Interaction between Chlorogenic Acid and Antioxidants

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Abstract. *The interaction between chlorogenic acid (CGA) and antioxidants was investigated by two different parameters: radical intensity and cytotoxicity induction. ESR spectroscopy shows that CGA produced radicals under alkaline condition. The CGA radical was scavenged by 100-300-fold lower concentrations of sodium ascorbate or N-acetyl-l-cysteine (NAC), whereas the ascorbate radical was not completely scavenged by CGA. The cytotoxic activity of CGA against human oral tumor cells (HSC-2, HSG) was completely eliminated by lower concentrations of sodium ascorbate or NAC, whereas that of sodium ascorbate or NAC was only slightly reduced by CGA. The present study demonstrated that CGA induces cytotoxicity by its radical-mediated oxidation mechanism and suggests the applicability of ESR spectroscopy for the screening of drug to drug interaction.*

We have reported a close relationship between radical generation and cytotoxicity in ascorbic acid (1), gallic acid (2) and dopamine (3) -related compounds. Only derivatives of these compounds, which produce radicals, were cytotoxic, whereas those which do not produce radicals were inactive. We recently found that CGA, a phenolic compound widely distributed into various plants (structure shown in Figure 1 in reference 4), induced apoptotic cell death (characterized by caspase activation and production of large DNA fragments) in human oral tumor cells. The cytotoxic action of CGA was significantly reduced by catalase (E. C. 1. 11. 1. 6.), which decomposes hydrogen peroxide (H_2O_2)(4). This suggests that CGA induces apoptosis by its prooxidant action. On the other hand, a lower concentration of CGA acts as an antioxidant, scavenging superoxide anion and hydroxyl radical (4-6) and inhibiting lipid peroxidation (7). CGA thus displays two different actions (antioxidant and prooxidant), depending upon its concentrations. We have recently found that lower molecular weight tannins (gallic acid, tannic acid, epigallocatechin gallate) reduced the radical intensity and cytotoxic activity, of sodium ascorbate, which in turn reduced those of tannins. On the other hand, high molecular weight lignin synergistically enhanced both the radical intensity and cytotoxic activity of sodium ascorbate (8). However, there has been no detailed study of the interaction between CGA and sodium ascorbate or other antioxidants. Therefore, we investigated here how two antioxidants, sodium ascorbate and N-acetyl-l-cysteine (NAC), modified the radical intensity (measured by ESR spectroscopy) and cytotoxic activity of CGA against human salivary gland tumor (HSG) and human squamous cell carcinoma (HSC-2) cell lines.

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Materials and Methods

Materials. The following reagents were obtained from the indicated companies: Dulbecco's modified Eagle medium (DMEM) (Gibco BRL, Grand Island, NY, USA); fetal bovine serum (FBS) (JRH Biosci, Lenexa, KS, USA); sodium ascorbate (Tokyo Kasei Kogyo, Ltd., Tokyo, Japan); CGA (5-caffeoylquinic acid hemihydrate) and dimethyl sulfoxide (DMSO) (Wako Pure Chem. Ind., Ltd., Osaka, Japan); 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), N-acetyl-L-cysteine (NAC) and cysteine (Sigma Chem. Ind., St. Louis, MO, USA).

Assay for radical intensity. The radical intensity of CGA and sodium ascorbate was determined at 25°C in 0.08 M $NaHCO_3/Na_2CO_3$ buffer, pH 9.5 or 0.08 M Tris-HCl buffer, pH 8.0, respectively, using ESR spectroscopy (JEOL JES RE1X, X-band, 100 kHz modulation frequency). Instrument settings: center field, 336.0 ± 5.0 mT; microwave power, 8 mW; modulation amplitude, 0.1 mT; gain, 500; time constant, 0.1 second; scanning time, 4 minutes. Radical intensity was defined as the ratio of peak height of these radicals to that of MnO (8,9).

Assay for cytotoxic activity. The cells were incubated in DMEM supplemented with 10% heat-inactivated FBS. The cytotoxic activity of CGA was determined by the MTT method and the relative viable cell number was expressed as absorbance at 540 nm of MTT-stained cells (4). 50% cytotoxic concentration (CC_{50}) was determined from the dose-response curve.

Results

ESR spectroscopy showed that CGA produced seven peaks of radicals under alkaline condition (Figure 1). Although the radical intensity of CGA increased with pH, with an optimum at 10.5 (4), pH 9.5 was used to minimize the degradation of sodium ascorbate (9). The intensity of CGA radical increased with incubation time up to 10 minutes. Changes in the radical