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Capillary Electrophoretic Determination of Theanine, Caffeine, and Catechins in Fresh Tea Leaves and Oolong Tea and Their Effects on Rat Neurosphere Adhesion and Migration

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Theanine, caffeine, and catechins in fresh tea leaves and oolong tea were determined by using capillary electrophoresis (CE). CE separated these tea polyphenols from three other tea ingredients, namely, caffeine, theophylline, and theanine, within 8 min. The young leaves (apical bud and the two youngest leaves) were found to be richer in caffeine, (−)-epigallocatechin gallate (EGCg), and (−)-epicatechin gallate (ECg) than old leaves (from 5th to 7th leaves). On the other hand, the old leaves (from 8th to 10th leaves) contained higher levels of theanine, (−)-epigallocatechin (EGC), and (−)-epicatechin (EC). Results from a comparison of fresh young tea and oolong tea compositions indicated oolong tea contained more theanine and catechins than fresh young tea. Furthermore, it was found that the levels of theanine, EGC, and EGCg in young leaves rose markedly with the withering process. Caffeine did not markedly change. However, fully or partially fermented teas (oolong tea or pauchong tea) have a common initial step in the withering process. Fresh tea leaves or oolong tea extract (0.1%, w/v) markedly inhibited neurosphere adhesion, cell migration, and neurite outgrowth in rat neurospheres. Theanine (348 μg/mL) and caffeine at high concentration (50 μg/mL) did not inhibit neurosphere adhesion or migration activities, but EGCg at 20 μg/mL effectively inhibited neurosphere adhesion for 24 h. These results indicated that EGCg might affect neural stem cell survival or differentiation.

KEYWORDS: Tea; CE; catechins; theanine; caffeine; neurospheres; EGCg

INTRODUCTION

Tea is a very popular beverage, and ∼2.5 million tons of it are produced in the world annually (*1*). Most of the tea produced in Japan is green tea, whereas Taiwan and China produce both green and black teas and several different types of teas, including oolong, pauchong, and pu-er. Catechins are the most abundant polyphenols in the leaves of tea (*Camellia sinensis*). The major catechins in tea are (+)-catechin (C), (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-catechin gallate (Cg), (−)-epicatechin gallate (ECg), and (−)-epigallocatechin gallate (EGCg). All are illustrated in **Figure 1**. These compounds are potential antioxidant agents (*2*, *3*) and have numerous potentially beneficial pharmacological properties, including suppression of carcinogenesis (*4*) and tumorigenesis (*5*) and the inhibition of tumor growth or tumor metastasis (*6*).

The role of tea consumption and tea polyphenols in the prevention of cancer and cardiovascular disease has received a great deal of attention (*7*−*10*). Recently, many reports have indicated that the total catechin content is highest in young leaves and decreases markedly in old leaves (*11*). Over 300 different kinds of tea are now produced, but there are only three general forms of tea: unfermented green tea; partially fermented oolong tea, pauchong tea, and pu-er tea; and fermented black tea. Green tea is manufactured by drying fresh tea leaves and preventing oxidation of the tea polyphenols (*12*). The manufacture of black tea is characterized by a high degree of fermentation, which produces a series of chemical condensations (*13*). Oolong or pauchong tea is partially fermented, and the tea composition is partially similar to that of green tea.

Generally, fermented or partially fermented teas (oolong tea or pauchong tea) have in common the withering process. This can decrease the water level and help operation. Three things support the withering process: (a) increased quality of tea, (b) increased catechins and enzyme to form complexes, and (c)

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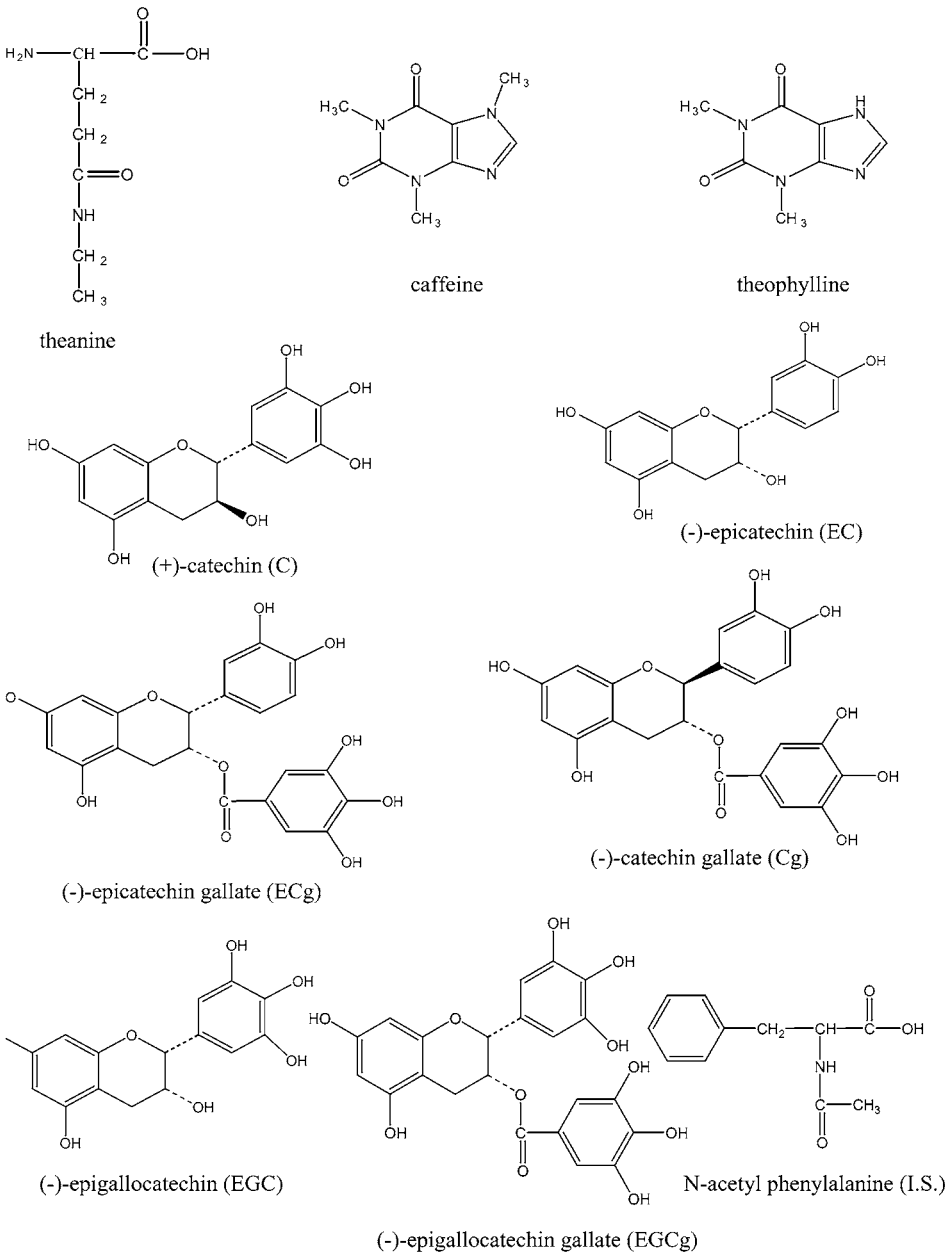

Figure 1. Chemical structures of tea components.

fermentation, which influences tea composition (*14*). The withering process is essential to the manufacture of high-quality tea. On the other hand, the withering process and fermentation bring about marked chemical changes (*15*) including (a) protein degradation to produce amino acids, (b) sugar as substrate to promote biochemical reactions, (c) increased polyphenol enzyme activity, (d) chlorophyll degradation and influence on tea color, and (e) condensations of catechins to form theaflavins, bisflavanols, and thearubigins.

Current interest in the health effects of tea and the investigation of natural materials as a source of chemopreventative agents have led to development of faster analytical methods for the determination of tea compositions. In the literature, several methods have been established based upon reversed-phase high-performance liquid chromatography (HPLC) or capillary electrophoresis (CE) for tea component analysis (*16*, *17*).

Neural stem cells are multipotential progenitor cells that are undifferentiated and capable of proliferation, self-renewal, and the production of many differentiated functional progenies (*18*). A single neural stem cell is capable of generating various kinds of cells within the CNS, including neurons, astrocytes, and oligodendrocytes. Many scientists are interested in investigating neural stem cells or neural progenitor cells from the aspects of both basic development biology and therapeutic applications for neuronal degeneration diseases. Several endogenous neurotrophic factors, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), neurotrophic factor-3 (NT-3), and platelet-derived growth factor (PDGF), have been identified. However, the therapeutic use of these neurotrophic factors was limited and unable to reach the brain after systemic administration. However, natural products (small molecules) with the ability to increase or decrease neurotrophic signaling might provide another therapeutic strategy.

In the present study, we have investigated the composition of theanine, caffeine, and tea polyphenols in various parts of fresh tea (young leaves and old leaves) in summer and in commercial oolong tea by CE analysis. We also studied tea composition change during the withering process. Furthermore, we also investigated neural stem cell adhesion and migration

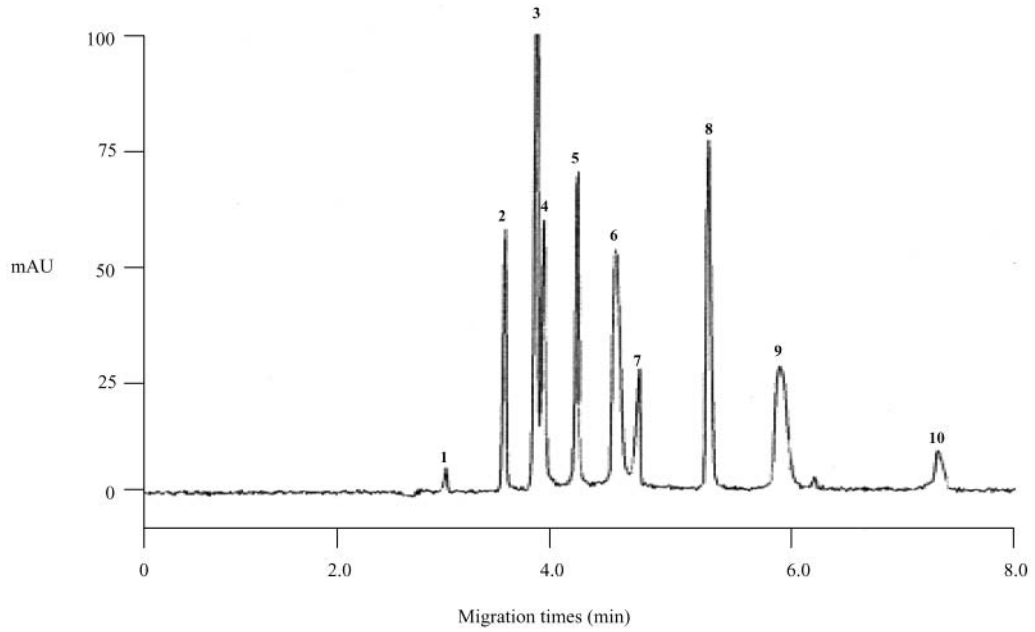

Figure 2. CE separation of standard authentic compounds. Peaks: 1, theanine (250 μg/mL); 2, catechin (250 μg/mL); 3, theophylline (250 μg/mL); 4, caffeine (250 μg/mL); 5, EGC (125 μg/mL); 6, EGCg (250 μg/mL); 7, *N*-acetylphenylalanine (internal standard, 1×10^{-6} mol/mL); 8, EC (125 μg/mL); 9, ECg (125 μg/mL); 10, Cg (25 μg/mL). Running conditions: 100 mM SDS, 25 mM phosphate, 6% (v/v) methanol, pH 7.0; separation carried out at 12.5 kV; fused-silica capillary; total length, 37 cm; length to detector, 30 cm; temperature, 20 °C; injection time, 5.0 s; detection at 214 nm.

assay by tea composition. We found that EGCg might affect neural stem cell survival or differentiation.

MATERIALS AND METHODS

Instrumentation. Electrophoresis was carried out using the Beckman P/ACE 5510 (Beckman Instruments, Fullerton, CA) with on-column detection. An uncoated fused-silica capillary column from Beckman with an internal diameter of 50 μm and total length of 37 cm was used. The effective separation length was 30 cm. The operating temperature was 20 °C. Detection was effected by measurement of diode array detector at 214 nm.

Chemicals and Reagents. Theanine, C, theophylline, caffeine, EGC, EGCg, *N*-acetylphenylalanine, EC, ECg, and Cg (the chemical structures of which are depicted in **Figure 1**) were obtained from Sigma (St. Louis, MO). Sodium dodecyl sulfate (SDS) was obtained from E. Merck (Darmstadt, Germany). The SDS was of analytical grade suitable for electrophoresis. All buffer salts were of analytical grade. All solutions were prepared with distilled water deionized with a Milli-Q system (Millipore, Bedford, MA).

Collection of Tea Leaves. In summer, we plucked fresh young tea leaves (containing the apical bud and the two youngest leaves), old leaves (from 5th to 7th leaves), and other (bud to 10th leaf) TTE12 at the Wun Shan Branch, Tea Research and Extension Station, Council of Agriculture (Taipei, Taiwan). The collected tea leaves were dried at 70 °C overnight in an electric oven with a rotating fan to keep the heat evenly distributed. The weight of the tea leaves was checked from time to time until a constant weight was reached. Several commercial oolong tea brands from different cities located in Taiwan and China were analyzed.

Preparation of Samples and Standard. Tea liquors were prepared from Taiwan oolong tea and China oolong tea samples and dried fresh tea leaves (TTE12) as 2% (w/v) tea solutions. The water extracts of dried tea leaves and oolong tea leaves were made by shaking them for 10 min in boiling hot water in a thermal flask. The extracts were then filtered through a Millex-GS 0.22 μm filter (Millipore, Malsheim, France) to remove particulate matter. All samples were spiked with the internal standard to give a final concentration of 1×10^{-6} mol/mL *N*-acetylphenylalanine. *N*-Acetylphenylalanine (30 μL, 1×10^{-5} mol/mL) was added to oolong tea samples and dried fresh tea leaves of tea solutions (270 μL) at a final concentration of 1×10^{-6} mol/mL before analysis. A separate standard solution was prepared for each analyst. All standards were dissolved in water. Theanine, caffeine, theophylline, catechin, and EGCg were prepared at 10 mg/mL and EC, EGC, and ECg at 5 mg/mL; CG was prepared at 1 mg/mL and *N*-acetylphenylalanine (internal standard) at 1×10^{-5} mol/mL.

Analytical Conditions (*17*). Dried tea leaves and oolong tea samples were analyzed with a running buffer of 100 mM SDS, 25 mM phosphate, and 6% (v/v) methanol, pH 7.0. All samples were injected pneumatically (0.5 psi) for 5 s and were analyzed with an applied voltage of 12.5 kV. At the beginning of each day the capillary was regenerated by rinsing for 5 min with water, for 10 min with 0.1 N NaOH, and for 5 min with water. Repeating five analyses of the standards after the regeneration equilibrated the column. Before each analysis, the capillary was rinsed with running buffer for 2 min. After each analysis, the capillary was rinsed for 2 min with 0.1 N NaOH and for 2 min with water. The capillary was stored in water overnight.

Neural Stem Cell (Neurosphere) Culture. Rat embryonic cortical neurons were obtained from time pregnant Wistar rats at 17 days (E17) of gestation. Embryos were recovered by C-section under Nembutal anesthetic. Dissociated cells were obtained by enzymatic digestion of minced tissues by 0.1% trypsin for 1 min at 25 °C. Following three washes with PBS, the cortex showed subsequent mechanical dissociation and was filtered through a 70 μM nylon cell strainer (Falcon, Fisher Scientific, Pittsburgh, PA). The cells were then suspended into culture medium. The growth medium consisted of B27-supplemented neurobasal medium (Gibco), penicillin G, streptomycin sulfate (1:100, Gibco), and 0.5 mM L-glutamine. The cell suspension was diluted in the B27-supplemented neurobasal medium and plated on six plastic plates to obtain a final density of 1×10^6 cells/mL. These cells were cultured at 37 °C in a humidified 5% CO_2/95% air atmosphere. Neural stem cells were grown as free-floating aggregates (neurospheres). By 4 days, primary neurospheres were generated that could undergo further migration assay.

Neural Stem Cell Adhesion Assay. Primary generated neurospheres were plated as whole spheres on six-well plastic plates coated with poly(D-lysine) (50 μg/mL, Sigma) in the presence or absence of dried fresh tea liquors and oolong tea liquors (0.1%, w/v), theanine (43.5−348 μg/mL), caffeine (10−50 μg/mL), EGCg (10−30 μg/mL), and EGF (10 ng/mL, as positive control) for 24 h. The growth medium consisted of B27-supplemented neurobasal medium (Gibco), penicillin G, streptomycin sulfate (1:100, Gibco), and 0.5 mM L-glutamine.

Table 1. Comparison of the Composition (Milligrams per Gram) of TTE 12 from Different Plucking Positions (Dried Fresh Tea Leaf from a Bud to 10th Leaf) in Summer (August 2001)[a,b]

	plucking position										
tea product	bud	1st leaf	2nd leaf	3rd leaf	4th leaf	5th leaf	6th leaf	7th leaf	8th leaf	9th leaf	10th leaf
theanine	47.3 ± 4.3	42.3 ± 2.5	26.4 ± 2.0	18.9 ± 1.4	14.2 ± 1.3	28.0 ± 1.8	49.4 ± 4.3	38.2 ± 2.2	58.4 ± 3.9	35.3 ± 2.2	54.6 ± 4.7
catechin	3.0 ± 0.2	5.1 ± 0.3	6.5 ± 0.5	5.5 ± 0.3	4.8 ± 0.3	3.3 ± 0.2	4.1 ± 0.3	3.4 ± 0.2	3.6 ± 0.3	2.8 ± 0.2	3.3 ± 0.2
theophylline	1.3 ± 0.2	1.8 ± 0.2	ND[c]	ND	ND	ND	ND	ND	ND	ND	ND
caffeine	41.7 ± 2.6	49.7 ± 4.3	45.5 ± 3.3	40.0 ± 3.8	31.3 ± 2.9	27.6 ± 2.4	31.4 ± 1.8	27.2 ± 2.3	30.3 ± 2.4	26.6 ± 1.8	28.0 ± 2.6
EGC	2.3 ± 0.05	4.1 ± 0.3	4.0 ± 0.3	2.7 ± 0.3	4.3 ± 0.3	4.1 ± 0.2	8.8 ± 0.5	7.1 ± 0.4	10.3 ± 0.7	6.2 ± 5.7	9.8 ± 0.6
EGCg	29.4 ± 1.8	27.1 ± 2.2	21.0 ± 1.3	11.6 ± 0.8	8.6 ± 0.5	8.0 ± 0.4	13.3 ± 0.8	10.5 ± 0.6	12.4 ± 0.9	9.3 ± 0.6	12.3 ± 1.1
EC	0.06 ± 0.01	1.3 ± 0.1	1.6 ± 0.2	0.5 ± 0.05	0.8 ± 0.05	0.1 ± 0.02	1.9 ± 0.2	1.3 ± 0.1	2.4 ± 0.2	0.9 ± 0.1	2.4 ± 0.3
ECg	5.2 ± 0.4	5.7 ± 0.3	4.9 ± 0.4	2.9 ± 0.2	1.0 ± 0.2	1.1 ± 0.1	1.7 ± 0.2	1.1 ± 0.1	1.3 ± 0.2	0.8 ± 0.05	ND
Cg	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

[a] Samples were prepared as described under Materials and Methods. [b] Values are averages, $n = 3$, and are given in 1 g of dry tea leaf on 50 mL of water, or 2% (w/v). [c] Not detectable.

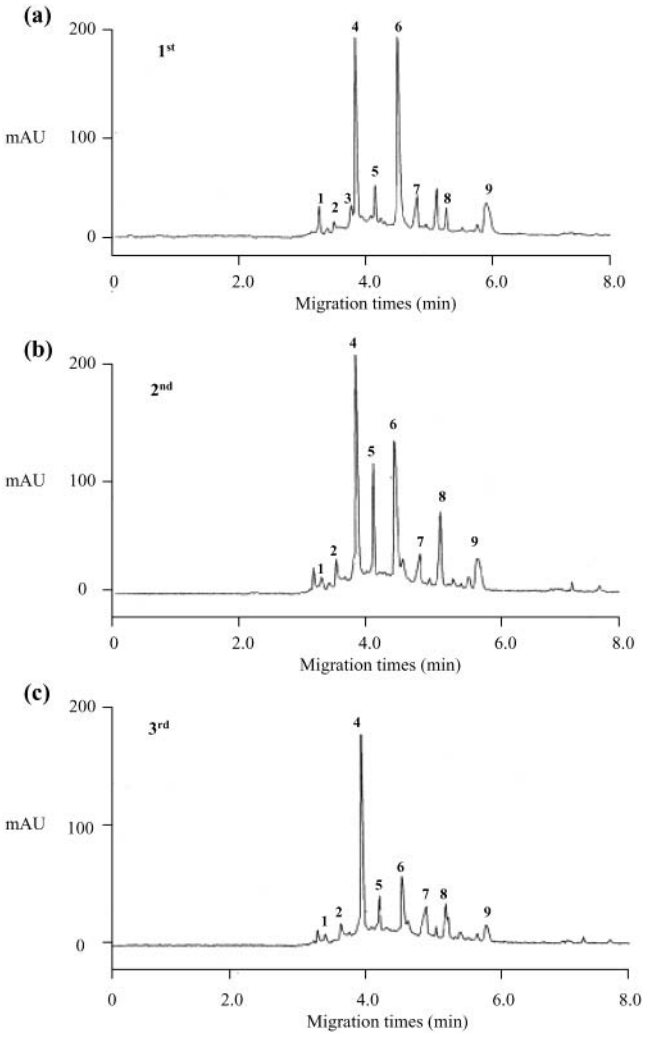

Figure 3. Electropherogram of a fresh tea leaf extract prepared from first to third leaves: (**a**) first leaf; (**b**) second leaf; (**c**) thirrd leaf. Peak identification and running condition are the same as in **Figure 2**.

Migration Assay for Neurite Outgrowth from Neurospheres. Primary generated neurospheres were plated as whole spheres on poly(D-lysine)-coated six-well plastic plates for 72 h in the presence or absence of dried fresh tea liquors and oolong tea liquors (0.1%, w/v), theanine (43.5−348 μg/mL), caffeine (10−50 μg/mL), EGCg (10−30 μg/mL), and EGF (10 ng/mL, as positive control). The growth medium consisted of B27-supplemented neurobasal medium (Gibco), penicillin G, streptomycin sulfate (1:100, Gibco), and 0.5 mM L-glutamine.

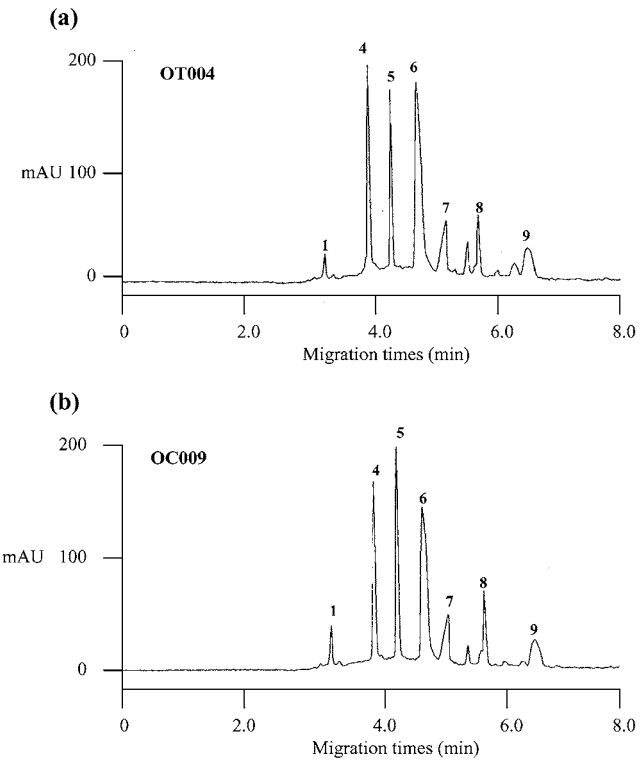

Figure 4. Analysis of Taiwan and China oolong teas: (**a**) one of the three different oolong tea-producing cities in Taiwan; (**b**) one of the three different oolong tea-producing cities in China. Peak identification and running condition are the same as in **Figure 2**.

RESULTS

Separation of Authentic Standard Tea Polyphenols, Methylxanthine, and Theanine by CE. A mixture of six catechins, including C, EGC, EGCg, EC, ECg, and Cg, two methylxanthines including theophylline and caffeine, theanine, and the internal standard (*N*-acetylphenylalanine) was separated by CE as described above, and a baseline resolution was achieved (**Figure 2**). The CE separation of these tea constituents was accomplished in 8 min.

CE Separation of Catechins, Methylxanthines, and Theanine in Fresh Tea Leaves (First to Third) of TEE12. The CE profiles of fresh tea leaves (first to thirrd) are shown in **Figure 3**. We found that from the first leaf to the third leaf the level of theanine and EGCg markedly decreased. Furthermore, we determined the tea composition change in fresh tea leaves (bud to 10th leaf) of TEE12. As shown in **Table 1**, the results indicate that theanine concentration markedly decreased between

Table 2. Levels of Theanine, Caffeine, and Catechins in Taiwan and China Oolong Teas (Milligrams per Gram of Tea)

tea	theanine	caffeine	EGC	EGCg	EC	ECg
OT004	52.8 ± 4.1	39.7 ± 3.1	12.5 ± 1.1	32.6 ± 3.0	4.0 ± 0.3	4.8 ± 0.3
OT009	60.1 ± 3.8	37.6 ± 3.5	21.8 ± 1.6	19.4 ± 0.9	6.4 ± 0.4	2.6 ± 0.2
OT011	69.9 ± 4.7	39.7 ± 4.1	26.4 ± 2.2	27.1 ± 2.5	7.8 ± 0.5	3.3 ± 0.4
OC005	20.4 ± 1.6	37.8 ± 2.7	5.8 ± 0.3	11.7 ± 1.2	0.8 ± 0.05	1.9 ± 0.2
OC009	92.0 ± 7.1	39.5 ± 3.2	18.6 ± 1.6	27.6 ± 1.8	5.9 ± 0.4	4.3 ± 0.4
OC033	80.1 ± 6.4	40.6 ± 2.7	22.4 ± 2.6	28.3 ± 2.1	7.2 ± 0.5	4.4 ± 0.3

[a] Samples were prepared as described under Materials and Methods. OT004, OT009, and OT011 are oolong tea samples purchased from Taiwan; OC005, OC009, and OC033 are oolong tea samples purchased from China. [b] Values are averages, $n = 3$, and are given in 1 g of dry tea on 50 mL of water, or 2% (w/v).

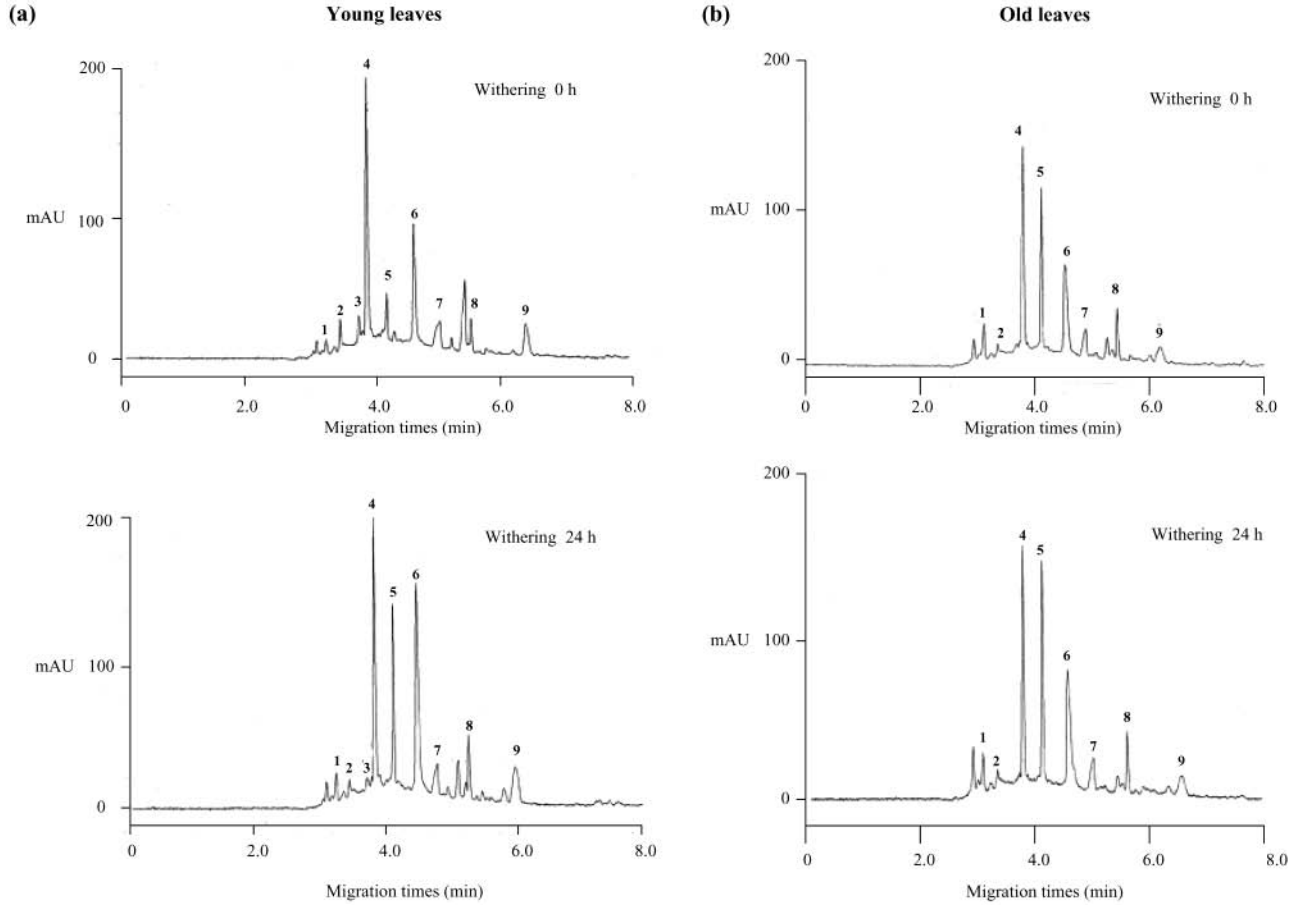

Figure 5. Analysis of fresh young leaf tea and old leaf tea composition change during a 24 h withering process: (**a**) fresh young leaves; (**b**) old leaves. Peak identification and running condition are the same as in **Figure 2**.

the bud and the 3rd leaf but markedly increased from the 8th to the 10th leaf. C concentration increased markedly from between the bud and the third leaf. Theophylline was not detectable. Caffeine decreased significantly between the bud and the fourth leaf. EGC increased markedly between the bud and the sixth leaf. EGCg decreased markedly between the bud and the fourth leaf. EC showed no significant change from the bud to the 6th leaf but markedly increased from the 8th to the 10th leaf. Cg was not detectable.

CE Analysis of Oolong Tea. Oolong tea is the most popular tea consumed in Taiwan and southern China because of its attractive aroma and taste. We chose three products from different cities located in Taiwan and China. As shown in **Figure 4**, Taiwan oolong tea (OT004) and China oolong tea (OC009) were separated by CE. From a comparison of the three different Taiwan oolong tea compositions, shown in **Table 2**, it appears that the levels of theanine and catechins including EGC, EGCg, EC, and ECg were significantly higher in comparison with fresh tea leaves (**Figure 3**). However, OC005 had the lowest level of theanine and catechins. Partially fermented tea (oolong tea or pauchong tea) undergoes an important withering process. We will further evaluate this important process that causes composition change in tea.

Evaluation of Tea Composition Change during Withering Process by CE. Fresh young tea leaves (containing the apical bud and the two youngest leaves) and old leaves (from the fifth to the seventh leaf) were collected. The fresh tea leaves were followed through the indoor withering process for 24 h. The collected tea leaves were dried at 70 °C overnight in an electric oven with a rotating fan to keep the heat evenly distributed. As shown in **Figure 5a**, the levels of theanine, EGC, EC, ECg, and EGCg increased markedly in young tea leaves during the withering process, but caffeine did not change significantly. Furthermore, in old tea leaves the levels of catechins did not significantly change, as shown in **Figure 5b**. We think only EGC partially increased. These data suggested that the withering process could be regarded as most critical to the quality of partially fermented tea product.

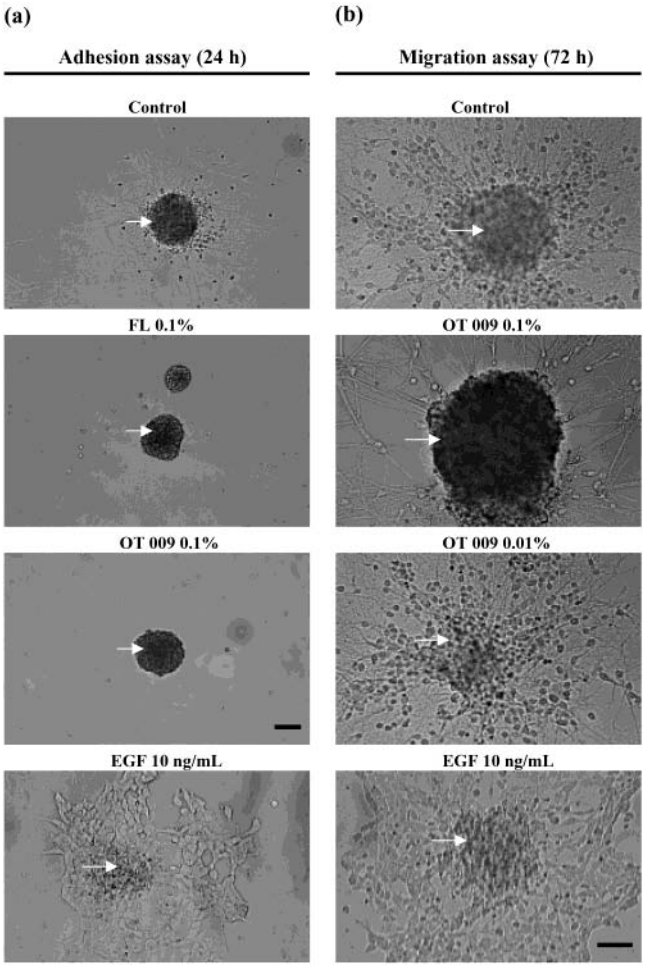

Figure 6. Fresh young leaf tea and oolong tea (OT009) extract liquors significantly inhibited neural stem cell (neurospheres) adhesion and migration from neurospheres: (**a**) neurospheres were treated with EGF (10 ng/mL, as positive control), fresh young leaf tea (FL), and oolong tea (OT009) extract liquors at a final concentration of 0.1% (w/v) for 24 h to evaluate cell adhesion; (**b**) neurospheres were plated on a poly(D-lysine)-coated, six-well plate and cultured in a serum-free B27-supplemented neurobasal medium for 24 h. They were then treated with EGF (10 ng/mL, as positive control) and a final concentration of 0.1–0.01% (w/v) oolong tea extract liquors for 72 h to evaluate cell migration. The arrow indicates the neurospheres. Scale bar = 50 μm.

Fresh Young Tea Leaves and Oolong Tea Compositions Can Influence Neural Stem Cell (Neurosphere) Adhesion and Differentiation. We isolated whole primary neurospheres and plated them on a poly(D-lysine)-coated six-well plate and cultured them in a serum-free B27-supplemented neurobasal medium containing a final concentration of 0.1% (w/v) tea liquors. We found that the untreated group can attach to the bottom of the plate and induce neurosphere cell migration and neurite outgrowth as shown in **Figure 6a**. However, fresh tea leaves or oolong tea (OT009) extract liquors markedly inhibited neurosphere attachment to the bottom of the plate and did not induce cell migration or neurite outgrowth. We used EGF (10 ng/mL) as positive control. However, OT009 (0.1%, w/v), contained a tea composition including theanine (60.1 μg/mL), caffeine (37.6 μg/mL), EGC (21.8 μg/mL), EGCg (19.4 μg/mL), EC (6.4 μg/mL), and ECg (2.6 μg/mL) as shown in **Table 2**. We evaluated the effect of oolong tea liquors on neural stem cells by migration assay. Our results indicated that OT009 at a concentration of 0.1% (w/v) significantly inhibited neurons or astrocytes or oligodendrocyte migration from neural stem cells and found neurite larger than control as shown in **Figure 6b**. However, at the lowest concentration (0.01%, w/v) we observed no influence on cell migration from neural stem cells. In this experiment, we used EGF as positive control. We remain interested in what kind of tea compositions can inhibit neurosphere adhesion or migration. We will continue to seek resolution of this question.

Inhibition of Neural Stem Cell Adhesion and Migration by EGCg. To further investigate what tea compositions can influence neural stem cell differentiation fates, we considered the possible involvement of theanine, catechins, or methylxanthine. Treatment of neural stem cells with 0−50 μg/mL of EGCg for 24 h resulted in dramatic inhibition of cell adhesion at a concentration of 20 μg/mL. However, caffeine did not influence cell adhesion at the high concentration of 50 μg/mL, as shown in **Figure 7a**. We next investigated whether EGCg has similar effects on migration activities. Our results indicated that EGCg markedly inhibited cell migration from neurospheres at concentrations of 20−30 μg/mL. However, caffeine did not inhibit neurosphere differentiation into neurons, astrocytes, and oligodendrocytes at a concentration of 50 μg/mL, as shown in **Figure 7b**. We next investigated whether theanine has similar effects on adhesion assay. Our results indicated that theanine did not inhibit neurosphere attachment to the bottom of the plate or induce neuroaphere migration or neurite outgrowth at concentrations of 43.5−348 μg/mL, as shown in **Figure 7c**. The results obtained in these experiments suggest that EGCg might influence neural stem cell differentiation fates and have a partial cytotoxicity effect at the level of 20−30 μg/mL.

DISCUSSION

Tea is one of the most popular beverages in the world because of its taste, aroma, and, lately, reported health benefits. Hundreds of different teas are now produced, mainly in Asia, including China, Taiwan, Japan, India, and Southeast Asia, and Central Africa and are exported throughout the world. Tea has been considered a crude medicine in China for more than 4000 years. Recent literature has indicated the health protective characteristics of tea, generally associated with the high level of flavonoids in the leaves and extracts, and this has contributed to the public's general attitude toward the beverage (*19*). Tea flavonoids have been reported to exhibit different kinds of pharmacological properties such as antioxidant activities (*20*, *21*), regulation of carcinogen metabolism, inhibition of cell proliferation (*22*, *23*), induction of cell apoptosis (*10*), and cell cycle arrest (*24*).

Gradient reversed-phase HPLC is the most frequently used method for the analysis of tea compositions (*25*, *26*). CE has been used to analyze compounds in tea samples, and the resolution of the analytes was much better with micellar electrokinetic chromatographic (MEKC) than with capillary zone electrophoresis (CZE) (*17*). However, the CZE method was developed to determine both catechins and theaflavins (TFs). Poor sample stability and high relative standard deviation (RSD) values were encountered when TFs were analyzed according to this method (*27*). In general, the MEKC methods provide better separation, resolution, and quantitation for a larger number of catechins than do the rudimentary CZE methods (*28*). In this paper, we modified the MEKC method from that of Aucamp et al. (*17*), which achieves the separation of the tea catechins, theanine, caffeine, and thephylline. It also provided the shortest analysis time (8 min) with good resolution than the MEKC method from Aucamp et al.

We studied the nature and variation of tea compositions, including catechins, caffeine, and theanine in the growing tea

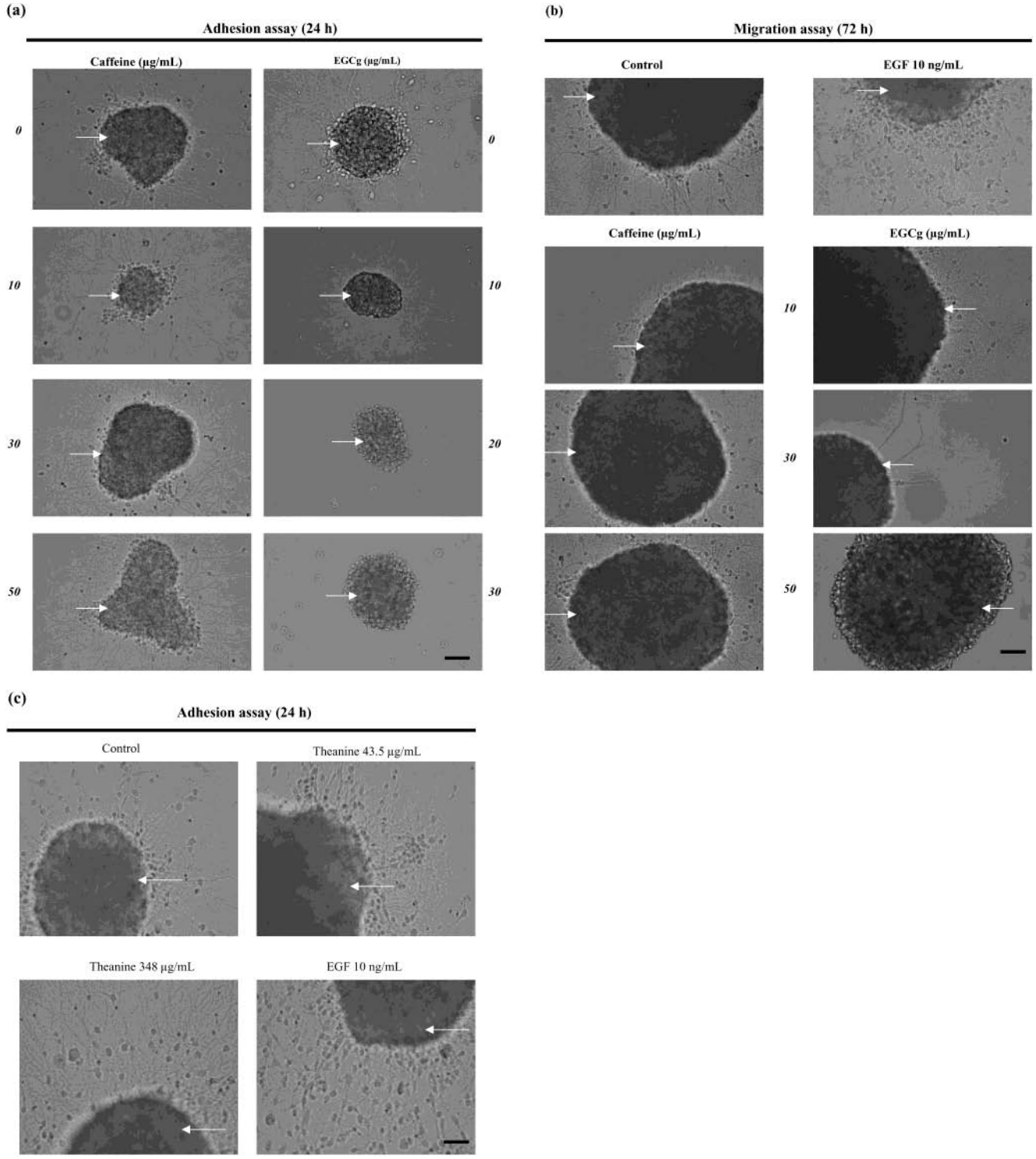

Figure 7. Dose dependence of the inhibition of adhesion and migration in neurospheres by EGCg: (**a**) neurospheres were plated on poly(D-lysine)-coated, six-well plate and cultured in a serum-free B27-supplemented neurobasal medium and then treated with caffeine or EGCg at various concentrations for 24 h to evaluate adhesion; (**b**) neurospheres were plated on poly(D-lysine)-coated, six-well plate and cultured in a serum-free B27-supplemented neurobasal medium for 24 h and then treated with EGF (10 ng/mL, as positive control), caffeine, or EGCg at a final concentration of 0–50 μg/mL for 72 h to evaluate cell migration; (**c**) neurospheres were plated on poly(D-lysine)-coated, six-well plate and cultured in a serum-free B27-supplemented neurobasal medium and then treated with theanine at various concentrations for 24 h to evaluate adhesion. The arrow indicates the neurospheres. Scale bar = 50 μm.

tree, within different parts of the plant and their level differences during processing of the unfermented green tea, the partially fermented oolong, pauchong, and pu-er teas, and the fermented black tea. We were interested in determining the tea composition change for tea leaves between the bud and 10th leaf and used CE for analysis. Our data indicated that theanine decreased markedly between the bud and 4th leaf but markedly increased from the 8th to 10th leaves. C increased markedly between the bud and 2nd leaf. Caffeine decreased markedly between the bud and 4th leaf. EGC increased significantly between the bud and 6th leaf. EGCg decreased markedly between the bud and 3rd leaf. EC showed no significant change, and ECg decreased

markedly between the bud and 4th leaf, as shown in **Table 1**. These results suggested that the tea flush (two leaves and a bud) contained high levels of theanine, C, caffeine, EGCg, and ECg. In Taiwan, the manufacture of oolong tea or pauchong tea was chosen for collection of tea flush. The amount and proportion of various tea compositions, depending on the leaf age, are directly correlated with the quality of the final beverage. We think that the finest teas are made from young tea (containing the apical bud and the two youngest leaves) shoots, which contain the highest theanine, caffeine, and catechin, including EGCg and ECg, levels.

It is estimated that ~80% of harvested tea is consumed as oolong tea in Taiwan. We used CE to analyze six commercial Taiwan and China oolong tea products. Our data indicated that only OC005 contained lower amounts of theanine and catechins than other oolong teas. We also found oolong tea contained higher amounts of catechins and theanine than fresh young tea as shown in **Figures 3** and **6**. However, caffeine did not significantly change. Interestingly, fresh young tea compositions were similar to that of the unfermented green tea; furthermore, the effect of the withering process on the manufacture of oolong tea was determined. Our results indicated that the young leaves contained EGC and EGCg levels that increased markedly during the withering process, as shown in **Figure 6a**. The old leaves contained the lowest catechins but partially increased EGC and EGCg amounts during the withering process. The results suggested that the withering process was a very important step, which helped increase the quality of the tea product.

A recent study had reported that theanine has a neuroprotective effect against glutamate-induced cell death in cultured rat cortical neurons (*29*, *30*). On the other hand, green tea extract and its main polyphenol constituent, (−)-epigallocatechin-3-gallate (EGCg), possess potent neuroprotective activity in cell culture and a mouse model of Parkinson's disease (*31*, *32*). All results suggest that the neuroprotective mechanism of EGCg against oxidative stress-induced cell death includes stimulation of PKC and modulation of cell survival/cell cycle genes. In this paper, we were interested in the effect of tea extract on neural stem cell differentiation.

Cells isolated from the embryonic CNS divide in response to epidermal growth factor (EGF) and fibroblast growth factor 2 (bFGF) while retaining the ability to differentiate into neurons, astrocytes, and oligodendrocytes. These cultures can be grown in aggregates termed neurospheres, which contain a heterogeneous mix of both multipotent stem cells and more restricted progenitor cells (*33*, *34*). We investigated the neural stem cell (neurosphere) differentiation fate treated with fresh tea leaves and oolong tea extract liquors at a concentration of 0.1% (w/v). Our data indicated that both liquors inhibited markedly neurosphere adhesion, limited cell migration from neurospheres, and inhibited neurite outgrowth as shown in **Figure 7**. Furthermore, we next questioned what kind of tea compositions influence neurosphere differentiation fates. We treated neurospheres with various concentrations of caffeine or EGCg to evaluate the adhesion or migration. Our data indicated that both theanine and caffeine at a high dose (348 and 50 μg/mL, respectively) did not inhibit neurosphere adhesion or migration activities but that EGCg efficiently inhibited neurosphere adhesion for 24 h at a concentration of 20 μg/mL. In the neurosphere migration assay, EGCg treated for 72 h significantly limited cell migration and neurite outgrowth from neurospheres. All of theses results demonstrated that EGCg might influence neural stem cell survival or neural stem cell differentiation fates at the level of 20−30 μg/mL.

The chemical composition of green tea, with regard to its major components, is similar to that of the fresh leaves of the tea plant. It contains many polyphenolic compounds, which account for up to 30% of the dry weight of green tea leaves. One cup of brewed green tea contains up to 200 mg of EGCg. A recent bioavailability study showed that frequent consumption of green tea results in high levels of EGCg in various body organs (*35*). In this paper, fresh tea leaves or oolong tea extract (0.1%, w/v) markedly inhibited neurosphere adhesion, cell migration, and neurite outgrowth in rat neurospheres. However, the oolong tea extract (OT009, 0.1%) contained EGC (21.8 μg/mL), EGCg (19.4 μg/mL), EC (6.4 μg/mL), and ECg (2.6 μg/mL) as shown in **Table 2**. The dose is close to a light brew and occurs only in the gastrointestinal tract, but not in other parts of human organs. On the other hand, if EGCg entered into the circulation system in the human body, it would exist in the conjugated form, not in the free form. All of these reasons indicated that one cup of brewed green tea every day could not damage neural stem cells in the human brain.

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