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Green tea catechins (EGCG and EGC) have modulating effects on the activity of doxorubicin in drug-resistant cell lines

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The chemopreventive effect of polyphenols from green tea [e.g. (−)-epigallocatechin gallate (EGCG) and (−)-epigallocatechin (EGC)] against cancer has been demonstrated in several studies. The aim of this investigation was to prove whether these compounds modulate the activity of antineoplastic drugs. Therefore, the influence of EGCG and EGC was tested on doxorubicin-resistant murine sarcoma (S180-dox) and human colon carcinoma (SW620-dox) cell lines. Both substances showed a sensitizing effect on the cell lines if they had been treated with doxorubicin. These results suggest that protein kinase C may be inhibited by EGCG and EGC, and this may lead to a reduced expression of some drug resistance related proteins.

Key words: Doxorubicin, (−)-epigallocatechin gallate, (−)-epigallocatechin, green tea, modulation.

Introduction

Consumption of green tea, made from unfermented leaves of *Camellia sinensis* has been shown to afford protection against carcinogenesis of the esophagus, fore stomach, duodenum, colon, liver and lung[1–3] in humans. The chemopreventive effect against cancer has also been demonstrated in mouse and rat models.[4–6] The main responsible components of green tea are some polyphenols, especially flavonols from the catechin-type, in particular (−)-epigallocatechin gallate (EGCG) and (−)-epigallocatechin (EGC). It has been assumed in several studies that these compounds reveal their chemopreventive activity by inducing antioxidant and free radical scavenging enzymes, which protect the cell (i.e. DNA) from damage by peroxides and the superoxide anion. Furthermore, it has been reported that the polyphenolic compounds EGCG and EGC inhibit protein kinase C (PKC), which is also often induced after exposure to carcinogens.[7]

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Both radical scavenging enzymes and one of the key enzymes of signal transduction, PKC, are involved in the response of tumor and leukemic cells to antineoplastic drugs. Overexpression of radical scavenging enzymes (catalase, glutathione peroxidase and glutathione-S-transferase) has been shown to diminish the sensitivity of cells to antineoplastic agents, since these enzymes detoxify cytotoxic radical metabolites of the drugs.[8–10] PKC activates the transcription factor AP1 by N-terminal phosphorylation and C-terminal dephosphorylation of c-*jun*.[11,12] Several genes of drug resistance-related proteins contain an AP1-binding-sequence[13,14] and hence may be down-regulated if PKC is inhibited. The most important resistance protein, the membrane efflux pump P-glycoprotein (Pgp), the detoxifying enzyme glutathione-S-transferase π and the target protein of anthracyclines and epipodophyllotoxins topoisomerase IIα are substrates for phosphorylation of PKC, and the activity of these proteins may be altered by phosphorylation.[15–17] Therefore, the antioxidative components of green tea may reduce the activity of antitumor drugs by induction of radical scavenging enzymes; however, inhibition of PKC may lead to a reduced expression or activity of some drug resistance related proteins which may result in sensitizing cells to certain antineoplastic agents.

The aim of our study was to investigate whether the main phenolic components of green tea, i.e. EGCG and EGC, influence the activity of the antineoplastic drug doxorubicin. This drug is one of the most effective anthracycline antibiotics with a broad antitumor spectrum. Its effect is partly caused by production of radical toxic metabolites and through cleavage of DNA by formation of the ternary topoisomerase II–DNA–doxorubicin complex. Doxorubicin is also a substrate for the membrane pump Pgp and for glutathione-S-transferase π, and represents therefore an appropriate drug for our purpose.

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Material and methods

Drugs

Doxorubicin (Pharmacia, Freiburg, Germany) was used as antineoplastic drug and two polyphenolic components of green tea, i.e. EGCG and EGC (both from Sigma, München, Germany), were proved as modulators of doxorubicin resistance.

Tumor cells

A doxorubicin-resistant murine sarcoma 180 (S180-dox) cell line and a doxorubicin-resistant human colon carcinoma cell line (SW620-dox) were investigated. Cells were grown as monolayer cultures in RPMI 1640 medium (Biochrom, Berlin, Germany) supplemented with 10% fetal calf serum, 1% L-glutamine and antibiotics. Generation of the doxorubicin-resistant S180-dox subline has been described earlier.[18] SW620-dox cells were kindly provided by CC-K Chao (Chang Gung Medical College, Taoyuan, Taiwan). Resistance to doxorubicin was preserved by incubation of cells in 1 μg doxorubicin/ml culture medium (SW620-dox) or 50 μg doxorubicin/ml (S180-dox) 2 days after seeding for 2 days. Before an experiment, cells were grown at least for 7 days without doxorubicin.

Growth inhibition assays

Determination of the effect of EGCG and EGC on cell growth was done by seeding aliquots of 10 000 cells in 24-well plates (Becton Dickinson, Heidelberg, Germany). After 2 days EGCG and EGC at different concentrations were added. Seven days after application of EGCG or EGC the cells were electronically counted with a cell counter (Coulter, Krefeld, Germany). Three independent samples were each counted three times. For determination of the modulating effect of EGCG and EGC doxorubicin was added 2 days after seeding and EGCG or EGC at various times (24–48 h after seeding).

Results

In preliminary experiments (data not shown), we found that EGCG and EGC inhibit growth of S180 cells and SW620 cells (S180-dox and SW620-dox) which were resistant to doxorubicin. The growth of S180-dox was reduced at concentrations higher than 10 μg EGCG/ml and 5 μg EGC/ml, and completely stopped at concentrations higher than 50 and 40 μg/ml, respectively, 6 days later. For SW620-dox we found similar results. Preliminary experiments revealed also that the best modulating effect was achieved if cells had been treated with EGCG or EGC 24 h after seeding and 24 h prior doxorubicin application. Cells treated with EGCG or EGC 45 h after seeding (i.e. 3 h prior doxorubicin application) or 48 h after seeding (i.e. simultanous application of EGCG or EGC and doxorubicin) showed no effect.

Therefore, in further experiments EGCG and EGC were applied 24 h after seeding and 24 h later cells were treated with doxorubicin. Our investigations revealed that the appropriate doxorubicin therapy was 5 μg/ml for S180-dox and 1.5 μg/ml for SW620-dox. For the best modulating effects we used 20 μg EGCG for S180-dox and 10 μg EGCG for SW620-dox. Ten micrograms EGC were appropriate for S180-dox to obtain a modulating effect and 5 μg for SW620-dox cells. In S180-dox cells the number of EGCG and doxorubicin treated cells was 34% of the control versus 99% treated with doxorubicin and 56% with EGCG alone. A sensitizing effect was also found in EGC-treated cells. Samples treated with EGC and doxorubicin were diminished to a level of 10% of the control versus 83% treated with doxorubicin and 44% with EGC alone (Figure 1). These effects were obviously more than additive. Similar results were obtained for SW620-dox cells. A significant reduction in the growth of SW620-dox was observed after treatment with EGCG and doxorubicin (45% of the control) versus EGCG (75% of the control) or doxorubicin (91% of the control) alone. SW620-dox cells were also sensitized to doxorubicin by administration of EGC. The number of cells treated with EGC and doxorubicin was diminished to a level of 63% of the control versus 96% treated with EGC or 92% with doxorubicin alone (Figure 1).

Discussion

In this study we described the effect of the main green tea catechins EGCG and EGC on the proliferation of a murine S180 and a human SW620 drug-resistant cell line. We also investigated whether these substances modulate the resistance to the cytostatic effect of doxorubicin.

It has been frequently reported that green tea extracts and isolated components of green tea leaves have inhibitory effects on chemical-induced carcinogenesis and tumor growth. To our knowledge there exists no study about the effect of EGCG or EGC on

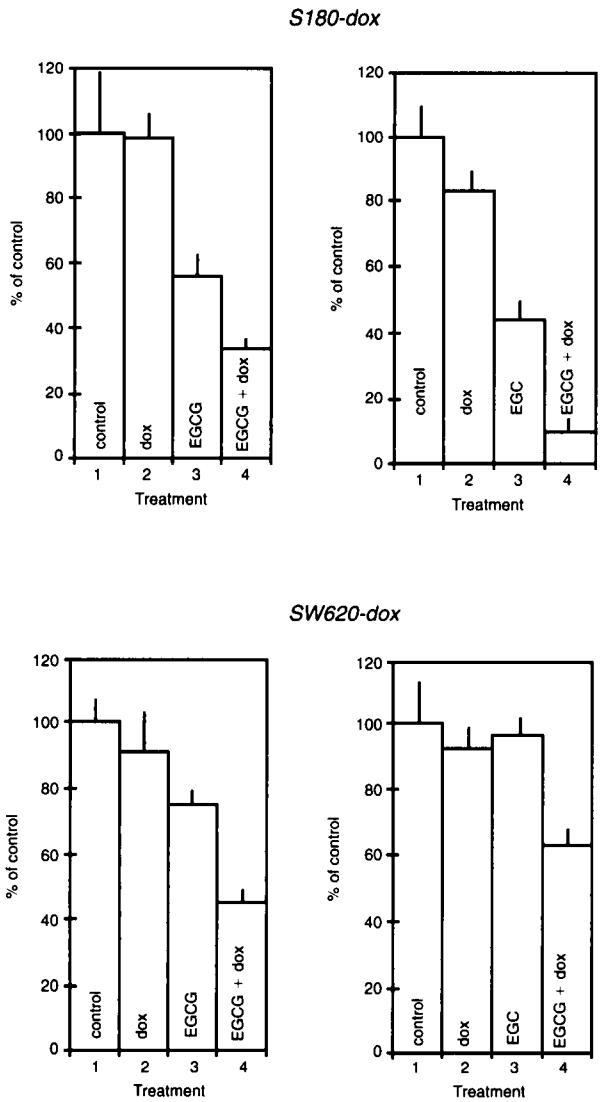

Figure 1. Modulation of the resistance to doxorubicin after EGCG or EGC application relative to the control ± SD. *S180-dox:* cells were treated 24 h after seeding with 20 μg/ml EGCG (or 10 μg/ml EGC) and 48 h after seeding with 5 μg/ml doxorubicin. *SW620-dox:* Cells were treated 24 h after seeding with 10 μg/ml EGCG (or 5 μg/ml EGC] and 48 h after seeding with 1.5 μg/ml doxorubicin.

the antineoplastic activity of doxorubicin from the biochemical modulation view point. Sadzuka *et al.* demonstrated that theanine, another but chemically not related component of green tea leaves, enhanced the inhibitory effect of doxorubicin on tumor growth.[19] We also found that EGCG and EGC sensitized S180 and SW620 doxorubicin-resistant cells to the cytostatic activity of doxorubicin.

However, we have to review these findings critically. In consideration of hitherto existing data we assume that application of green tea catechins is twoedged. On the one hand, EGCG increases expression of certain resistance-related enzymes and tea phenols itself shows antioxidative activity.[20–22] Additionally, the influence of the inhibitory effect of EGCG and EGC on proliferation has to be considered, since diminished proliferation is connected with reduced topoisomerase IIα expression and therefore with diminished cytotoxic potential of doxorubicin. These findings suggest that administration of EGCG and EGC might increase resistance to chemotherapeutic agents. On the other hand, inhibition of the PKC pathway might not only lead to reduced expression of Pgp and other resistance proteins but also to a diminished phosphorylation of these proteins, which results in less activity. It has also been shown that EGCG inhibits okadaic acid,[5] which is responsible for the inhibition of protein phosphatases 1 and 2A. Since these enzymes dephosphorylate Pgp[23] EGCG may sensitize cells to drugs over the okadaic acid pathway.

Taken together, we found a modulating effect of EGCG and EGC on the sensitivity of human SW620 and S180 doxorubicin-resistant cells to doxorubicin; however, further investigations about the mechanism of action are necessary. The intriguing results confirm us to extend our investigations to the expression and activity of proteins responsible for drug resistance (in particular Pgp, topoisomerase IIα, glutathione-S-transferase, glutathione peroxidase and catalase) after application of green tea catechins in several human tumor cell lines.

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