Supporting Information

Green tea catechins: inhibitors of glycerol-3-phosphate dehydrogenase
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Glycerol-3-phosphate dehydrogenase (GPDH) activity assay

Assays were performed at 25°C for 5 min in a final volume of 250 μL per vial containing 10 μL of rabbit muscle GPDH (1 unit activity) and 240 μL of substrate solution. The final substrate mixture per vial contained 100 mM triethanolamine/HCl buffer (pH 7.5), 2.5 mM EDTA, 0.1 mM β-mercaptoethanol, 0.12 mM NADH, 0.2 mM DHAP, and different concentrations of green tea catechins, such as EGCG, ECG, EGC, or EC. The 5-min change in absorbance at 340 nm for determination of the disappearance of NADH was followed with a recording spectrophotometer at 25°C. Initial reaction velocity was determined. To determine the concentration of EGCG that inhibited GPDH activity by 50%, the GPDH activity was calculated as 1 μmole NADH oxidized per minute per milligram of enzyme. To determine kinetic parameters of GPDH altered by EGCG, enzyme assays were carried out at different concentrations of the substrates either DHAP or NADH in the absence and presence of each green tea catechin. Double reciprocal plots were made to obtain apparent inhibition constant (Ki) [26, 27]. Each value given is the average of at least three separate determinations.

Statistical analysis

Data are expressed as the mean±SEM. Unpaired Student’s t test was used to examine differences between the control and treatment groups. One-way ANOVA followed by the Student-Newman-Keuls multiple-range test was used to examine differences among multiple groups. Differences were considered significant at p<0.05. Statistics were performed using SigmaStat (Jandel Scientific).
Results and discussion

In vivo, plasma concentrations of green tea EGCG were generally reported to be in the range of 0.2–244 μM in animals and humans. In a phase I pharmacokinetic study [1], the average maximum plasma concentration and time to reach the maximum plasma concentration were 0.96 μM and 4 h, respectively, after a single oral dose administration of 800 mg EGCG to healthy subjects. A plasma EGCG concentration of 1 μM would be similar to levels in man (70 kg) 1 h after drinking 2~10 cups (200 mL/cup) of decaffeinated green tea (1.2 g per cup containing 0.88 mg EGCG, 82 mg EGC, 33 mg ECG, and 32 mg EC) [2]. In a randomized, double-blind, placebo-controlled clinical trial in France, the maximal plasma EGCG concentrations in healthy men (with median body weight of 72 kg) ranged from 0.26 to 6.4 μM at 1.3~2.2 h after a single oral dose of EGCG capsule (50~1600 mg/capsule) [3]. The mean half-life values were observed between 1.8 and 5 h [2-4]. Blood contained the major free form of EGCG and a conjugated form of 4’,4’’-di-O-methyl-EGCG [4], which are primarily excreted through the bile into the colon and then metabolized. However, the ring-fission metabolites were detected in urine. To our knowledge, no studies of the EGCG concentrations have been reported in human organs. In animals, sixty minutes after intragastric administration of EGCG at a dose of 500 mg/kg body weight (bw) to rats, the levels of EGCG were 12 μM in the plasma, 48 μM in the liver, 0.5 μM in the brain, 565 μM in the small intestinal mucosa, and 69 μM in the colon mucosa [5], if the density of tissues is assumed to be 1 g/cm³. When EGCG was administered to rats 2 h after a single oral dose administration of 2500 mg/kg bw and 2 h after a single intravenous dose of 50 mg/kg bw, its levels were 244 and 206 μM, respectively [6]. When [4-3H]-EGCG was orally administered to rats, results indicated that a small amount of intact EGCG was absorbed and that many of its microflora-derivative metabolites, such as EGC, 4’,4’’-di-O-methyl-EGCG, 5-(5’-hydroxyphenol)-γ-valerolactone 3’-O-β-glucuronide, and
5-(3',5'-dihydroxyphenyl)-γ-valerolactone, were detected in the blood, bile, and/or feces [7, 8]. When EGCG was administered at a dose of 100 mg/kg bw to rats by intraperitoneal injection, the plasma levels of unmetabolized EGCG, determined by HPLC, were 24, 2, 1, and 1 μM at 0.5, 1, 2, and 24 h, respectively [9]. When [3H]-EGCG was administered directly into the mouse stomach within 24 h, radioactivity was found in the small intestine, stomach, colon, liver, lung, pancreas, mammary gland, skin, brain, kidney, uterus, ovary, and testes [10]. After either intravenous (10 mg/kg bw) or intragastric (75 mg/kg bw) administration of EGCG to mice for 5 min to 12 h, the maximal plasma EGCG concentrations were detected to be 2.7 or 0.28 μM, while the maximal tissue levels of EGCG determined in the small intestine, colon, lung, liver, kidney, spleen, and prostate ranged from 0.002 to 45.2 μM [11]. Taken together, these observations suggest that EGCG has systemic effects after the intestinal absorption and that EGCG has low bioavailability, which may be dependent on the tissues, the route of administration, the number of times administered, the duration and dosage of treatment, the presence of digestive enzymes, the rate of degradation, and the techniques and assay models employed. Accordingly, the doses (1~100 μM) of EGCG (the IC50 value of this study is 20 μM) used in our study were a little bit higher than its circulating levels, but corresponded to higher tissue EGCG levels.

Supporting references
