MRP), the ATP-dependent efflux transporters (14). EGCG is a substrate for phase II enzymes including UDP-glucuronosyltransferase, sulfotransferase, and catechol-*O*-methyltransferase, and is converted to EGCG glucuronide, sulfur, or methyl-conjugates, respectively, which are easily excreted forms (15, 16).

Certain dietary factors also affect cellular EGCG level and bioavailability of EGCG (14, 17). Previously, we reported that curcuminoids and isoflavones increased cellular level of EGCG (14). It was also observed that piperine affected EGCG metabolism in HT-29 cells and increased bioavailability of EGCG in mice (17). Since our normal diet consists of many different constituents, there are likely to be many potential interactions that affect cellular uptake and bioavailability of physiologically active dietary compounds including EGCG. However, there is limited information available on these interactions and consequences. In the present study, we investigated possible interactions of EGCG with other tea constituents and EGCG metabolites in regulating cellular level of EGCG. Our results indicate that black tea theaflavins and theasinensin A (TsA), an EGCG oxidative metabolite, could serve as a potential dietary modulators in regulating cellular levels of EGCG.

## Materials and Methods

**Chemicals and cell lines** [$^3$H]-EGCG (13 Ci/mmol) was synthesized as described previously (18) and was generously provided by Dr. Yukihiko Hara of Mitsui Norin Co., Ltd. (Fujieda City, Japan). Unlabeled EGCG and other green tea catechins were a generous gift from Unilever Best Foods (Englewood Cliffs, NJ, USA). EGCG 4"-glucuronide, 4"-methyl EGCG, and 4',4"-dimethyl EGCG were enzymatically and chemically synthesized, respectively, in our laboratory (16, 19). Black tea polyphenols including theaflavin, theaflavin-3'-gallate, and theaflavin-3',3"-digallate (TFdiG), and TsA were generously provided by Dr. Chi-Tang Ho (Rutgers University, NJ, USA). The structures of EGCG, its metabolites, thea-

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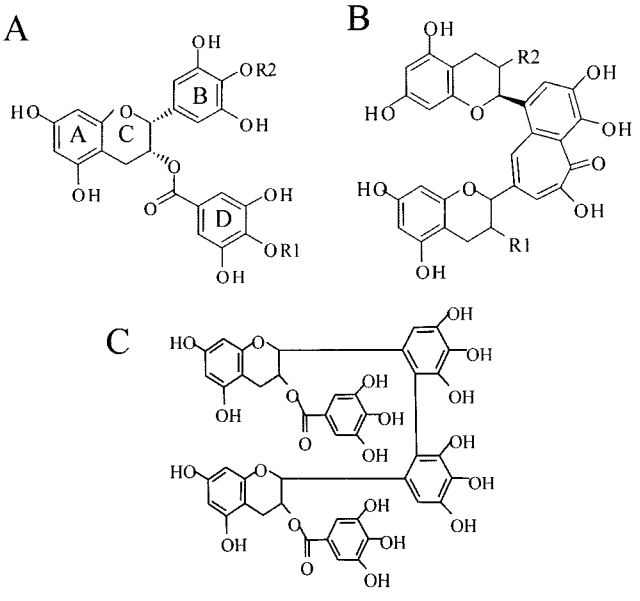
flavins, and TsA in the current study were shown in Fig. 1. HT-29 human colon cancer cell line was obtained from American Type Culture Collection (Rockville, MD, USA). The cells were maintained in McCoy's 5A medium supplemented with 10% fetal bovine serum, 100 unit/mL penicillin, and 0.1 mg/mL streptomycin, at 37°C in 95% humidity and 5% $CO_2$. All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

**Analysis of cellular uptake of [$^3$H]-EGCG by HT-29 cells** HT-29 cells were plated into a 24 well culture plate. When cells reached approximately 90% confluence, the cells were preincubated with Hank's balanced salt solution (HBSS) for 2 hr. The cells were then incubated with [$^3$H]-EGCG (10 μM, 0.5 μCi/mL) in 400 μL HBSS containing 100 μM ascorbic acid with various constituents or vehicle (0.05% DMSO). After 2 hr incubation, the cells were washed three times with ice-cold phosphate buffered-saline. The attached cells were lysed by adding 200 μL of 0.2N NaOH and the well was washed with 300 μL of distilled water twice, which were combined as total cell lysates. The radioactivity from total cell associated EGCG was analyzed by a scintillation counter (Model LS3801; Beckman Coulter Inc., Fullerton, CA, USA).

To analyze changes in cellular accumulation of EGCG in response to the different temporal exposure to TFdiG or TsA, [$^3$H]-EGCG (10 μM, 0.5 μCi/mL), or TFdiG or TsA (10 μM) alone was first incubated with cells in HBSS containing 100 μM ascorbic acid. After 30 min incubation, the first media were removed and replaced with media containing TFdiG or TsA, or [$^3$H]-EGCG (10 μM, 0.5 μCi/mL). After 30 min, cells were prepared for analysis as described above.

**Data analysis** Statistical significance was evaluated using the Student's *t*-test. One-way analysis of variance and the Tukey's honestly significant difference (HSD) test were also used for comparing the effects of test compounds.




| A. EGCG and its metabolites | R1 | R2 |
|---|---|---|
| EGCG | H | H |
| 4"-MeEGCG | $CH_3$ | H |
| 4'4"-DiMeEGCG | $CH_3$ | $CH_3$ |
| EGCG 4"-Glucuronide | Glucuronic acid | H |
| **B. Black tea polyphenols** | **R1** | **R2** |
| Theaflavin | OH | OH |
| Theaflavin-3'-gallate | OH | Gallate |
| Theaflavin-3',3"-digallate | Gallate | Gallate |
| **C. Theasinensin A (an EGCG oxidative dimer)** | | |

**Fig. 1. Chemical structures of tea constituents used in the present study.**

## Results and Discussion

**Effects of tea catechins, caffeine, and EGCG metabolites on cellular level of EGCG** Cellular uptake of EGCG was analyzed using radiolabeled [$^3$H]-EGCG. After a 2 hr incubation of HT-29 cells with 10 μM [$^3$H]-EGCG, total cell associated EGCG was 150-200 pmol per mg cell protein. Because EGCG is unstable under cell culture condition, 100 μM ascorbic acid was added. There was no significant degradation of EGCG observed after ascorbic acid addition. In order to analyze the effects of (-)-epicatechin (EC), (-)-epigallocatechin (EGC), and (-)-epicatechin-3-gallate (ECG) on uptake of EGCG by HT-29 cells, [$^3$H]-EGCG (10 μM) was incubated simultaneously with an equal concentration of each catechin. After 2 hr incubation, the amount of cell-associated EGCG was not affected significantly by EC and EGC. ECG decreased EGCG levels slightly, but it was not statistically significant (Fig. 2A). Indomethacin, a positive control as a MRP inhibitor, increased EGCG levels by 2.5-fold. Previously we reported that MRP1 and 2 transporters were involved in EGCG efflux, and cellular EGCG levels increased by several folds in the presence of inhibitors of these transporters such as indomethacin (14, 20). We also reported that tea catechins including EC, EGC, and ECG did not affect cellular accumulation of EGCG in Madin-Darby canine kidney cells overexpressing MRP1 (14).

The present findings are consistent with previous observations indicating that cellular levels of EGCG were not changed by other tea catechins in HT-29 cells. The uptake and/or efflux of EC, EGC, and ECG in HT-29 cells may occur independently with those of EGCG. The effect of EGCG metabolites on EGCG uptake by HT-29 cells was also analyzed. EGCG is metabolized to methyl EGCG and EGCG glucuronides by catechol-*O*-methyltransferase and UDP-glucuronosyltransferase, respectively (15, 16). It was previously shown that levels of intracellular EGCG methyl metabolites and EGCG glucuronides increased in the presence of inhibitors of MRP1 and 2 (20), suggesting that they are also substrates for these transporters. Accordingly, it is possible that these metabolites modulate uptake and/or efflux of EGCG and therefore affect EGCG cellular levels. Cellular levels of EGCG, however, were not changed when co-incubated with EGCG metabolites including 4"-methyl EGCG, 4',4"-dimethyl EGCG, or

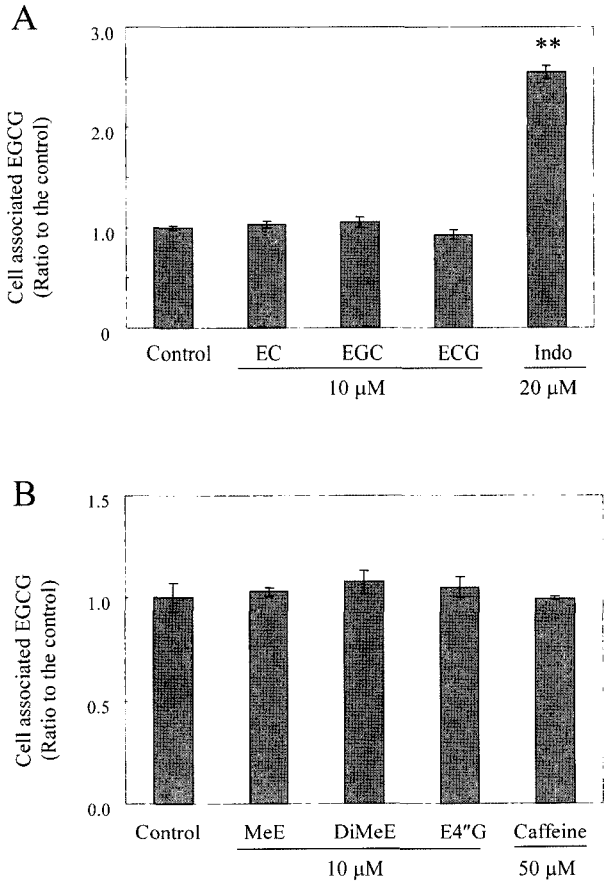



**Fig. 2. Effects of tea constituents (A) and EGCG metabolites (B) on the accumulation of [$^3$H]-EGCG in HT-29 cells.** The results are the mean±SD (n=4-8). **Significantly different from control according to Student's *t* test ($p<0.01$).

EGCG 4"-glucuronide (Fig. 2B). Caffeine, one of important tea constituents, did not affect cellular EGCG level. The results suggest that there is little competition between EGCG and its metabolites for the influx and efflux of EGCG.

**Effects of theaflavins and TsA on cellular level of EGCG** The effect of black tea theaflavins on cellular accumulation of EGCG was also investigated. Theaflavins are converted from green tea catechins during the fermentation process, which account for 0.3-2% in black tea on dry weight basis (2). Interestingly, we found that theaflavins significantly decreased cellular levels of EGCG. The effect of theaflavins with gallate groups on cellular EGCG levels was more prominent than that of theaflavin (Fig. 3). TsA, an EGCG oxidative dimer, also significantly decreased intracellular EGCG levels. The effect of TsA was comparable to that of TFdiG. Cellular intake of EGCG mainly occurs through passive diffusion, whereas intracellular EGCG can be transported out of cell by efflux pumps (14, 20).

In order to understand which process is involved in decreasing intracellular EGCG levels by TFdiG and TsA, two different treatment schemes of TFdiG or TsA addition before or after EGCG incubation were applied. Intracellular EGCG levels significantly decreased in both

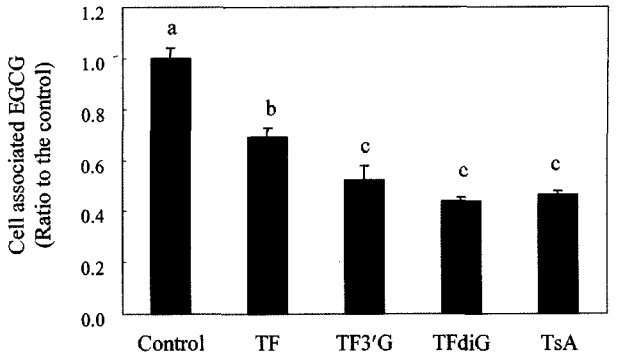



**Fig. 3. Effects of black tea theaflavins and TsA (each 10 μM) on the accumulation of [$^3$H]-EGCG in HT-29 cells.** The results are the mean±SD (n=4-8). Different letters indicate a significant difference ($p<0.01$) based on one-way analysis of variance and the Tukey's HSD test.

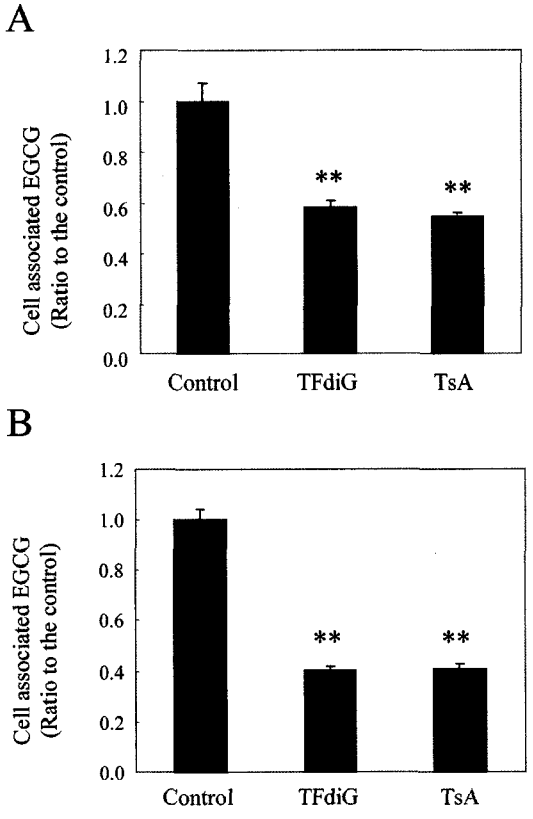



**Fig. 4. Effects of TFdiG and TsA (each 10 μM) on the accumulation of [$^3$H]-EGCG (10 μM) in HT-29 cells by different sequence of treatment.** (A) EGCG was incubated first for 30 min, medium was then replaced with one containing TFdiG or TsA and incubated further 30 min. (B) EGCG was incubated for 30 min after incubation of TFdiG or TsA for 30 min. Each bar represents the mean±SD (n=4-8). **Significantly different from control according to Student's *t* test ($p<0.01$).

schemes. The effects of TFdiG or TsA appeared to be more pronounced when cells were exposed to TFdiG or TsA before EGCG addition (54-58%) as compared with EGCG preincubation (40-41%) (Fig. 4). The results suggest that TFdiG or TsA interferes with influx of EGCG

into cells. Previously we reported that TsA, a dimer of EGCG, is one of the major EGCG oxidative products in our cell culture system, and it is predominantly located in the membrane fraction (20). TsA located in membrane surface of cells might interfere with diffusion of EGCG into cells. A similar mechanism might explain the effect of TFdiG that is formed through oxidative dimerization among quinines from gallocatechins.

Considering the results from preincubation of EGCG, EGCG already taken by cells was also relocated out of cells after adding TFdiG or TsA. The phenomenon could be explained as a result of the stimulation of EGCG efflux or cellular EGCG binding sites were replaced by TFdiG or TsA. Since the absorption through the intestinal epithelium is one of the major processes that affects the bioavailability of many dietary constituents, our findings may provide basic information for modulation of EGCG bioavailability through interaction with other dietary factors. Tea catechins including EC, EGC, EGCG, and ECG are converted to black tea polyphenols such as theaflavins and higher molecular thearubigens during fermentation process. A certain amount of EGCG, however, still remains in black tea. Our results indicate that EGCG uptake in the gastrointestinal epithelium might be hampered by theaflavins (and probably thearubigens) when black tea is consumed. The formation of TsA, one of major EGCG oxidative products, was also observed in mild alkaline solution as well as in culture media (21). It is believed that pH and oxygen are critical factors for oxidation of EGCG, and its oxidative products are produced mainly above neutral pH in the presence of molecular oxygen. Although there is no evidence that EGCG oxidation occurs in body under oxygen-limited circumstances, it is still possible that green tea solution with a neutral or mild alkaline pH can generate significant amounts of TsA, which may interfere with cellular absorption of EGCG in the gastrointestinal epithelium.

In conclusion, the present findings provide evidence that theaflavins and TsA can affect cellular accumulation of EGCG. Their effects may decrease the bioavailability of EGCG *in vivo*. The precise mechanisms involved in decreasing intracellular level of EGCG by theaflavins or TsA need to be explored further.

## References


1. Yang CS, Landau JM. Effects of tea consumption on nutrition and health. J. Nutr. 130: 2409-2412 (2000)
2. Balentine DA, Wiseman SA, Bouwens LC. The chemistry of tea flavonoids. Crit. Rev. Food Sci. 37: 693-704 (1997)
3. Yang CS, Maliakal P, Meng X. Inhibition of carcinogenesis by tea. Annu. Rev. Pharmacol. 42: 25-54 (2002)
4. Khan N, Afaq F, Saleem M, Ahmad N, Mukhtar H. Targeting multiple signaling pathways by green tea polyphenol (-)-epigallocatechin-3-gallate. Cancer Res. 66: 2500-2505 (2006)
5. Chung JY, Park JO, Phyu H, Dong Z, Yang CS. Mechanisms of inhibition of the Ras-MAP kinase signaling pathway in 30.7b Ras 12 cells by tea polyphenols (-)-epigallocatechin-3-gallate and theaflavin-3,3'-digallate. FASEB J. 15: 2022-2024 (2001)
6. Sah JF, Balasubramanian S, Eckert RL, Rorke EA. Epigallocatechin-3-gallate inhibits epidermal growth factor receptor signaling pathway. Evidence for direct inhibition of ERK1/2 and AKT kinases. J. Biol. Chem. 279: 12755-12762 (2004)
7. Shimizu M, Deguchi A, Hara Y, Moriwaki H, Weinstein IB. EGCG inhibits activation of the insulin-like growth factor-1 receptor in human colon cancer cells. Biochem. Bioph. Res. Co. 334: 947-953 (2005)
8. Berger SJ, Gupta S, Belfi CA, Gosky DM, Mukhtar H. Green tea constituent (-)-epigallocatechin-3-gallate inhibits topoisomerase I activity in human colon carcinoma cells. Biochem. Bioph. Res. Co. 288: 101-105 (2001)
9. Nam S, Smith DM, Dou QP. Ester bond-containing tea polyphenols potently inhibit proteasome activity *in vitro* and *in vivo*. J. Biol. Chem. 276: 13322-13330 (2001)
10. Naasani I, Oh-Hashi F, Oh-Hara T, Feng WY, Johnston J, Chan K, Tsuruo T. Blocking telomerase by dietary polyphenols is a major mechanism for limiting the growth of human cancer cells *in vitro* and *in vivo*. Cancer Res. 63: 824-830 (2003)
11. Hong J, Yang CS. Effect of purified green tea catechins on cytosolic phospholipase $A_2$ and arachidonic acid release in human gastrointestinal cancer cell lines. Food Sci. Biotechnol. 15: 799-804 (2006)
12. Chen L, Lee MJ, Li H. Yang CS. Absorption, distribution, elimination of tea polyphenols in rats. Drug Metab. Dispos. 25: 1045-1050 (1997)
13. Lee MJ, Maliakal P, Chen L, Meng X, Bondoc FY, Prabhu S, Lambert G, Mohr S, Yang CS. Pharmacokinetics of tea catechins after ingestion of green tea and (-)-epigallocatechin-3-gallate by humans: formation of different metabolites and individual variability. Cancer Epidem. Biomar. 11: 1025-1032 (2002)
14. Hong J, Lambert JD, Lee SH, Sinko PJ, Yang CS, Involvement of multidrug resistance-associated proteins in regulating cellular levels of (-)-epigallocatechin-3-gallate and its methyl metabolites. Biochem. Bioph. Res. Co. 310: 222-227 (2003)
15. Lu H, Meng X, Yang CS. Enzymology of methylation of tea catechins and inhibition of catechol-O-methyltransferase by (-)-epigallocatechin gallate. Drug Metab. Dispos. 31: 572-579 (2003)
16. Lu H, Meng X, Li C, Sang S, Patten C, Sheng S, Hong J, Bai N, Winnik B, Ho CT, Yang CS. Glucuronides of tea catechins: enzymology of biosynthesis and biological activities. Drug Metab. Dispos. 31: 452-461 (2003)
17. Lambert JD, Hong J, Kim DH, Mishin VM, Yang CS. Piperine enhances the bioavailability of the tea polyphenol (-)-epigallocatechin-3-gallate in mice. J. Nutr. 134: 1948-1952 (2004)
18. Kohri T, Nanjo F, Suzuki M, Seto R, Matsumoto N, Yamakawa M, Hojo H, Hara Y, Desai D, Amin S, Conaway CC, Chung FL. Synthesis of (-)-[4-$^3$H] epigallocatechin gallate and its metabolic fate in rats after intravenous administration. J. Agr. Food Chem. 49: 1042-1048 (2001)
19. Meng X, Sang S, Zhu N, Lu H, Sheng S, Lee MJ, Ho CT, Yang CS. Identification and characterization of methylated and ring-fission metabolites of tea catechins formed in humans, mice, and rats. Chem. Res. Toxicol. 15: 1042-1050 (2002)
20. Hong J, Lu H, Meng X, Ryu JH, Hara Y, Yang CS. Stability, cellular uptake, biotransformation, and efflux of tea polyphenol (-)-epigallocatechin-3-gallate in HT-29 human colon adenocarcinoma cells. Cancer Res. 62: 7241-7246 (2002)
21. Yoshino K, Suzuki M, Sasaki K, Miyase T, Sano M. Formation of antioxidants from (-)-epigallocatechin gallate in mild alkaline fluids, such as authentic intestinal juice and mouse plasma. J. Nutr. Biochem. 10: 223-229 (1999)