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Effects of interactions of EGCG and Cd^{2+} on the growth of PC-3 cells and their mechanisms

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Abstract

The preventive and therapeutic effects of a major component of catechins of green tea, epigallocatechin-3-gallate (EGCG), on prostate cancer have been demonstrated in many studies. It is well known that metal ions are necessary for human health, but an imbalance in metal ions metabolism can lead to many diseases including prostate cancer. Understanding the interactions of EGCG with metal ions might elucidate its mechanism in preventing and curing prostate cancer. The present study focused on the effects of Cd^{2+} and EGCG on the growth of androgen-insensitive prostate cancer cell PC-3 investigated by MTT assay, the effects of EGCG and Cd^{2+} on absorption of Cd^{2+} and Zn^{2+} by PC-3 cells were detected by atomic absorption spectroscopy (AAS), and the interactions of EGCG with Cd^{2+} were determined by distribution coefficient and UV–Vis spectroscopy detection. The results showed that Cd^{2+} suppressed viability of PC-3 cells in concentration- and time-dependent manner, and EGCG enhanced the effect of Cd^{2+} on PC-3 cells. EGCG was shown to decrease the absorption Cd^{2+} and increase the absorption of Zn^{2+} by PC-3 cells, while the effects of Cd^{2+} on the absorption of Cd^{2+} and Zn^{2+} were opposite to that of EGCG. In the presence of both EGCG and Cd^{2+}, absorption of Cd^{2+} and Zn^{2+} by PC-3 cells was dependent on concentrations of EGCG, Cd^{2+} and its order of addition. Results from the distribution coefficient determination and UV–Vis spectroscopy analysis indicated that Cd^{2+} might affect conformation of EGCG, while no complex of EGCG with Cd^{2+} was observed in the system.

Keywords: EGCG; Cd^{2+}; Zn^{2+}; Prostate cancer

1. Introduction

Prostate cancer is a disease threatening our health heavily. Epidemiological studies, animal tests, and cell culture system researches have demonstrated the putative chemopreventive effects of catechins of green tea, especially EGCG, on prostate cancer (Chung et al., 2001; Bhatia and Agarwal, 2001; Liao et al., 1995; Srivastava and Dalela, 2004; Allen et al., 2004). Some reports have indicated mechanisms of EGCG inhibiting growth of prostate cancer cells and regulating their metabolism (Yu et al., 2004; Siddiqui et al., 2004; Fang et al., 2003; Hastak et al., 2003). The proposed mechanisms have included induction of apoptosis, regulation of cell signal and cell cycle, effect on gene expression, and modulation of the structure and activities of some kinds of enzymes.

Metal ions are necessary to our health, but an imbalance in its metabolism can lead to many diseases including prostate cancer. Modulation of metal ions metabolism might be an important way to prevent and cure prostate cancer, although there are few investigations in this aspect. Many researchers have focused on the interactions of catechins with metal ions. They found that catechins could result in redox reactions or chelate with metal ions, especially

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transitional metal ions, under different conditions (Navarro et al., 2005; Hayakawa et al., 2004; Tang et al., 2004; Kumamoto et al., 2001; Yu et al., 2005). Interactions of catechins with metal ions also changed their bioactivities and metabolisms, which might be an important way that catechins could prevent or cure prostate cancer. Investigations on the effects of interactions of catechins with metal ions on the growth of prostate cancer cells might be a novel aspect determining the mechanism of catechins in preventing or curing prostate cancer.

Some metal ions, such as zinc ion, cadmium ion, selenium ion and copper ion have been linked to prostate cancer (Feustel et al., 1987; Waalkes et al., 1999; Nayak et al., 2003; Venkateswaran et al., 2004). The concentration of Zn^{2+} is known to be higher in prostate than other organs, and has been shown to be lower in cancerous prostate glands (Bataineh et al., 2002). Some papers have reported that Zn^{2+} could inhibit the growth of prostate cancer cells and prostate tumors (Liang et al., 1999; Feng et al., 2000; Iguchi et al., 1998). Cd^{2+} is toxic metal ion and an inducer to prostate cancer, and its bioactivity is contrary to Zn^{2+} in prostate gland (Martin et al., 2002; Ye et al., 2000). In normal prostate glands, the metabolisms of Zn^{2+} and Cd^{2+} are in a balance, which when disturbed might lead to prostate diseases. Zn^{2+} and Cd^{2+} have closely associated with the happening of prostate cancer. It might be one of important pathways during initiation, promotion and progression stages of prostate cancer that absorption and metabolism of Zn^{2+} and Cd^{2+} were not normally carried out in the body. But, so far it has not been clearly elucidated whether EGCG plays a critical role in the process by adjusting their absorptions, metabolisms and interactions yet. It is important to understand the mechanism of catechins in preventing and curing prostate cancer by analyzing the effects of catechins on the balance of Zn^{2+} and Cd^{2+} metabolism. In present paper, we studied the effects of EGCG and Cd^{2+} on the growth of PC-3 cells, the modulation of EGCG on absorption of Zn^{2+} and Cd^{2+} by PC-3 cells, and the mechanisms of EGCG interacting with Cd^{2+}.

2. Materials and methods

2.1. Materials

Purified preparation of EGCG was obtained from Sigma (St. Louis, MO). PC-3 cells were purchased from the Shanghai Institute of Cell Biology, Chinese Academy of Sciences. All other chemicals were of analytical grade.

2.1.1. Cell culture

PC-3 cell line was cultured in F-12 medium (GIBCO, Invitrogen Corporation, Carlsbad, CA) supplemented with 10% fetal bovine serum and 100 U/mL penicillin–streptomycin. The cells were maintained at 37 °C and 5% CO_2 in a humid environment (Shellab, Cornelius, OR).

2.2. Viability of PC-3 cells in the presence of EGCG and Cd^{2+}

We performed MTT assays to determine the effects of EGCG and Cd^{2+}on PC-3 cells growth. EGCG or $CdSO_4$ (Cd^{2+}) was dissolved in double-distilled water. Cells were plated in 96-well tissue culture plates and were treated with varying doses of EGCG or Cd^{2+} for 12, 24 and 36 h. After completion of the treatment, cells were washed with PBS and 20 μL of 5 mg/mL MTT (3-[4,5-dimethythiazol-2-yl]-2,5-diphenyltetrazolium bromide) was added to the cells and incubated for 4 h at 37 °C. The precipitated formazan was subsequently dissolved in dimethyl sulfoxide (DMSO). The absorbance (ABS) in each well was then recorded at 490 nm through a micro-plate reader (Thermal Lab system, Finland), and percentage cell viability was calculated against untreated cells:

$$\text{Cell viability } (\%) = \frac{\text{ABS}_{(\text{treatment, with cells})} - \text{ABS}_{(\text{treatment, without cells})}}{\text{ABS}_{(\text{control, with cells})}} \times 100$$

In addition, we estimated the interactions between EGCG and Cd^{2+}. The cells were incubated with either EGCG or Cd^{2+} for 30 min, thereafter, the other was added to cells, and incubated for 24 h at 37 °C continuously. Their viability was determined by MTT assay.

2.3. Effects of EGCG and Cd^{2+} on absorption of Cd^{2+} and Zn^{2+} in PC-3 cells

Cells (1×10^5) were seeded onto $2 \times 25\ cm^2$ plates and incubated with different concentrations of EGCG and Cd^{2+} for 24 h. Following this treatment, the cells were washed twice with PBS, pH 7.4, and 1 ml trypsinase was added; after 5 min, cells were collected by centrifugation (1500 r/min) at 4 °C for 5 min, and washed twice with PBS. Cells were digested with HNO_3 and $HClO_4$, and then concentrations of Cd^{2+} and Zn^{2+} were detected by flame atomic absorption spectrometry (AAS) (Spectrum Instruments, Shanghai, PR China).

2.4. Distribution coefficient and UV–Vis spectroscopy determination

Distribution coefficients of EGCG were determined with phosphate buffers and *n*-octanol in the presence of different concentrations of Cd^{2+}. EGCG was dissolved in PBS (pH 7.0) treated with different concentrations of Cd^{2+}, and vigorously mixed with *n*-octanol (w/w = 1:1). After centrifugation at 1000 r/min, EGCG contents in PBS layer were measured by HPLC (LC-2010A Shimadzu, Japan). Distribution coefficients of EGCG in the presence of different concentrations of Cd^{2+} were calculated. UV–Vis spectrum determination (Hewleet Packard, HP8453) of the PBS layer was conducted at the same time.

3. Results

3.1. Viability of PC-3 cells in the presence of EGCG and Cd^{2+}

Viability of PC-3 cells treated with different concentrations of Cd^{2+} was observed by MTT assay. Cd^{2+} suppressed growth of PC-3 cells in a concentration- and time-dependent manner (Fig. 1). At 36 h, PC-3 cells were hardly detected when concentration of Cd^{2+} was over 10 μM, and 24 h was chosen as the best time to evaluate effects of Cd^{2+} on PC-3 cells. According to our previous report, EGCG exerts optimal bioactivity in PC-3 cell culture system at 24 h, and also EGCG decreased the viability of PC-3 cells concentration- and time-dependently (Fig. 2).

3.2. Effects of interaction of EGCG with Cd^{2+} on growth of PC-3 cells

The viability of PC-3 cells reduced significantly when both EGCG and Cd^{2+} were added (Figs. 3 and 4),

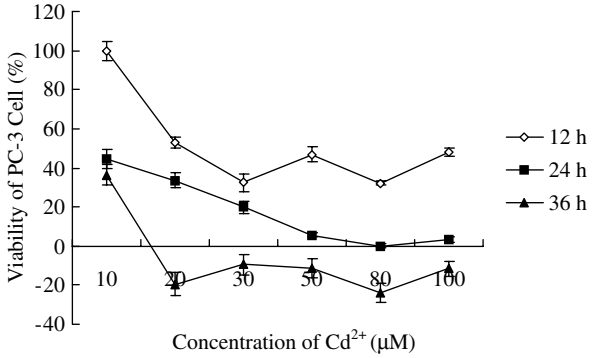

Fig. 1. Cytotoxicity of Cd^{2+} on PC-3 cells by MTT assay ($n = 7$).

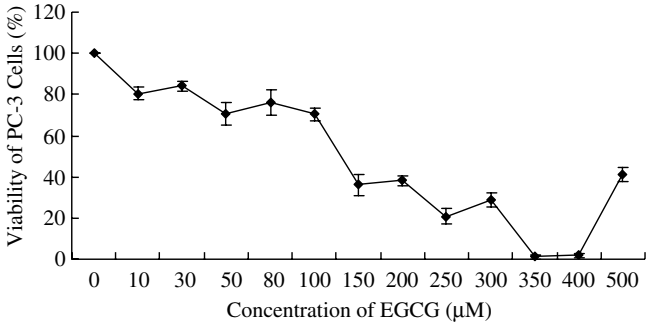

Fig. 2. Cytotoxicity of EGCG on PC-3 cells by MTT assay ($n = 7$).

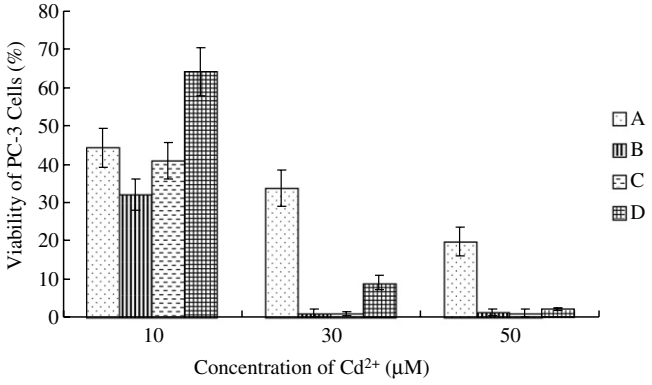

Fig. 3. Effects of Cd^{2+} on the viability of PC-3 cells in the presence of 30 μM EGCG. A, treated with different concentrations of Cd^{2+} only; B, added 30 μM EGCG, then mixed with different concentrations of Cd^{2+}; C, 30 μM EGCG and different concentrations of Cd^{2+} were added into PC-3 cells at the same time; D, added different concentrations of Cd^{2+}, then mixed with 30 μM EGCG ($n = 7$).

although their effects on the growth of PC-3 cells depended on concentration and order of addition. The toxicity of Cd^{2+} to PC-3 cells was strengthened in the presence of EGCG.

3.3. Effects of EGCG and Cd^{2+} on absorption of Cd^{2+} and Zn^{2+} in PC-3 cells

The regulation of metal ions absorption is an important aspect of the interactions of catechins with metal ions.

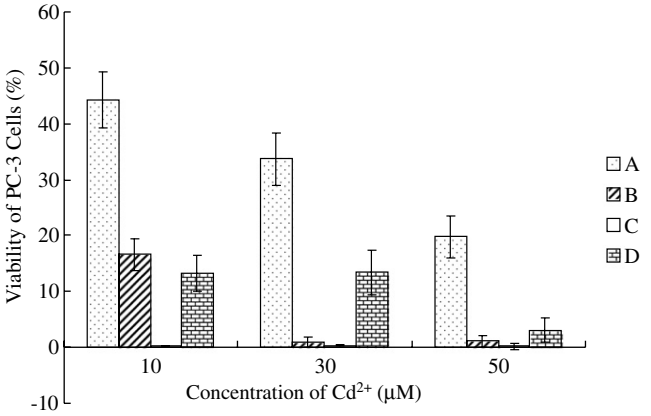

Fig. 4. Cytotoxicity of Cd^{2+} on PC-3 cells when 50 μM EGCG was added. A, treated with different concentrations of Cd^{2+} only; B, added 50 μM EGCG, then mixed with different concentrations of Cd^{2+}; C, 50 μM EGCG and different concentrations of Cd^{2+} were added into PC-3 cells at the same time; D, added different concentrations of Cd^{2+}, then mixed with 50 μM EGCG; ($n = 7$).

Cd^{2+} and Zn^{2+} are related to the initiation and progression of prostate cancer, and an imbalance in their metabolisms might be an important reason. EGCG were shown in previous reports to prevent prostate cancer, and may be involved in important mechanism that regulates the absorption and metabolism of Cd^{2+} and Zn^{2+} in PC-3 cells. We, therefore, determined the Cd^{2+} and Zn^{2+} contents in PC-3 cells treated with different concentrations of EGCG and Cd^{2+}. Interestingly, as shown in Figs. 5 and 6, Cd^{2+} treatments increase the absorption of Cd^{2+} and decrease the absorption of Zn^{2+} in a concentration-dependent manner. In addition, EGCG strongly inhibit absorption of Cd^{2+} and evidently promote absorption of Zn^{2+} in PC-3 cells concentration-dependently.

Compared to treatment with 150 μM EGCG, Cd^{2+} contents in PC-3 cells were enhanced in the presence of 50 μM Cd^{2+} (Fig. 7). Also, concentrations of Zn^{2+} in PC-3 cells treated with 150 μM EGCG and 50 μM Cd^{2+} were less than those treated with 150 μM EGCG alone and were higher than those treated with 50 μM Cd^{2+}. Cd^{2+} and

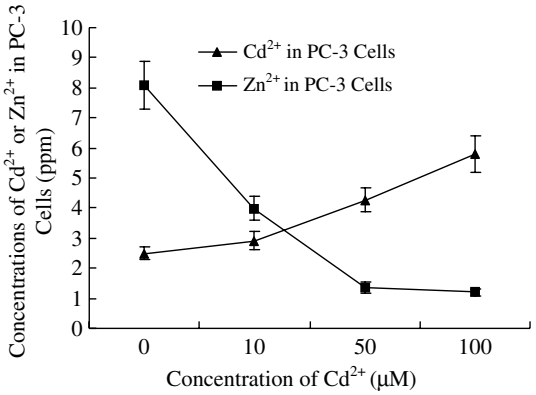

Fig. 5. Effects of different concentrations of Cd^{2+} on absorption of Cd^{2+} and Zn^{2+} in PC-3 cells ($n = 3$).

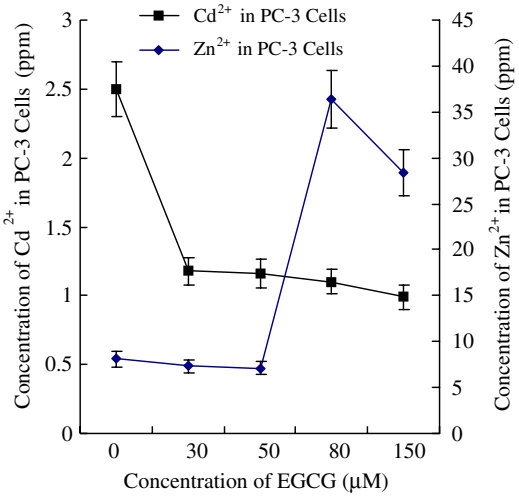

Fig. 6. Effects of different concentrations of EGCG on absorption of Cd^{2+} and Zn^{2+} in PC-3 cells ($n = 3$).

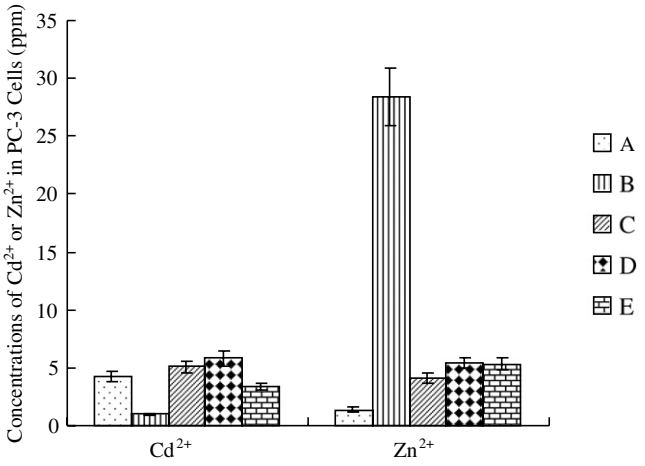

Fig. 7. Effects of EGCG and Cd^{2+} on absorption of Cd^{2+} and Zn^{2+} in PC-3 cells. A, treated with 50 μM Cd^{2+} only; B, treated with 150 μM EGCG only; C, added 50 μM Cd^{2+}, then mixed with 150 μM EGCG; D, added 150 μM EGCG, then mixed with 50 μM Cd^{2+}; E, 150 μM EGCG and 50 μM Cd^{2+} were added into PC-3 cells at the same time ($n = 3$).

Zn^{2+} contents in PC-3 cells were also shown to depend on the order of addition for EGCG and Cd^{2+}.

3.4. Distribution coefficient and UV–Vis spectroscopy analysis

The distribution coefficient of EGCG was measured in a *n*-octanol/PBS system with or without Cd^{2+}. Results showed that the distribution coefficient of EGCG was reduced in the presence of Cd^{2+}, and the effects of Cd^{2+} on it depended on concentration of Cd^{2+} (Table 1), but, in the presence of 50 μM Cd^{2+}, the distribution coefficient of EGCG was higher than in the presence of 10 μM Cd^{2+}.

Table 1
Distribution coefficient of EGCG in the presence of Cd^{2+} ($n = 5$)

	$\log K$
200 μM EGCG	1.08 ± 0.05
200 μM EGCG + 10 μM Cd^{2+}	0.79 ± 0.02
200 μM EGCG + 50 μM Cd^{2+}	0.98 ± 0.03

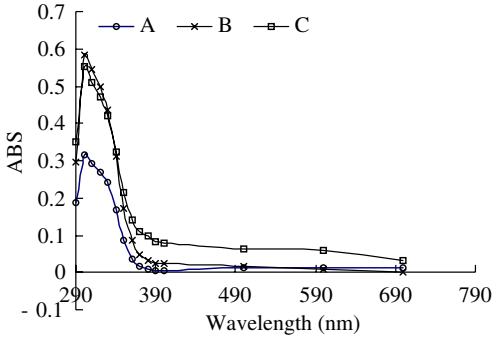

Fig. 8. UV–Vis spectroscopy of EGCG in the presence of different concentrations of Cd^{2+}. A, 200 μM EGCG; B, 10 μMCd^{2+} +200 μM EGCG; C, 50 μMCd^{2+} +200 μM EGCG.

UV–Vis measurement of EGCG dissolved in PBS system (pH 7.0) in the presence of Cd^{2+} was made. UV–Vis spectrum of EGCG was not obviously changed after Cd^{2+} was added into the system, indicating that a chelating reaction might not occur between EGCG and Cd^{2+} (Fig. 8).

4. Discussion

Cd^{2+} and its compounds were classified as "carcinogenic to humans" by IARC in 1993. This is supported by epidemiological and animal studies suggesting their carcinogenic potential on the prostate gland (Zeng et al., 2004; Misra et al., 2003; Achanzar et al., 2001). Contradictory to this notion, this paper has shown that Cd^{2+} suppressed growth of androgen-insensitive prostate cancer cell PC-3. We inferred that Cd^{2+} might exert different bioactivities with different kinds of prostate cells, including normal prostate cell, androgen-sensitive prostate cancer cell and androgen-insensitive prostate cancer cell. Past reports have pointed out that Cd^{2+} was able to activate the reporter in the absence of androgen, and this activation depended on the presence of androgen receptor (Ye et al., 2000). Cd^{2+} could enhance the androgen response when both androgen and Cd^{2+} were applied together to the reporter-transfected cells. Activation of the reporter by Cd^{2+} was not associated with cell proliferation or interleukin 6 (IL-6) production, which was proposed to be involved in cadmium-induced carcinogenesis in other experimental systems. The results indicated that Cd^{2+} had an androgen-like activity in prostate epithelial cells. Therefore, the mechanisms of Cd^{2+} affecting prostate cancer were thought to be complex, and concentrations of Cd^{2+} in prostate cancer cells or normal cells were not the sole factor.

Many reports have displayed evidences of a chemo-preventive effect of EGCG on prostate cancer (Jian et al., 2004; Thomas and Kim, 2005; Brusselmans et al., 2003). In our studies, EGCG and Cd^{2+} exerted coordinated effects

on inhibiting growth of PC-3 cells. The interactions of catechins with metal ions included two aspects: (1) affecting absorption of metal ions; (2) reacting with each other directly. Our results suggested that EGCG affected absorption and metabolism of Cd^{2+} in PC-3 cells. EGCG reduced concentration of Cd^{2+} and increased concentration of Zn^{2+} in PC-3 cell, indicating that disruption of the metabolic balance of Cd^{2+} and Zn^{2+} might be a mechanism of EGCG to inhibit growth of PC-3 cells. When EGCG and Cd^{2+} were coexistent, Cd^{2+} contents in PC-3 cells were increased and viability of PC-3 cells was declined, indicating that the mechanism of EGCG in inhibiting growth of PC-3 cells might change in the presence of Cd^{2+}. Also, distribution coefficients of EGCG were changed in the presence of Cd^{2+}, which was not induced by ion intensity because it is not concentration-dependent. Through UV–Vis spectroscopy, no complex was detected in the system. Cd^{2+} might affect conformation and absorption of EGCG.

Prostate epithelial cells accumulated the highest zinc levels compared to other cells in the body. Evidence suggests that Zn^{2+} plays a critical role in the normal function and pathology of the prostate gland, and might be a good marker for diagnosing prostate cancer (Lekili et al., 1991). Regulating absorption and metabolism of Zn^{2+} through EGCG and Cd^{2+} could be an important strategy to affect growth of PC-3 cells. Cd^{2+} could decrease Zn^{2+} contents in PC-3 cells, while the concentration of Zn^{2+} was increased in the presence of EGCG. Regardless of Cd^{2+} or EGCG was used to treat the cells, the viability of PC-3 cells was decreased. This might imply that EGCG and Cd^{2+} affected the growth of PC-3 cells, but in different ways.

Therefore, we concluded that EGCG and Cd^{2+} significantly inhibited the growth of prostate cancer cells, and the inhibition by EGCG and Cd^{2+} might be mediated mainly through the suppression of absorption of Zn^{2+} and Cd^{2+}. More particularly, in the presence of EGCG and Cd^{2+} together the inhibitory effects were quiet different from that of their effects presented alone. It might have something to do with the formation of new compounds between EGCG and metal ions. This finding provided another possible mechanism for the effects of EGCG on the preventive protection against prostate cancer. Further studies are needed to characterize the interactions between EGCG and metal ions.

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