Collagen gel contraction by fibroblasts is inhibited by (-)-epigallocatechin gallate.

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Summary

In 1979, Bell *et al.* reported that fibroblasts incorporated in collagen gel induce a progressive contraction of the gel (1). This phenomenon has been considered as an *in vitro* equivalent of the connective tissue contraction that occurs during wound healing. Fibroblast-driven contraction of collagen gel is stimulated not only by serum but also by platelet derived growth factor (PDGF) (2), transforming growth factor β (3), endothelin (4), and thrombin (5).

We examined the effects of five kinds of green tea catechin on the collagen gel contraction by fibroblasts. (-)-Epigallocatechin gallate (EGCG) and (-)-epigallocatechin in the culture medium were found to inhibit the collagen gel contraction. Affinity chromatography revealed the binding affinities of EGCG for collagen gel contraction factors in serum and for human recombinant PDGF. These data indicate that EGCG inhibits collagen gel contraction by blocking the activity of PDGF and other serum factors, and indicate that EGCG is a potential agent in the treatment of the pathological contraction of scars.

Keywords
Epigallocatechin gallate (EGCG), collagen gel contraction, platelet derived growth factor (PDGF)

Introduction

Green tea catechins have been reported to show pharmacological effects such as an anticarcinogenic (6), antimetastatic (7), antioxidant (8), and antimicrobial activities (9). The green tea constituents, such as EGCG have been the major subjects of investigation on those activities. We have reported EGCG inhibits cancer cell adhesion to the endothelial cells (10), fibronectin (6), and laminin (11). It has been revealed that EGCG binds to fibronectin, fibrinogen, and histidine rich glycoprotein in blood plasma (12). EGCG suppresses cell proliferation through epidermal growth factor (EGF) receptor binding in human A431 epidermoid carcinoma cells (13). EGCG inhibits tyrosine phosphorylation of PDGF β-receptor of A172 human glioblastoma (14), and the PDGF-BB induced signaling transduction pathway in vascular smooth muscle cells (15).

In this study, we examined the effects of EGCG on collagen gels contraction induced by fibroblasts and demonstrated interaction between EGCG and PDGF.

Material and Methods

Collagen Gel Culture

A solution of acid soluble type I collagen in dilute HCl (pH 3) at concentration of 3 mg/ml was obtained from Nitta Gelatin Co. (Osaka). Solution of 16.7 volume of collagen solution (3 mg/ml), 8.3 volume of 3-fold concentrated Dulbecco's modified Eagle's medium (DMEM), 35 volume of DMEM, 20 volume of fetal bovine serum (FBS), 20 volume of the cell suspension in DMEM (2×10^5 cells/ml) were gently mixed at 4 ℃ giving the final density of 4×10^4 cells/ml. The mixed solution (4 ml) containing cells and collagen was incubated in a 6 cm culture dish at 37 ℃. Collagen gel formed was scraped off at the periphery at 60 min after starting the culture to release gels from the well. They received 4 ml of test catechin solutions at various concentrations.

Measurement of gel contraction

To measure collagen gel diameter, dishes were placed on top of a metric ruler on a dark background. Optimal visibility of gel edges was obtained by irradiating white light horizontally against the edge of the dish. Contracted gels gave well-formed disks; they showed very slight differences of diameter at various points. The average of the major and minor axes was taken as the diameter.

Serum

EGCG immobilized on agarose gel was prepared as described previously (12). FBS was loaded onto an EGCG-Sepharose 4B column. EGCG-unbound serum was collected. Serum concentration was determined by absorbance at 280 nm. After being washed with PBS, the column was eluted with PBS containing 4 M urea and 1 M NaCl, and fractions were collected. The EGCG-bound fractions thus obtained were dialyzed against 50 mM $NaHCO_3$ and were freeze-dried.

Affinity Chromatography

The binding between human recombinant PDGF-BB and EGCG was examined by affinity chromatography as follows. A PDGF-BB solution in PBS was loaded onto an EGCG-Sepharose 4B column. After being washed with PBS, the column was eluted with PBS containing 4 M urea and 1 M NaCl. PDGF in the eluates was then monitored by enzyme-linked immunoassay and Western blotting using goat anti-human PDGF immunoglobulin G, and peroxidase-conjugated rabbit anti-goat immunoglobulin G, essentially according to the method described previously (16).

Result and Discussion

We evaluated the effects of various tea catchins on collagen gel contraction by fibroblasts. The starting diameter of collagen gel was 52 mm. In the absence of catechins, gel diameter was reduced to 22.0 mm after incubation for 24 hours. In the presence of (+)-catechin, (-)-epicatechin, (-)-epicatechin gallate, (-)-epiallocatechin, and EGCG at 25 μ M, the gel diameter was reduced to 20.8, 21.7, 22.5, 24.8, and 30.3 mm, respectively. EGCG inhibited strongly serum-stimulated collagen gel contraction induced by fibroblasts. In the presence of EGCG at 0, 6.3, 12.5, 25, and 50 μ M, gel diameter was reduced to 24.3, 26.5, 27.5, 34.0, 46.0 mm, respectively. Thus, EGCG inhibited collagen gel contraction by fibroblasts dose-dependently. In this study, EGCG did not decrease the cell number in the collagen gel after 24 hours of culture (data not shown). These data suggest that inhibitory effects of EGCG on collagen gel contraction are not due to inhibition of cell growth.

In the next experiment, FBS was loaded onto an EGCG-column and separated an EGCG-bound fraction from an EGCG-unbound fraction. We evaluated the effects of these fractions on collagen gel contraction. In the presence of 5% FBS (control), the EGCG-unbound serum fraction, and the EGCG-unbound serum fraction plus the EGCG-bound serum components (375 μ g/ml), gel diameters were reduced to 30.8, 39.2, and 30.8 mm after incubation for 24 hours, respectively. The EGCG-bound serum components restored gel contraction to the same degree of control. These results indicated that EGCG bound to some collagen gel contraction factor(s) in serum.

Similarly, human recombinant PDGF-BB was loaded onto an EGCG-Sepharose column and the bound fractions were eluted with the buffer containing 4 M urea 1 M NaCl. The effluents were monitored by the enzyme-linked immunoassay (ELISA) using goat anti-human PDGF antibody. The results indicated that PDGF-BB was bound by the column (Table 1), demonstrating the binding interaction between PDGF-BB and EGCG.

Finally, we examined whether EGCG inhibited PDGF-stimulated collagen gel contraction. The starting diameter of collagen gel was 22 mm. In the absence of serum, the PDGF-BB (100

ng/ml)-stimulated collagen gel diameter was reduced to 12.8 mm after incubation for 24 hours. When PDGF-BB-stimulated collagen gel was incubated with EGCG (25 μ M) in the presence of fibroblast (2×10^5 cells/ml) for 24 hour, collagen gel diameter was only reduced to 21.0 mm. Thus, EGCG also inhibited PDGF-stimulated collagen gel contraction, and these results suggest that the binding of EGCG to PDGF prevents collagen gel contraction induced by fibroblasts.

Green tea contains significant amounts of catechins. Approximately 10-15 % of the dry weight of the green tea are catechins. Furthermore, a cup of green tea contains approximately 200 mg EGCG and the concentration of EGCG in the plasma was 0.3 μ M after drinking two to three cups of tea (17). However, it is possible that catechins are able to accumulate in tissues thereby resulting in locally and temporarily high concentrations of catechins. It may be expected that green tea and EGCG are potential drugs in the treatment of the pathological contraction of scars.

Table 1. Binding of PDGF-BB to EGCG immobilized on agarose as evidenced by ELISA.

Fraction Number	1	2	3	4	5
Absorbance (450 nm)	0.005	0.003	0.005	0.098	1.010
Fraction Number	6	7	8	9	10
Absorbance (450 nm)	0.812	0.797	0.716	0.453	0.24
Fraction Number	11	12	13	14	15
Absorbance (450 nm)	0.298	0.256	0.200	0.038	0.063

Acknowledgements

This study was supported in part by a Grant-in-aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan. Y. Suzuki is recipient of Research Fellowship for Young Scientists (10756).

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