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Inhibition of Chlorogenic Acid-induced Cytotoxicity by $CoCl_2$

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Abstract. *Chlorogenic acid (CGA) induced apoptotic cell death in human oral squamous cell carcinoma (HSC-2) and salivary gland tumor (HSG) cell lines. CGA exhibited oxidation potential in the culture medium, as demonstrated by NO monitor. Both cytotoxic activity and oxidation potential were significantly reduced by the addition of $CoCl_2$. ESR spectroscopy showed that CGA produced seven peaks of radicals under alkaline condition, while addition of $CoCl_2$ altered the spectral pattern and diminished the radical intensity of CGA. $CoCl_2$ accelerated the CGA-induced coloration of the culture medium and modified the difference spectrum at around 325 nm, an absorption maximum characteristic of CGA. These data suggest that $CoCl_2$ induced conformational changes in the CGA molecule.*

Chlorogenic acid (CGA) has shown diverse biological activities, including anti-HIV activity (1), antioxidant activity (2-4), anticarcinogenic activity (5-8), modulating activity of cytochrome P450-linked enzyme (9, 10) and antiallergic activity (11). We have recently reported that CGA induced cytotoxicity against human oral tumor cells (human oral squamous cell carcinoma HSC-2, human salivary gland tumor HSG) (12). The cytotoxic activity of CGA was significantly reduced by various antioxidants (catalase, sodium ascorbate, N-acetyl-L-cysteine) (13), suggesting that CGA induced cytotoxicity by its pro-oxidant action. However, the mechanism of cytotoxicity induction by CGA has not yet been elucidated. We have recently found that the cytotoxic activity of CGA was significantly reduced by $CoCl_2$ (12). At present, there are at least two possibilities: one is that $CoCl_2$ may directly interact with CGA and transform it into an inactive form, while a second one is that $CoCl_2$ may induce transcription factors (4) which stimulate the gene expression of glycolytic enzymes and various growth factors necessary for cell survival. This study was undertaken to test the first possibility.

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Key Words: Chlorogenic acid, oral tumor cells, $CoCl_2$, cytotoxicity, radical, ESR, absorption spectrum, oxidation potential.

Materials and Methods

Materials. The following chemicals and reagents were obtained from the indicated companies: Dulbecco's modified Eagle medium (DMEM) (GibcoBRL) and RPMI-1640 medium (Gibco, Grand Island, NY, USA); fetal bovine serum (FBS) (JRH Biosci, Lenexa, KS, USA); CGA (5-caffeoylquinic acid hemihydrate), $CoCl_2 \bullet 6H_2O$, hydrogen peroxide (H_2O_2) and dimethyl sulfoxide (DMSO) (Wako Pure Chem. Ind., Ltd., Osaka, Japan); 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) and 2-methoxyestradiol(2-ME) (Sigma Chem. Co., St. Louis, MO, USA); herbimycin A, farnesyl thiotriazole, phorbol 12-myristate 13 acetate (PMA), 4-H-Bromo-1,2,4-oxadiazolo[3,4-d]benz(b) oxazin-1-one and 9-(tetrahydro-2′-furyl)adenine (Funakoshi Co., Ltd., Tokyo, Japan).

Cell culture. HSC-2 and HSG cell lines were maintained as monolayer cultures at 37°C in DMEM medium supplemented with 10% heat-inactivated FBS in a humidified 5% CO_2 atmosphere. When the cells were treated with CGA, they were replaced with fresh DMEM medium supplemented with 10% FBS. Human promyelocytic leukemic cells (HL-60) were maintained in RPMI -1640 medium supplemented with 10% FBS, in a humidified 5% CO_2 atmosphere.

Assay for cytotoxic activity. The cells were incubated for 24 hours in DMEM supplemented with 10% heat-inactivated FBS with various concentrations of CGA in the presence or absence of $CoCl_2$. 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 (12). The 50% cytotoxic concentration (CC_{50}) was determined from the dose-response curve.

Assay for radical intensity. The radical intensity of CGA and $CoCl_2$ was determined at 25°C in 0.1 M $NaHCO_3/Na_2CO_3$ buffer, pH 9.5, 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, 250; 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 (13, 14).

Spectral analysis. Absorption spectrum and difference spectra were measured, using the Shimazu spectrophotometer UV-2200.

Assay for oxidation potential. The indicated concentrations of samples were added to 10 ml of DMEM medium supplemented with 10% FBS