

Bioavailability and biological activity of tea polyphenols.

Chung S. Yang, Hong Lu, Xiaofeng Meng, Jie Liao, Guang-yu yang, Mao-Jung Lee, Pius Maliakal, and Chuan Li

Laboratory for Cancer Research, College of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, New Jersey 08854-8020, U.S.A.

Abstract

Tea polyphenols have anti-cancer activities including modulation of key signal transduction pathways *in vitro*. The possible significance of these activities *in vivo* depends on the bioavailabilities of the polyphenols. After oral administration of tea to the rat, about 14% of EGC, 31% of EC, and <1% of EGCG appeared in the blood. After *i.v.* administration, the $t_{1/2}$ of EGC and EC were 40-45 min, and that of EGCG was about 5 times longer. EGC and EC were excreted from the urine, but EGCG was excreted through the bile to the intestines. In the mouse, the bioavailability of EGCG was higher. Prolonged oral administration of green tea to rats and mice changed the profile of plasma and urinary levels of catechins. After administration of 3 g of decaffeinated green tea solids (in water) to humans, the C_{max} for EGCG, EGC, and EC (reached between 1.4-2.4 h) were 0.57, 1.60, and 0.6 μM respectively. The corresponding $t_{1/2}$ values were 5.0, 2.8, and 3.4 h. Tea catechins undergo extensive *O*-methylation, glucuronidation, and sulfation. These reactions, the efflux pumps, and other transporters may play key roles in determining the bioavailability of tea catechins. These metabolites as well as their ring fission metabolites of catechins (formed by intestinal microflora) were identified by LC-MS, and were found in significant quantities in the blood and urine. The biological activities of these tea catechin metabolites could be very interesting and remain to be studied.

Key Words: Tea, polyphenols, pharmacokinetics, and cancer prevention

Biological Activities

The inhibitory activity of tea constituents against tumorigenesis has been demonstrated in many studies with animal models (1). Nevertheless, the molecular mechanisms of such inhibition are not clearly understood. Many investigators have used cell lines to elucidate the biological activities that may be related to the inhibition of carcinogenesis and shown that EGCG, the major polyphenol in tea, has the following activities (reviewed in (1)):

1) Inhibition of MAP-kinase related signal transduction pathways as well as transcription factor (such as AP-1) activities; 2) preventing the degradation of I κ B and thus the activation of NF κ B; 3) modulating cell cycle regulation by affecting the levels or activities of cdk2/4, Rb, p16^{INK4a}, p21^{WAF1}, and p53; 4) interfering the ligands binding to receptors in system such as EGF, PDGF, FGF, and protein kinase C; 5) inhibiting cell proliferation and enhancing apoptosis; and 6) inhibiting angiogenesis and tumor cell invasion. Nevertheless, the relevance of some of these activities to the inhibition of tumorigenesis *in vivo* is uncertain, because of the rather high concentrations of EGCG (usually >20 μM) used in comparison to the plasma and tissue levels of EGCG observed in humans and animals (usually <1 μM) after tea ingestion. A clear understanding of the

Abbreviations: EGCG, (-)-epigallocatechin-3-gallate; EGC, (-)-epigallocatechin; EC, (-)-epicatechin; COMT, catechol-*O*-methyltransferase; UGT, UDPG-glucuronosyl transferase.

bioavailability, biotransformation, and biological activities of tea constituents is vital for elucidating the mechanisms of inhibition of carcinogenesis by tea.

Recent studies in our laboratory demonstrated some of the aforementioned activities in a lung tumorigenesis model in A/J mice. In this study, A/J mice were injected a dose of 4-methylnitrosamino-1-(3-pyridyl)-1-butanone (NNK) (*i.v.*, 100 mg/kg) and were given green tea (0.6% green tea solid) as the sole source of drinking fluid. After 16 weeks, the mean number of lung tumors formed per mouse was decreased by 40% in the tea treated group. The number of microvessels (as determined by immunohistochemistry with Factor VIII-related antibody) formed per tumor was significantly lower in the tea treated group (4.70 ± 0.43 vs. 9.40 ± 0.60). It was also significantly lower when the number of microvessels were scored per unit area of the tumor. The VEGF staining score was also lower in the tea treated group (0.98 ± 0.17 vs. 1.43 ± 0.07). Enhanced apoptosis due to tea treatment was observed as the apoptotic index was higher in the tea treated group than the control ($2.51 \pm 0.18\%$ vs. $1.57 \pm 0.11\%$) based on morphological analysis and ($5.38 \pm 0.64\%$ vs. $2.78 \pm 0.61\%$) based on the TUNEL assay). The immunochemical staining for phosphorylated c-jun in the lung tumors was less intense in the tea treated group than the control, suggesting the inhibition of the phosphorylation of c-jun by the tea treatment. These preliminary results are interesting. Experiments are being planned to determine, in short term experiments, whether these activities are due to the direct action of tea constituents.

Pharmacokinetics of Tea Polyphenols

After *i.v.* administration of decaffeinated green tea to rats, the $t_{1/2}$ of EGC and EC (the total concentrations of the conjugated and non-conjugated forms) were 40-45 min, and that of EGCG was about 5 times longer (2). EGC and EC were excreted from the urine, but EGCG was excreted through the bile to the intestine. With oral administration, about 14% EGC, 31% of EC, and <1% EGCG appeared in the blood. In the mouse, the bioavailability of EGCG was much higher, but it was still lower than EGC. Prolonged oral administration of green tea to rats and mice changed the profile of plasma and urinary levels of catechins, possibly due to enzyme induction (3). After administration of 3 g of decaffeinated green tea solids (in water) to humans, the C_{max} for EGCG, EGC, and EC (reached between 1.4-2.4 h) were 0.57, 1.60, and 0.6 μM respectively. The corresponding $t_{1/2}$ values were 5.0, 2.8, and 3.4 h (4). Additional studies indicated that the plasma EGCG are mostly in the non-conjugated form, whereas EGC and EC are mostly in the conjugated (mostly glucuronide) forms (5).

Biotransformation of Tea Catechins

Because of the polyphenolic structure, tea catechins do not undergo appreciable Phase I metabolism by enzymes such as cytochromes P450 in animals. Tea catechins, however, undergo Phase II metabolism extensively. After ingestion of tea, over 50 metabolites (mostly in methylated, glucuronidated, and sulfated forms) could be detected by LC-MS (6). The COMT-catalyzed methylation and UGT-catalyzed glucuronidation have been extensively characterized in mice and rats. For EGC, the major methylation sites are the 4'- and 3'-positions of the B ring, and the major glucuronidation sites are the 7-position of the A ring and 3'-position of the B-ring (Fig. 1). The methylation of 4'-position has no apparent effect on the subsequent glucuronidation, whereas methylation of the 3'-position would prevent glucuronidation at this position. On the other hand, glucuronidation of the 3'-position would inhibit the methylation at the 3'- and 4'-positions, whereas glucuronidation at the A-ring does not affect the methylation at the B-ring. As judged by the V_{max} and catalytic efficiency (V_{max}/K_m), the mouse liver microsomes are more efficient, than the rat liver

microsomes, in catalyzing the glucuronidation of EGC both at the 7- and 3'-positions (Table 1). The rat liver appears to be more active, than the mouse liver, in the methylation of EGC.

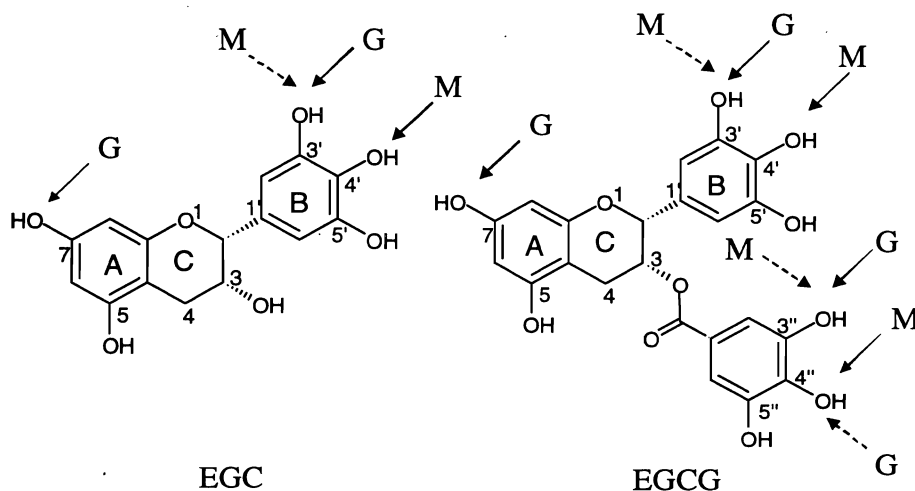


Fig. 1 Glucuronidation (G) and Methylation (M) of Tea Catechins

Table 1. Glucuronidation and methylation of catechins in the liver and small intestine

		<i>V</i> _{max} (pmol/mg/min)/K _m (μM)			
		Rats		Mice	
		Liver	Intestine	Liver	Intestine
UGT	EGCG	$\frac{26.2}{376} = 0.07$	Trace	$\frac{77.2}{262} = 0.3$	$\frac{64.8}{107} = 0.6$
	EGC 7-O	$\frac{127}{755} = 0.17$	Trace	$\frac{311}{420} = 0.74$	Trace
	EGC 3'-O	$\frac{5.9}{277} = 0.02$	Trace	$\frac{353}{547} = 0.65$	Trace
COMT	4''-O-MeEGCG	$\frac{196}{4.2} = 46.5$	$\frac{35.4}{0.6} = 56.1$	$\frac{67.9}{1.9} = 35.9$	$\frac{21.0}{1.1} = 18.7$
	4'-O-MeEGC	$\frac{4211}{24.9} = 169$	$\frac{370}{26.2} = 14.1$	$\frac{2296}{15.0} = 153$	$\frac{83}{21.2} = 3.9$

For EGCG, in addition to the above described A- and B-rings, the 3''- and 4''-positions of the D-ring are sites for methylation and glucuronidation (Fig. 1). The mouse liver and intestine have higher activities, than the rat counter part, in catalyzing the glucuronidation of EGCG. Of particular interest is the rather high activity in the mouse intestine vs. the very low level of activity in the rat intestine. This may be related to the higher bioavailability of EGCG in the mice than rats. For the methylation of EGCG, the rats have higher activities than the mice. In both species, the methyltransferase activity (V_{max}) toward EGC is 20-30 times higher than toward EGCG in the liver. However, EGCG is a much stronger inhibitor of COMT.

Tea catechins are also degraded in the intestine by microflora. Several microbial metabolites, including 4-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, 3-methoxy-4-hydroxy-hippuric acid, and 3-methoxy-4-hydroxybenzoic acid, were observed in human urine samples (7). 5-(3',4'-Dihydroxyphenyl)- γ -valerolactone and 5-(3',4',5'-trihydroxyphenyl)- γ -valerolactone were identified in the human urine as the ring fusion products of EGC and EC, respectively (8). Both metabolites (mainly in the conjugated form) were detected in the urine and plasma in amounts several fold higher than their respective precursors in some individuals. Judging from the di(tri)-hydroxyphenyl and valerolactone structures, these metabolites may possess interesting biological activities.

Concluding Remarks

The antioxidative properties and biological activities of tea polyphenols have been studied extensively *in vitro*. Their biological activities after oral ingestion of tea, however, are determined by the bioavailability and biotransformation. The blood, tissue, and urine levels of tea catechins and their metabolites are beginning to be understood. The theaflavins from black tea have also been shown to inhibit lung tumorigenesis, even though they have not been convincingly detected in the blood. It is possible that degradation products of theaflavins (and perhaps thearubigens), formed by intestinal microflora, is absorbed and display biological activities. Further studies on the biological activities of these compounds will enhance our understanding on the health effects of tea consumption.

References

1. Yang, C. S., Maliakal, P., and Meng, X. Inhibition of carcinogenesis by tea, *Annu. Rev. Pharmacol. Toxicol.* **42**: 25-54, 2002.
2. Chen, L., Lee, M.-J., Li, H., and Yang, C. S. Absorption, distribution, and elimination of tea polyphenols in rats, *Drug Metab. Dispos.* **25**: 1045-1050, 1997.
3. Kim, S., Lee, M.-J., Hong, J., Li, C., Smith, T. J., Yang, G.-Y., Seril, D. N., and Yang, C. S. Plasma and tissue levels of tea catechins in rats and mice during chronic consumption of green tea polyphenols, *Nutr. & Cancer.* **37**: 41-48, 2000.
4. Yang, C. S., Chen, L., Lee, M.-J., Balentine, D., Kuo, M. C., and Schantz, S. P. Blood and urine levels of tea catechins after ingestion of different amounts of green tea by human volunteers., *Cancer Epidemiol. Biomark. Prev.* **7**: 351-354, 1998.
5. Chow, H. H., Cai, Y., Alberts, D. S., Hakim, I., Dorr, R., Shahi, F., Crowell, A., Viner, J., and Yang, C. S. Phase I pharmacokinetic study of tea polyphenols following single-dose administration of epigallocatechin gallate and polyphenon E, *Cancer Epidemiol. Biomark. Prev.* **10**: 53-58, 2001.
6. Li, C., Meng, X., Winnik, B., Lee, M.-J., Lu, H., Sheng, S., Buckley, B., and Yang, C. S. Analysis of urinary metabolites of tea catechins by liquid chromatography/electrospray ionization mass spectrometry, *Chem. Res. Toxicol.* **14**: 702-702, 2001.
7. Pietta, P. G., Simonetti, P., Gardana, C., Brusamolino, A., Morazzoni, P., and Bombardelli, E. Catechin metabolites after intake of green tea infusions, *Biofactors.* **8**, 1998.
8. Li, C., Lee, M.-J., Sheng, S., Prabhu, S., Winnik, B., Huang, B., Meng, X., Chung, J. Y., Yan, S., Ho, C.-J., and Yang, C. S. Structural identification and characterization of two metabolites of catechins in human urine and blood after tea ingestion, *Chem. Res. Toxicol.* **13**: 177-184, 2000.