

by endothelial and smooth muscle cells.³ Inter-cellular cell adhesion molecule-1 (ICAM-1) is another adhesion molecule that participates in leukocyte adhesion.⁴ The expression of VCAM-1 and ICAM-1 are elevated at sites of inflammation.^{5,6} Induction of these adhesion molecules in endothelial cells by inflammatory cytokines, such as IL-1 β and TNF α , is regulated at the level of gene transcription and requires the transcription factor of nuclear factor- κ B (NF κ B).^{7,8}

Green tea polyphenols consist of (-)-epigallocatechin-3-gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG), (-)-epicatechin (EC), (+)-gallocatechin (GC), and catechin (C). EGCG is the major polyphenol component of green tea. Several reports have demonstrated that green tea polyphenols have potent inhibitory properties on tumor promotion and inflammation, especially (-)-epigallocatechin-3-gallate (EGCG).⁹⁻¹¹ However, recent reports have demonstrated that black tea can be as effective as green tea in cancer chemoprevention.¹²⁻¹⁵ During the manufacture of black tea, the "fermentation" process causes green tea polyphenols to oxidize and form oligomeric flavanols, including theaflavins, thearubigin, and other oligomers. Theaflavins are a mixture of theaflavin (TF-1), theaflavin-3-gallate (TF-2a), theaflavin-3'-gallate (TF-2b), and theaflavin-3,3'-digallate (TF-3). Thearubigins comprise the most abundant phenolic fraction of black tea, and their structures are not well characterized. Among black tea components, theaflavins are generally considered to be the effective components for cancer chemoprevention. In this study, we used both black tea and green tea polyphenols to assess their inhibitory effects on IL-1 β -induced adhesion molecules in human umbilical vein endothelial cells (HUVECs). To further understand the mechanisms that underlie the inhibitory actions, the individual polyphenols, ECG, EGCG, and TF-3, were used to study their effects on IL-1 β -induced VCAM-1 protein and mRNA levels, and IKK and NF κ B activities in HUVECs.

MATERIALS AND METHODS

Materials and Cell Culture

Individual theaflavins and EGCG were purified as previously described.^{16,17} Human umbilical vein endo-

thelial cells (HUVECs) were cultured in medium 199, supplemented with 20 mmol/L HEPES, 100 mg/mL endothelial cell growth substance (Upstate, Lake Placid, NY), and 20% FBS (GIBCO, Grand Island, NY), supplemented with 5 U/mL heparin, 100 IU/mL penicillin, and 0.1 mg/mL streptomycin. Human promyelocytic leukemia HL-60 cells were grown in 85% RPMI 1640 and 15% FBS (GIBCO), supplemented with 2 mM glutamine (GIBCO). Cultures were maintained at 37 °C with a gas mixture of 5% CO₂ and 95% air.

Western Blotting

Equal amounts of total cellular protein (50 μ g) were resolved by SDS-polyacrylamide gel electrophoresis (PAGE), and transferred onto Immobilon-P membranes (Millipore, Bedford, MA) as described previously.¹⁸ The membrane was then incubated with anti-VCAM-1 or anti-ICAM-1 antiserum (Santa Cruz Biotechnology, Santa Cruz, CA), and subsequently probed with anti-mouse IgG antibody conjugated to horseradish peroxidase (Santa Cruz Biotechnology) and visualized using enhanced chemiluminescence kits (ECL; Amersham, Arlington, IL). Densities of the bands were quantitated by a computer densitometer (IS-1000 Digital Imaging System, Alpha Innotech Corp., San Leandro, CA).

Endothelial Cell-leukocyte Adhesion Assay

HL-60 cells were labeled with [³H]-thymidine by incorporating [³H]-thymidine into its DNA for 2 h, after which cells were washed by centrifugation. Confluent HUVECs in 6-well plates were washed 3 times, and labeled HL-60 cells were added to each well of the HUVECs. HL-60 cells were allowed to adhere to HUVECs by incubation at 37 °C for 0.5 h, after which unbound HL-60 cells were removed by washing. HL-60 cells bound to HUVECs were lysed with 50 Mm Tris, pH 8.0, containing 0.1% SDS, and the radioactivity (cpm) was counted using a Beckman L-600 scintillator.

Probe and Northern Blotting

Total RNA was isolated from cultured cells, and cDNA was prepared as previously described.¹⁸ Amplification of VCAM-1 cDNA was performed using the following oligonucleotide primers: 5'-AGATA-