Published in final edited form as:

Pharm Res. 2010 June ; 27(6): 1066–1071. doi:10.1007/s11095-010-0056-3.

Effect of dietary Polyphenon E and EGCG on development of benzo[a]pyrene induced lung tumors in A/J mice

Qi Zhang¹, Huijing Fu², Jing Pan¹, Jun He¹, Seto Ryota³, Yukihiro Hara³, Yian Wang¹, Ronald A. Lubet⁴, and Ming You^{1,*}

¹Department of Surgery, Campus Box 8109, The Alvin J Siteman Cancer Center, Washington University School of Medicine, 660 South Euclid Avenue, St Louis, MO 63110, USA

²Department of Energy, Environmental and Chemical Engineering, Washington University of St Louis, One Brookings Drive, Box 1180, St. Louis, Missouri 63130, USA

³Mitsui Norin Co., Ltd., Shizuoka 426-01, Japan

⁴Chemoprevention Agent Development Research Group, National Cancer Institute, Bethesda, MD 20892, USA

Abstract

In this study, we compared the chemopreventive activities of Polyphenon E (Poly E), (–)-epigallocatechin-3-gallate (EGCG) and Polyphenon E without EGCG (Poly E-EGCG) on the development of benzo(a)pyrene (B(a)P)-induced lung tumors in A/J mice. One week after carcinogen treatment, A/J mice were fed with 0.975% (wt/wt) EGCG, 0.525% (wt/wt) Poly E-EGCG or 1.5% (wt/wt) Poly E in AIN-76A purified powder diet for 24 weeks. Poly E significantly decreased both tumor multiplicity (52.2%, $p < 0.001$) and tumor load (64.0%, $p < 0.001$), while EGCG or Poly E-EGCG did not significantly inhibit lung tumor multiplicity. EGCG was more stable in a complex mixture (Poly E) than as a pure compound. EGCG was ineffective when administered by diet likely due to its instability. Thus, the efficacy of EGCG on lung tumorigenesis requires the presence of other tea catechins.

Keywords

Chemoprevention; lung tumorigenesis; polyphenon E; EGCG; degradation

Introduction

Green tea, produced from the leaves of the plant *Camellias sinensis*, is one of the most widely consumed beverages in the world. Polyphenon E (Poly E) is a well-defined pharmaceutical-grade mixture of green tea polyphenols that contain different catechins such as epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), gallic acid (GCG), and (–)-epigallocatechin-3-gallate (EGCG) [1]. Poly E has various biological

*Corresponding author: Ming You, Department of Surgery and Alvin J Siteman Cancer Center, Washington University in St. Louis, 660 South Euclid Avenue, Campus Box 8109, 10130 Wohl Clinic, St Louis, MO 63110, Phone: 314-362-9294, Fax: 314-362-9366, youm@msnotes.wustl.edu.

activities including antioxidation, modulation of enzyme systems for metabolizing chemical carcinogens and inhibition of tumor promotion [2]. Poly E has been investigated extensively in terms of its cancer chemopreventive activities. Poly E administered by drinking water, diet or aerosol can inhibit carcinogenesis-induced lung (Table 1). Poly E administered by aerosol or diet significantly inhibited benzo(a)pyrene (B(a)P)-induced lung tumorigenesis in A/J mice [3–6]. Poly E by drinking water inhibited the progression of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced lung tumor in A/J mice [7]. The National Cancer Institute has assisted the development of Poly E as a candidate for human chemoprevention studies.

EGCG is the most abundant catechin found in green tea, and comprises 65% of Poly E (Supplementary Table 1). EGCG inhibits cell proliferation, induces apoptosis, induces cell cycle arrest, stimulates angiogenesis, affects cell signaling pathways, and inhibits epidermal growth factor, other kinases and DNA methyltransferase in different cell lines [8–11]. The effect of EGCG on lung tumorigenesis has been reported by several groups. This includes lung tumorigenesis induced by NNK, cisplatin and tobacco smoke. EGCG inhibited lung tumorigenesis when delivered by drinking in NNK-induced lung tumorigenesis model in A/J mice [12,13]. EGCG by drinking also reduced the numbers of cisplatin-induced lung tumors in A/J mice [13]. In contrast, aerosol EGCG did not decrease tumor multiplicities or tumor load in B(a)P, NNK or tobacco smoke-induced lung tumorigenesis [5,14]. No studies on the effect of EGCG alone administered by diet have been reported.

The present study was designed to compare the chemopreventive activities of EGCG, Poly E and Polyphenon E without EGCG (Poly E-EGCG) administered by diet, and determine to what extent EGCG is responsible for the chemopreventive efficacy of Poly E.

Materials and Methods

Reagents

Benzo(a)pyrene (B(a)P, 99% pure) and tricapylin were purchased from Sigma Chemical Co. (St. Louis, MO). B(a)P was dissolved in tricapylin immediately before use in animal bioassays. EGCG, Poly E-EGCG and Poly E were obtained from Tokyo Food Techno Co., Ltd. (Tokyo, Japan). The main difference between Poly E and Poly E-EGCG was the content of EGCG. Poly E contained 65% EGCG while Poly E-EGCG contained only 1.4% EGCG (Supplementary Table 1).

Animals and *in vivo* treatment

Female A/J mice at 6 weeks of age were obtained from Jackson Laboratories (Bar Harbor, ME). Animals were housed with wood chip bedding in environmentally controlled, clean-air room with a 12-hour light-dark cycle and a relative humidity of 50%. Drinking water and diet were supplied *ad libitum*. The study was approved by the Washington University's Institutional Animal Care and Use Committee. Female A/J mice were given a single intraperitoneal injection of B(a)P (100 mg/kg body weight) in 0.2 ml of tricapylin. One week after B(a)P injection, the mice were randomly divided into four groups with 10 mice per group. Animals received special diets for 24 weeks (Figure 1). Mice were fed AIN-76A

purified powder diet (Dyets, Inc., Bethlehem, PA) containing 0.975% (wt/wt) EGCG, 0.525% (wt/wt) Poly E-EGCG or 1.5% (wt/wt) Poly E. Diets were supplemented with 3% sucrose as previous described [7]. All diets were prepared weekly, and fresh diet in the cages changed daily. Diets were prepared with a KitchenAid mixer (St. Joseph, MI) mixing for at least 1 hour. The body weights of mice were measured every week for the duration of the study. To control the dose of these chemopreventive reagents, we monitored the consumption of diet by measuring them daily. The health conditions of mice were also monitored daily during the study duration. We did not observe any sign of toxicity and loss of body weight. Mice were sacrificed 25 weeks after exposure to the carcinogen B(a)P.

Plasma and tissue collection

Blood samples from the retroorbital plexus of each animal were collected in EDTA-treated tubes. The blood was centrifuged at 950×g for 10 min at 4°C. The obtained plasma was kept at -80°C for further analysis. Lungs from each mouse were fixed in Tellyesniczky's solution overnight [15], and then stored in 70% EtOH. The fixed lungs were evaluated under a dissecting microscope to obtain fixed surface tumor count and individual tumor size. Tumor volume was calculated based on the following formula: $V = 4\pi r^3/3$ [16]. The total tumor volume in each mouse was calculated as the sum of all tumors. Tumor load was determined by averaging the total tumor volume of each mouse in each group.

Determination of EGCG in diet or plasma by HPLC

Approximately 0.2 g EGCG, Poly E-EGCG or Poly E diet, prepared freshly (fresh) or licked by mice then exposed in the air for 5 days (old), were dissolved in 20 ml water, and filtered with 0.22 µm steriflip Millipore filter (Millipore corp. MA). The concentration of each catechin in diet samples was determined using an HPLC. HPLC 1100 series system, which consists of autosampler, binary pump, thermostatted column compartment, and diode array detector for fresh and old diet and variable wavelength detector for plasma (Agilent Tech, Santa Clara, CA). For diet analysis, the HPLC column was 4.6 × 75 mm Zorbax SB-C18 3.5 µm column, the wavelength was 244 nm. The mobile phase consisted of ammonium acetate:acetonitrile (80:20), the column temperature was 40 °C, and the flow rate of the mobile phase was 1 ml/min. For plasma analysis, a Synergi 4µ Max-RP (Phenomenax, Torrance, CA) column was equilibrated with Buffer A, consisting of 0.05 M sodium phosphate, pH 7.2 in 25 % methanol. After injection, Buffer B (methanol: water: tetrahydrofuran = 70:30:3) was increased from 0 to 8% by a linear gradient between 0 and 3min, maintained at 8% between 3 and 18 min, and at 55% between 18 and 35 min. The flow rate was 0.8 ml/min. Quantification of EGCG and other catechins were based on calibration curves derived from 5 standards (EGCG, C, EGC, ECG and EC) ranging from 1.8 µg/ml to 1000 µg/ml.

Statistical Analysis

We hypothesized that B(a)P -induced lung tumors are more likely to occur in the carcinogen control group than in the treatment groups. Accordingly, Student's *t* test was used to test the differences between the control group and the treatment groups. Means and standard deviations were calculated for analysis of tumor multiplicity and load.

Results

Poly E, but not Poly E- EGCG decreased tumor multiplicity and tumor load

Lung tumor incidence was 100% in all groups. B(a)P induced an average of 22.6 ± 5.0 tumors per mouse in control group, and tumor load was $25.9 \pm 5.3 \text{ mm}^3$. Mice treated with Poly E diet showed a significant decrease in both tumor multiplicity (52.2% inhibition, 10.8 ± 4.2) and tumor load (64.0% inhibition, $9.3 \pm 5.2 \text{ mm}^3$) when compared to the control group. Poly E-EGCG decreased tumor load (27.0%, $18.9 \pm 5.7 \text{ mm}^3$) but not tumor multiplicity (Figure 2).

The concentration of EGCG in EGCG group is similar with Poly E group, and the concentration of other catechins in poly E-EGCG group is similar with poly E group. The main difference between Poly E and Poly E-EGCG was the content of EGCG. Poly E contained 65% EGCG while Poly E-EGCG contained only 1.4%. The difference in tumor multiplicity and tumor load between Poly E group and Poly E-EGCG group was therefore attributed to the presence of EGCG. This implied that EGCG might be the most effective component on chemoprevention. But interestingly, mice fed EGCG alone did not have a reduction in either tumor multiplicity or tumor load.

Stability of EGCG, Poly E-EGCG and Poly E by diet

The degradation of EGCG was widely reported [17], and during the experiment, we observed an obvious color change in EGCG diet suggesting oxidation or degradation of EGCG in the diet, we therefore conducted a HPLC analysis to detect the concentration of EGCG in diet, tried to find whether EGCG lost its function on chemoprevention because of degradation. We therefore conducted an HPLC analysis to detect the concentration of EGCG in diet under various conditions. Fresh diet made from each group and also old diet after mice licked, dissolved the fresh and old diet in 25% ethanol water solution, filtered and analyzed EGCG and other catechins by HPLC. For fresh diets, both the EGCG group and Poly E group had similar EGCG concentration, 79.9 and 78.4 $\mu\text{g/ml}$, respectively (Figure 3). For old diets, the concentration of EGCG was decreased to 1.7 $\mu\text{g/ml}$ in the EGCG group and 29.8 $\mu\text{g/ml}$ in the Poly E group. The degradation rate of EGCG was 62.0% in Poly E group and 97.7% in EGCG group. At the same time, the degradation rate of EC in Poly E-EGCG or EGCG group is only 11.7% and 8.3% (data not shown), indicated that EGCG was the most unstable component in Poly E. We also measured the concentration of EGCG in EGCG and Poly E group after exposed in air 10 days, and no significant degradation was found (data not shown). This analysis showed that old diet degraded EGCG much faster than EGCG store on air, and EGCG is more stable when its presence in a complex mixture (Poly E) than EGCG alone.

Stability of EGCG in plasma

The concentration of EGCG in plasma harvested from each group was determined. In the EGCG group, we detected 2 new peaks (7.2–7.3 and 9.9–10.0 min) other than EGCG peak (9.1–9.2 min) appeared on the HPLC spectra (Figure 4A). According to Sang *et al*'s report, the peak appeared at 9.9 min might be the dimer of EGCG, and the peak 7.2 minute was unknown but it was reported as the metabolites of EGCG [18,19]. For 10 plasma samples

from EGCG group, EGCG peak was detectable only in one sample, but all 10 samples presented the unknown 7.2 minute peak, and 5 out of 10 samples showed the 9.9 min dimer peak. In the Poly E group, all 10 plasma samples presented the EGCG peak, but only 4 out of 10 samples presented unknown peak, and no dimer peak was found in any sample. The EGCG concentration in plasma was 73.6 ± 232.7 ng/ml in the EGCG group, and 1065.2 ± 978.2 ng/ml in the poly E group (Figure 4B). This result further confirmed that EGCG is not stable when present without other catechins of tea polyphenols in plasma, and it might be degraded to metabolite or form dimer, therefore the bioavailability was decreased in mice treated with EGCG alone.

Discussion

Poly E is a well-defined pharmaceutical-grade mixture of tea polyphenols containing different catechins that inhibits the development of lung cancer in several animal models [13,15,20–22]. EGCG is the most abundant catechin found in green tea, and it has received most of the attention because it can inhibit cell proliferation in many cancer cell lines through different mechanisms. [8–11].

EGCG showed to have efficacy in the chemoprevention of mouse lung tumorigenesis when administered by drinking water [12,13]. However, no study of EGCG administered by diet has been previously reported. In the present study, we tested the chemopreventive efficacy of dietary EGCG on B(a)P-induced lung tumorigenesis. Poly E-EGCG has a similar catechin composition as Poly E with the exception of EGCG. The chemopreventive efficacy of EGCG and Poly E-EGCG was compared to Poly E using doses that delivered similar amounts of EGCG (Poly E versus) or similar amounts of other tea catechins (Poly E versus Poly E-EGCG).

Our results showed that administered by diet, Poly E decreased tumor multiplicity and tumor load. Poly E-EGCG decreased tumor load, but did not have a significant effect on tumor multiplicity. The main difference between the chemical composition of Poly E and Poly E-EGCG was the content of EGCG. Poly E contains 65.0% EGCG, while Poly E-EGCG contains only 1.4% EGCG. Although there were differences in the ratios among other tea catechins in Poly E and Poly E-EGCG, the absolute amounts of each catechin given at the doses used were similar. The difference in tumor multiplicities and tumor loads between Poly E group and Poly E-EGCG group were therefore attributed to the presence of EGCG. EGCG by diet would be the most effective compound in Poly E chemoprevention based on the results got from Poly E and Poly E-EGCG group. But interestingly, we did not observe any significant decrease in neither tumor multiplicity nor tumor load in EGCG group.

EGCG is relatively unstable under neutral or alkaline conditions, and could be rapidly degraded by deprotonation of hydroxyl group on the phenol rings. Methylation, glucuronidation and sulfation resulted in reduced biological activities *in vivo* [23–25]. During the experiment, we observed an obvious color change in EGCG diet and therefore hypothesized that degraded in the diet. Our HPLC results indicated that EGCG is more stable in the Poly E mixture than when present alone in the purified diet (97.7% degradation in the EGCG diet group versus 62.0% in the Poly E group). We also measured the

degradation of EGCG in EGCG diet and Poly E diet after storing them in air for 10 days, and neither of them has been degraded significantly (data not shown). This observation indicated that the degradation of EGCG might be induced by mice saliva. Because diets need mix with saliva before swallow and digestion, almost all EGCG was degraded when present alone. It is appeared that the degradation by saliva might be the reason that EGCG lost its function on chemoprevention by diet. For Poly E group, because of the presence of other catechins, the degradation of EGCG was delayed, and may be the reason for improved chemopreventive efficacy.

Such differences were also found in plasma. The EGCG peak seemed to be eliminated more readily in the plasmas collected from EGCG alone group than from Poly E group. Glucuronidation and sulfation of tea Polyphenols was reported to be the major elimination pathways for EGCG. The competition among tea polyphenols for glucuronosyltransferase and sulfotransferase may affect EGCG elimination [17]. Shimizu et al reported Poly E may be preferable to EGCG because the mixture of catechins may exert synergistic growth inhibitory effect [26]. The presence of other tea catechins might affect the absorption, biologic activity, or other properties of EGCG [21]. Because tea catechins have similar chemical structure, conversion of EGCG may be retarded and the residence time of EGCG may increase when administered with other catechins compared to pure EGCG.

In summary, this study confirmed that Poly E was an effective chemopreventive reagent on B(a)P-induced lung tumors in female A/J mice. Pure EGCG administered in the diet was not an effective chemopreventive agent, likely due to preferential degradation of pure EGCG compared to EGCG present in catechins mixture. The chemopreventive effect of EGCG was reported could also be caused by cooperation or synergy with other tea components [26]. Modifications of EGCG that improve its bioavailability will be an interesting strategy to pursue to improve its pharmaceutical profile.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

1. Chang PY, Mirsalis J, Riccio ES, Bakke JP, Lee PS, Shimon J, Phillips S, Fairchild D, Hara Y, Crowell JA. Genotoxicity and toxicity of the potential cancer-preventive agent polyphenon E. *Environ Mol Mutagen*. 2003; 41:43–54. [PubMed: 12552591]
2. Haque AM, Hashimoto M, Katakura M, Tanabe Y, Hara Y, Shido O. Long-term administration of green tea catechins improves spatial cognition learning ability in rats. *J Nutr*. 2006; 136:1043–7. [PubMed: 16549472]
3. Fu HJ, He J, Mei L, Zhang Q, Hara Y, Ryota S, Rubet RA, Chen R, Chen DR, You M. Anti-lung cancer effect of epigallocatechin-3-gallate is dependent on its presence in a complex mixture (Polyphenon E). *Cancer Prev Res*. 2009
4. Yan Y, Wang Y, Tan Q, Hara Y, Yun TK, Lubet RA, You M. Efficacy of polyphenon E, red ginseng, and rapamycin on benzo(a)pyrene-induced lung tumorigenesis in A/J mice. *Neoplasia*. 2006; 8:52–8. [PubMed: 16533426]
5. Yan Y, Cook J, McQuillan J, Zhang G, Hitzman CJ, Wang Y, Wiedmann TS, You M. Chemopreventive effect of aerosolized polyphenon E on lung tumorigenesis in A/J mice. *Neoplasia*. 2007; 9:401–5. [PubMed: 17534445]

6. Anderson MW, Goodin C, Zhang Y, Kim S, Estensen RD, Wiedmann TS, Sekar P, Buncher CR, Khoury JC, Garbow JR, You M, Tichelaar JW. Effect of dietary green tea extract and aerosolized difluoromethylornithine during lung tumor progression in A/J strain mice. *Carcinogenesis*. 2008; 29:1594–600. [PubMed: 18515278]
7. Lu G, Liao J, Yang G, Reuhl KR, Hao X, Yang CS. Inhibition of adenoma progression to adenocarcinoma in a 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung tumorigenesis model in A/J mice by tea polyphenols and caffeine. *Cancer Res*. 2006; 66:11494–501. [PubMed: 17145898]
8. Lu YP, Lou YR, Xie JG, Peng QY, Liao J, Yang CS, Huang MT, Conney AH. Topical applications of caffeine or (–)-epigallocatechin gallate (EGCG) inhibit carcinogenesis and selectively increase apoptosis in UVB-induced skin tumors in mice. *Proc Natl Acad Sci U S A*. 2002; 99:12455–60. [PubMed: 12205293]
9. Hu C, Kitts DD. Evaluation of antioxidant activity of epigallocatechin gallate in biphasic model systems in vitro. *Mol Cell Biochem*. 2001; 218:147–55. [PubMed: 11330830]
10. Lin JK, Liang YC, Lin-Shiau SY. Cancer chemoprevention by tea polyphenols through mitotic signal transduction blockade. *Biochem Pharmacol*. 1999; 58:911–5. [PubMed: 10509743]
11. Hou Z, Lambert JD, Chin KV, Yang CS. Effects of tea polyphenols on signal transduction pathways related to cancer chemoprevention. *Mutat Res*. 2004; 555:3–19. [PubMed: 15476848]
12. 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. [PubMed: 1617663]
13. Mimoto J, Kiura K, Matsuo K, Yoshino T, Takata I, Ueoka H, Kataoka M, Harada M. (–)-Epigallocatechin gallate can prevent cisplatin-induced lung tumorigenesis in A/J mice. *Carcinogenesis*. 2000; 21:915–9. [PubMed: 10783312]
14. Witschi H, Espiritu I, Ly M, Uyeminami D, Morin D, Raabe OG. Chemoprevention of tobacco smoke-induced lung tumors by inhalation of an epigallocatechin gallate (EGCG) aerosol: a pilot study. *Inhal Toxicol*. 2004; 16:763–70. [PubMed: 16036746]
15. Zhang Z, Liu Q, Lantry LE, Wang Y, Kelloff GJ, Anderson MW, Wiseman RW, Lubet RA, You M. A germ-line p53 mutation accelerates pulmonary tumorigenesis: p53-independent efficacy of chemopreventive agents green tea or dexamethasone/myo-inositol and chemotherapeutic agents taxol or adriamycin. *Cancer Res*. 2000; 60:901–7. [PubMed: 10706103]
16. Wang Y, Zhang Z, Kastens E, Lubet RA, You M. Mice with alterations in both p53 and Ink4a/Arf display a striking increase in lung tumor multiplicity and progression: differential chemopreventive effect of budesonide in wild-type and mutant A/J mice. *Cancer Res*. 2003; 63:4389–95. [PubMed: 12907609]
17. Chen L, Lee MJ, Li H, Yang CS. Absorption, distribution, elimination of tea polyphenols in rats. *Drug Metab Dispos*. 1997; 25:1045–50. [PubMed: 9311619]
18. Sang S, Lee MJ, Hou Z, Ho CT, Yang CS. Stability of tea polyphenol (–)-epigallocatechin-3-gallate and formation of dimers and epimers under common experimental conditions. *J Agric Food Chem*. 2005; 53:9478–84. [PubMed: 16302765]
19. Raneva VG, Shimizu Y, Shimasaki H. Antioxidant activity in plasma and tissues distribution of (–)-epigallocatechin gallate after oral administration to rats. *J Oleo Sci*. 2005; 54:289–298.
20. Liao J, Yang GY, Park ES, Meng X, Sun Y, Jia D, Seril DN, Yang CS. Inhibition of lung carcinogenesis and effects on angiogenesis and apoptosis in A/J mice by oral administration of green tea. *Nutr Cancer*. 2004; 48:44–53. [PubMed: 15203377]
21. Yang CS, Yang GY, Landau JM, Kim S, Liao J. Tea and tea polyphenols inhibit cell hyperproliferation, lung tumorigenesis, and tumor progression. *Exp Lung Res*. 1998; 24:629–39. [PubMed: 9659588]
22. Cao J, Xu Y, Chen J, Klaunig JE. Chemopreventive effects of green and black tea on pulmonary and hepatic carcinogenesis. *Fundam Appl Toxicol*. 1996; 29:244–50. [PubMed: 8742322]
23. Lu H, Meng X, Yang CS. Enzymology of methylation of tea catechins and inhibition of catechol-O-methyltransferase by (–)-epigallocatechin gallate. *Drug Metab Dispos*. 2003; 31:572–9. [PubMed: 12695345]

24. Chen Z, Zhu QY, Tsang D, Huang Y. Degradation of green tea catechins in tea drinks. *J Agric Food Chem.* 2001; 49:477–82. [PubMed: 11170614]
25. Landis-Piwowar KR, Huo C, Chen D, Milacic V, Shi G, Chan TH, Dou QP. A novel prodrug of the green tea polyphenol (–)-epigallocatechin-3-gallate as a potential anticancer agent. *Cancer Res.* 2007; 67:4303–10. [PubMed: 17483343]
26. Shimizu M, Deguchi A, Lim JT, Moriwaki H, Kopelovich L, Weinstein IB. (–)-Epigallocatechin gallate and polyphenon E inhibit growth and activation of the epidermal growth factor receptor and human epidermal growth factor receptor-2 signaling pathways in human colon cancer cells. *Clin Cancer Res.* 2005; 11:2735–46. [PubMed: 15814656]

Figure 1.

Figure 2.

(A)

(B)

	Concentration of EGCG ($\mu\text{g/ml}$)	Degradation rate
EGCG old	1.8	97.7 %
EGCG fresh	79.9	
Poly E old	29.8	62.0 %
Poly E fresh	78.4	

Figure 3.

(A)

(B)

Group	Unknown peak (7.2-7.3 min)	EGCG (9.1-9.2 min)	Dimer EGCG (9.9-10.0 min)
EGCG (ng/ml)	716.9 ± 356.1	73.6 ± 232.7	626.4 ± 659.5
Poly E (ng/ml)	464.5 ± 548.1	1065.2 ± 978.2	0

Figure 4.

Table 1

Chemoprevention of lung tumorigenesis by Poly E and EGCG

Reagent	Carcinogen	Animal model	Mode of treatment	Results	Ref.
EGCG	NNK	A/J mice	Drinking	Decreased tumor multiplicity	[12]
EGCG	Cisplatin or NNK	A/J mice	Drinking	Decreased tumor multiplicity	[13]
EGCG	Tobacco smoke or NNK	A/J mice	Aerosol	No effective	[14]
Poly E	B(a)P	A/J mice	Diet	Decreased tumor multiplicity and load	[5]
Poly E	B(a)P	A/J mice	Diet	Decreased tumor multiplicity and load	[6]
Poly E, EGCG	B(a)P	A/J mice	Aerosol	Poly E decreased tumor multiplicity and load, EGCG no effective	[5]
Poly E-EGCG, Poly E	B(a)P	A/J mice	Aerosol	Poly E decreased tumor multiplicity and load, Poly E-EGCG no effective	[3]
Poly E	NNK	A/J mice	drinking	Decreased tumor multiplicity	[7]

NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone