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Mini-review

Green tea: An effective synergist with anticancer drugs for tertiary cancer prevention

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ABSTRACT

Green tea is now an acknowledged cancer preventive in Japan. Based on evidence that colorectal adenomas and prostate cancer in humans have been prevented, we review here the concept that the combination of anticancer drugs with green tea catechin synergistically induces apoptosis of human cancer cells, inhibits tumor formation in mice, and enhances inhibition of tumor growth in xenograft mouse models. As a molecular mechanism by the combination, the induction of *growth arrest and DNA damage-inducible* 153 *(GADD*153, *CHOP)* gene expression is discussed in relation to death receptor 5 and TRAIL-apoptotic pathway. The combination of anticancer drugs with green tea could be a new cancer therapeutic strategy in humans.

1. Introduction

In 2002, we were pleased to publish a Mini-review entitled "green tea: cancer preventive beverage and/or drug" in this journal [1]. We demonstrated in 2008 that consuming the equivalent of 10 Japanese-size cups (120 ml/cup) of green tea daily (equivalent to 2.5 g of green tea extract) significantly (50%) prevented the recurrence of metachronous colorectal adenomas in patients: Our strategy consisted of a daily green tea beverage fortified by green tea tablets, a dried form of green tea infusion [2]. One tablet of 500 mg green tea extract contains 52.5 mg (-)-epigallocatechin gallate (EGCG), 34.6 mg (-)-epigallocatechin, 12.3 mg (-)-epicatechin, 11.1 mg (-)-epicatechin gallate, and 15.7 mg caffeine [2]. The catechins found in 10 Japanese-size cups of green tea extract are a tolerable dose for most Japanese, and have not shown any adverse effects [3]. In the USA, a phase I trial of oral green tea extract in adult patients was designed to determine the maximum tolerable dose, which was 4.2 g/m^2 once daily (equivalent to 25–30 Japanese cups) or 1.0 g/m^2 three times daily (equivalent to 7–8 Japanese cups). The side effects of the dose were caffeine related, and included polydipsia and urinary frequency [4]. The maximal plasma EGCG concentrations of three people given green tea extract from 2.2 mg/m^2 to 4.2 mg/m^2 were 100–225 ng/ml at 1–3 h after its administration, which returned to 50% concentration after 6–8 h [4]. The prevention of prostate cancer development in patients with high-grade prostate intraepithelial neoplasia using capsules of green tea catechins was reported in Italy in 2006 [5]. And the results of a 2009 phase II clinical prevention trial reported that capsules of green tea extract effectively prevented high-risk oral premalignant leukoplakia in patients [6]. Since green tea has become an acknowledged cancer preventive in Japan, we raised the following two questions: Is it advantageous for Japanese cancer patients to take anticancer drugs and cancer preventive green tea together? Does the combination have the potential to enhance efficacy, and to decrease the adverse effects, of the drugs?

Our experiment first demonstrated that the combination of sulindac/tamoxifen with EGCG induced synergistic/additive effects, such as induction of apoptosis in human lung cancer cell line PC-9, inhibition of cell growth, and inhibition of tumor necrosis factor α (TNF-α) release from BALB/3T3 cells treated with a tumor promoter okadaic acid [7]. Since TNF-α is considered to be an endogenous tumor promoter, inhibitions of TNF-α release from cells and TNF-α gene expression in the cells are key mechanisms in cancer prevention [8]. Next we found significant inhibition of intestinal tumor formation in multiple intestinal neoplasia (Min) mice through the combination of sulindac with green tea extract [9]. Enhanced effects were also confirmed by the combination of 5-fluorouracil (5-FU) with EGCG, which inhibited the proliferation of human head and neck squamous cell carcinoma cell lines, YCU-N861 and YCU-H891 [10]. Recently the combination of anticancer drugs with EGCG has been well accepted by numerous research groups, which have reported *in vitro* molecular

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therapeutic effects on human cancer cell lines and on the inhibition of tumor growth in *in vivo* xenograft mouse models implanted using human cancer cell lines. The therapeutic effects of the combination have been further extended to estrogen receptor α negative (ERα−) and positive (ERα+) human breast cancer cells [11], and anticancer drug-resistant and -sensitive cells of human cancer [12,13].

This Mini-review presents our initial experimental results with the inhibition of tumor development through the combination of anticancer drugs with EGCG, followed by recent results on the synergistic growth inhibition of tumors in xenograft mouse models implanted using human cancer cell lines of the lung, breast, prostate, liver and stomach. We first found that the combination enhanced the synergistic induction of *growth arrest and DNA damage-inducible* 153 (*GADD*153, *CHOP*) gene expression, an essential molecular mechanism involved in synergistic enhancement [14]. This new molecular mechanism is further discussed in relation to death receptor (DR) and TNF-related apoptosis-inducing ligand (TRAIL)-apoptotic pathway. All of the results strongly indicate that the combination is a new cancer therapeutic strategy that will expand cancer therapeutic effects on various cancers following treatment for tertiary cancer prevention [15].

2. Synergistic induction of apoptosis and *GADD*153 gene expression through the combination of anticancer drugs with EGCG

The treatment of PC-9 by combining 10 μM celecoxib, a cyclooxygenase-2 selective inhibitor, with 100 μM EGCG for 40 h induced the cells of sub-G_1 (apoptosis-phase), 44.6% of apoptotic cells, whereas those with celecoxib alone, and EGCG alone, induced only 3.0% and 6.1%, respectively. Isobolographic analysis of these results clearly indicated that the combination synergistically induced apoptosis of PC-9 ($D = 0.75$), as well as human lung cancer cell lines A549 and ChaGo K-1 (data not shown). PC-9 has a p53 mutation and A549 has wild type p53 [16,17], so apoptosis is induced by p53-independent pathway. The treatment of PC-9 by combining 50 μM sulindac, a cyclooxygenease inhibitor, with 100 μM EGCG for 24 h induced 11.2-fold expression of *GADD*153 (*CHOP*) gene, whereas those with celecoxib alone, sulindac alone, and EGCG alone, resulted in only 1.3, 1.1, and 3.0-fold expression, respectively [18]. This finding is supported by evidence that high upregulation of *GADD*153 gene expression is directly involved in the regulation of apoptosis [19].

The treatment of PC-9 via a combination of 50 μM sulindac with 100 μM EGCG for 40 h induced apoptosis in 42.9% of the cells, whereas those with sulindac alone, and EGCG alone, induced only 4.1% and 6.1% [18].

3. Enhanced inhibition of tumor development through the combination of anticancer drugs with EGCG

3.1. Inhibition of intestinal tumor formation in Min mice by combining sulindac with green tea extract

Min mice have a germline mutation of the murine *adenomatous polyposis coli* (*Apc*) gene and spontaneously develop intestinal tumors similar to those of familial adenomatous polyposis (FAP) patients [20]. Treatment of Min mice with sulindac in drinking water (84 mg/L) resulted in a decreased average tumor load [21]. In our experiments, Min mice were fed CE-2 diet with 0.03% sulindac and took water containing 0.1% green tea extract for 10 weeks: All mice ingested about 5 mg sulindac and 10 mg of green tea extract per day. At 16 weeks of age, the average number of tumors per mouse in the control group that was fed CE-2 diet without sulindac and green tea extract was 72.3 ± 28.3, and those of the groups treated with the combination, sulindac alone, and green tea extract alone, were 32.0 ± 18.7, 49.0 ± 12.7, and 56.7 ± 3.5, respectively. The combination showed a decrease of 55.7% of the average number of tumors per mouse. Histologically, the control group produced 10.8% adenocarcinomas, whereas the three groups treated with the combination, sulindac alone, and green tea extract alone, induced only adenomas [9]. Min mice treated with the combination showed an increase in body weight, as did those treated with sulindac alone and green tea extract alone, indicating that the treatment with the combination did not have any toxic effects.

3.2. Inhibition of lung tumor formation in A/J mice by combining celecoxib with green tea extract

4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced pulmonary adenoma in A/J mice is an appropriate model for studying the cancer preventive activity of the compound in the lungs. It was reported in 1992 that treatments with 0.6% decaffeinated green tea extract or 0.6% decaffeinated black tea extract reduced the percentage of tumor-bearing mice with NNK from 96.3% to 65.5% or 89.3%, respectively [22]. In our experiments, female A/J mice were given an injection of NNK (100 mg/kg, i.p.), and 3 days later mice began treatment with a combination of 0.05% celecoxib in their diet and 0.3% green tea extract in their drinking water for 16 weeks. The tumor incidence and average number of tumors per mouse for the group treated with NNK alone were 100% and 3.2 at 16 weeks of the experiment. The combination significantly reduced tumor incidence from 100% to 73.3%, a 26.7% inhibition and the average number of tumors per mouse from 3.2 to 1.1, a 65.6% inhibition [15].

4. Enhanced inhibition of tumor growth in xenograft mouse models

4.1. Tumors implanted using human lung cancer cell lines

Human non-small cell lung carcinoma (NSCLC) cell line H460, which had acquired resistance to the epidermal growth factor receptor (EGFR) inhibitor erlotinib, was injected into male severe combined immunodeficient (SCID)/bg mice, in order to establish tumors in xenograft mouse model. Three days after implantation, the mice were treated with a combination of erlotinib (10 mg/kg, p.o.) and EGCG (15 mg/kg, p.o.), erlotinib alone, EGCG alone, and 2% Tween-80 vehicle as a control, via gavage, on dosing schedule of 5-days-on, 2-days-off. At day 22 of the experiment, the average tumor volumes of the mice treated with the combination, erlotinib alone, EGCG alone and the vehicle were 382, 625, 730 and 828 mm^3, and the average tumor weights were 0.35, 0.60. 0.56 and 0.64 g, respectively: The values were estimated from the published Fig. 6 (A and B) [13]. The combination significantly reduced both tumor volume ($P = 0.03$) and weight ($P = 0.011$) versus control, whereas administrations of erlotinib alone and EGCG alone did not show any statistically significant effects. Thus, EGCG seems to sensitize erlotinib-resistancy, resulting in the inhibition of tumor growth *in vivo*, and EGCG is known to inhibit cell proliferation of the erlotinib-sensitive cell line (H2122). The results with other human NSCLC cell lines showed that the combination induced greater inhibition of cell growth than EGCG alone in H2122 and H358 cells [13].

Athymic nude mice were treated with a combination of erlotinib (50 mg/kg) and EGCG (125 mg/kg), erlotinib alone, EGCG alone, and 1% Tween-80 vehicle control via gavage for 7 days, before the implantation of human squamous cell carcinoma of the head and neck (SCCHN) cell line Tu212 in a xenograft mouse model. The