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(-)-Epigallocatechin-3-gallate downregulates Pg-P and BCRP in a tamoxifen resistant MCF-7 cell line

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ABSTRACT

We investigated the anticancer effect of EGCG treatment on a breast carcinoma cell line resistant to tamoxifen (MCF-7Tam cells). As there are no reports about the molecular mechanisms implicated in EGCG treatment of tamoxifen resistant breast carcinoma cells, we studied the effects of EGCG treatment on three plasma membrane proteins that are involved in the mechanism of drug-resistance: Multidrug Resistance Protein (MRP1), P-glycoprotein (P-gp) and Breast Cancer Resistance Protein (BCRP). EGCG treatment (10-100 μg/ml for 24-72 hours) caused cell growth inhibition and dose-dependent apoptosis: after 100 μg/ml EGCG treatment for 24 hours, Bax expression increased and Bcl2 expression decreased ($p < 0.05$). Coherently, Annexin V-FITC apoptosis assay detected a significant increase in labelled cells ($p < 0.05$). EGCG did not affect MRP1: in contrast, 100 μg/ml EGCG administration caused P-gp decrease to 53% of control cells ($p < 0.001$) and this effect was not due to downregulation of P-gp gene expression. EGCG induced P-gp decrease even when MG132, a strong proteasome inhibitor, was given together with EGCG to MCF-7Tam cells. EGCG treatment also inhibited BCRP activity: mRNA transcription and protein level did not change after treatment, but mitoxantrone test demonstrated a strong inhibition of BCRP activity ($p < 0.001$). In conclusion, the present results showed that EGCG could down-regulate the activity of two molecules that play a key role in drug metabolism and transport and that are highly expressed in tamoxifen resistant breast carcinoma cells. The interaction of EGCG and drugs used in the therapy of estrogen sensitive breast carcinoma ought to be subject of studies and the potential use of EGCG in drug-resistant diseases ought to be better considered.

Introduction

Green tea (*Camellia sinensis*,) has been widely investigated for potential health beneficial effects, including prevention of cancer (Chen et al., 2008; Khan and Mukhtar, 2007; Nagle et al. 2006). Many *in vivo* studies have demonstrated the inhibition of carcinogenesis by tea: the mechanisms proposed included the modulation of signal transduction pathways, inhibition of cell proliferation, induction of apoptosis and inhibition of tumour invasion and angiogenesis (Carlson et al., 2007; Shankar et al., 2007). These potentially beneficial effects of green tea are attributed to the catechins, the major poliphenolic components and, particularly, to (-)-Epigallocatechin-3-gallate (EGCG), the most abundant and extensively studied catechin of green tea (Ramos, 2007; Khan and Mukhtar, 2008).

Together with the effects on delaying and inhibiting carcinogenesis, green tea, or EGCG alone, has been demonstrated to overcome resistance to drugs currently used in cancer therapy. EGCG treatment caused a dose-dependent decrease in growth and apoptosis in trastuzumab-resistant BT474 human breast cancer cells and JIMT-1 breast cancer cells, derived from patient who displayed clinical resistance to trastuzumab therapy (Eddy et al., 2007). EGCG had similar effects on drug-sensitive (H69) and drug-resistant (H69VP) Small-cell Lung Carcinoma (SCLC) cells (Sadava et al., 2007). Tea polyphenols and EGCG reverted the Multidrug Resistant (MDR) phenotype on *in vivo* and *in vitro* models (Mei et al. 2005). Drug-resistant cells often express proteins belonging to the ABC multidrug transporters family, which lower the effective drug concentration in a cell by pumping the drug out (Kuo, 2007). P-gp, a 170–180 kDa transmembrane glycoprotein encoded by the human multidrug resistance gene 1 (MDR1), is a member of the ATP-binding cassette (ABC) transporter family and functions as an efflux pump for various anticancer agents such as the *vinca* alkaloids, anthracyclines and taxans (Sharom, 2008). P-gp is one of the most important determinants of the effectiveness of chemotherapeutic agents in the treatment of cancer: recent reports have shown that P-gp is also expressed in breast

Abbreviations: EGCG, (-)-Epigallocatechin-3-gallate; MCF-7Tam, MCF-7 resistant to tamoxifen; MRP1, Multidrug Resistance Protein 1; P-gp, Glycoprotein P; BCRP, Breast Cancer Resistance Proteins; ERα, Estrogen Receptor alpha

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cancer cells and that it can modulate tamoxifen concentration in serum and tissue (Mutoh et al., 2006). Another protein, Breast Cancer Resistance Protein (BCRP) has also been proposed to mediate drug resistance. BCRP (ABCG2), also known as the mitoxantrone resistance gene (MXR), is a 72 kDa half-transporter with six trans-membrane domains and an N-terminal ATP-binding site (Sharom, 2008). This protein has been shown to be widely expressed in a range of cultured breast cancer cell lines such as MCF-7 (Zhang et al., 2006). Multidrug Resistance Protein 1 (MRP1) has also been demonstrated to be involved in mediating drug resistance to a broad spectrum of antitumor agents (Sharom, 2008). Elevated expression of these transporters has frequently been found in breast cancer and correlations with elevated expression of MRP1 to chemotherapeutic response and outcome have been observed (Larkin et al., 2004).

Tamoxifen is the first line treatment used in patients having hormone-sensitive early breast cancer. Tamoxifen can block the Estrogen Receptor (ER) pathway, thereby inhibiting estrogen dependent gene transcription and tumour growth. Despite its benefit in patients with all stages of ER-positive breast cancer, resistance can occur de novo in approximately 30% patients or after initial response and long term treatment (acquired resistance). The underlying mechanisms for tamoxifen resistance are probably multifactorial, involving a number of profound changes in the expression of genes (Riggins et al., 2007) also including MDR1 (Nagaoka et al., 2006).

As the MDR phenotype can be modulated by herbal constituents, including green tea catechins (Zhou et al. 2004) we developed a MCF-7 cell line resistant to tamoxifen (Farabegoli et al., 2007) and we investigated whether EGCG treatment could overcome the MDR phenotype by modulating MDR1, BCRP and MRP1. This approach might provide a new tool for overcoming multidrug resistance in tumour cells and improve the efficacy of cancer therapy.

Material and methods

Cell lines

MCF-7 cell line was obtained by American Type Culture Collection (ATCC) (Manassas, Va, USA). MCF-7Tam cell line was developed growing MCF-7 cells in Minimum Essential Medium Alpha (α-MEM) without phenol red, supplemented with 10% charcoal-stripped Fetal Bovine Serum (FBS), 100 U/ml penicillin/streptomycin, 2 mM L-glutamine, 1 mM Na pyruvate (all from Cambrex, Bioland, Maryland, USA); 10^{-7}M 4-OH-tamoxifen (Sigma-Aldrich, St. Louis, MO, USA) was added to the medium for 1 year before starting the experiments as previously reported (Farabegoli et al., 2007).

MTT assay

MCF-7Tam cells were seeded onto 96-well plates (15.000 cells for well) and allowed to grow overnight in α-MEM, with or without 10^{-7}M 4-OH-tamoxifen (Sigma-Aldrich, St. Louis, MO, USA). The day after the cells were incubated with 10, 20, 50, 100 μg/ml EGCG (Sigma-Aldrich, St Louis, MO, USA) for 24-72 h. Cell viability experiments were conducted in quadruplicate and three independent experiments were carried out. Ten μl 3-(4,5-Dimethylthyazol-2-l)-2,5-diphenyletrazolium bromide (MTT) (Sigma-Aldrich, St. Louis, MO, USA) was added to 100 μl α-MEM in each well from a stock solution (5 mg/ml in PBS) and incubated for 4 h at 37 °C. Then, the medium was aspirated and the reaction was stopped by addition of 100 μl of dimethyl sulfoxide (DMSO) (Merck, Darmstad, Germany). The absorbance in each well was then measured with a microplate reader (Beckton Dickinson, Boston, MD, USA) at 570 nm.

Annexin V-FITC assay

MCF-7Tam cells (1×10^6) were incubated with 10-100 μg/ml EGCG for 24 hours and then washed twice in PBS and once in binding buffer. After washing, MCF-7Tam cells were incubated with 5 μl of Annexin V-fluorescein isothiocyanate (AV-FITC) and 5 μl of propidium iodide (PI) in 450 μl of binding buffer (BD Biosciences, San Diego, CA) in the dark for 10 min at room temperature with gentle agitation. Stained cells were counted after visualization by a fluorescence microscope equipped with a FITC filter.

Western blot analysis

MCF-7Tam cells were plated and treated with EGCG as already described. The cells were treated by trypsin and centrifuged at 300 × g for 10 min. The pellets were resuspended in lysis buffer (20 mM Tris-HCl, pH 7.5, 0.5 mM EDTA, 0.5% Triton X-100, 5 mM Na_3VO_4) and sonicated on ice, in the presence of protease inhibitors. Protein concentration was determined by the method of Lowry. Cell lysates (50 μg of protein per lane) were size fractioned in 7.5% SDS-polyacrylamide gel, prior to transfer to Hybond TM-C Extra membranes (GE Healthcare, UK) by standard protocols. The membranes were incubated with 5% milk in transfer buffer saline (0.2 M Tris-HCl, 0.5 M NaCl, 1% Tween 20, pH 7.6) (TBS) for 2 h at room temperature. Then, they were incubated with 1:200 mouse anti-BRCP (Sigma, Saint Louis, USA), 1:200 mouse anti-MRP1 (Biodesignes Int, USA), 1:200 mouse anti-P-gp (Signet Lab, UK), 1:500 rabbit anti-Bcl2 (S.Cruz, USA) and 1:500 rabbit anti-Bax (Sigma, Saint Louis, USA) dissolved in TBS-5% milk at 4 °C overnight. After two washings with TBS-5% milk, the membranes were incubated with the corresponding anti-mouse and anti-rabbit peroxidase conjugated secondary antibody diluted 1:1000 in 5% milk in TBS-1% Tween for 1 h. The proteins were detected by luminol (GE Healthcare, UK). The bands were quantified by using a densitometric images analysis software (Image Master VDS, Pharmacia Biotech, Uppsala, Sweden). The amount of protein in each lane was the same, as confirmed by actin (Sigma, Saint Louis, USA).

RNA extraction and RT-PCR

RNA was extracted by guanidinium-phenol-isoamylic alcohol method according to Chomczynski and Sacchi with modifications (Chomczynski and Sacchi, 1987) RT-PCR reaction was performed using the cMaster RT plus PCR system kit according to the instruction of the supplier (Eppendorf, Hamburg, Germany). The following primers were used: P-gp**F**: 5'-CCCATCATTGCAA-TAGCAGG-3', P-gp**R**: 5'-GTTCAAACTTCTGCTCCTcA-3'. BCRP**F**: 5'-TTCGGCTTGCAACAACTATG-3', BCRP**R**: 5'-TCCAGACACACCACG-GATAA-3'. ß-actin was used as an internal control of the reaction. The sequence of the primers were: ACT**F**: 5'-GGCATCGTGATG-GACTCCG-3', ACT**R**: 5'-GCTGGAAGGTGGACAGCGA-3'. Annealing temperature was 58 °C for P-gp and 55 °C for BCRP primer, respectively. ß-actin and P-gp or BCRP primers were used together into the same tube. Ten μl of PCR products were loaded onto a 1% agatose gel, run into an electrophoresis chamber, stained by ethidium bromide and visualized with a UV-transilluminator. Bands were analyzed by Kodak Electrophoresis Detection and Analysis System (EDAS) 290 (Rochester, NY, USA).

Rhodamine 123 and mitoxantrone intracellular accumulation

Rhodamine 123 accumulation: 1×10^6 MCF-7 and MCF-7Tam cells were treated with 10-100 μg/ml EGCG for 24 h and then incubated with 100 nM rhodamine 123 for 30 min (Wuchter et al, 2000). Then cells were treated with trypsin, harvested, resuspended in ice-cold PBS and analysed.

Mitoxantrone accumulation: 1×10^6 MCF-7 and MCF-7Tam cells were treated with 10-100 μg/ml EGCG for 24 h and then incubated with 100 nM mitoxantrone for 30 min (Zhang et al., 2004). Then, the cells were treated with trypsin, harvested, resuspended in ice-cold PBS and analysed. We used LoVoMDR cells as a positive control and LoVo (wild type) as a negative control. The analysis was performed using a FACScalibur flow cytometer (BD, San Josè, CA, USA) equipped with an Argon ion laser turned at 488 nm. A minimum of 10.000 cells for sample was acquired in all the analysis and the median fluorescence intensity was calculated. Histograms were elaborated by the statistic program in Cell Quest software (BD, San Josè, CA, USA).

Statistic analysis

Experiments were performed at least three times, when not otherwise specified. Data were expressed as mean and standard deviation ($\pm$SEM). Statistically significant differences were assessed by ANOVA followed by Bonferroni's multiple comparison test, or two-tail Student's *t*-test, as appropriate, using the GraphPad Prism 5.1 software statistical package. The level for accepted statistical significance was $p < 0.05$.

Results

Growth inhibition of MCF-7Tam cells by EGCG treatment

We first evaluated the growth inhibition effect after 10-100 μg/ml EGCG treatment on MCF-7Tam cells in presence or absence of 10^{-7} M 4-OH-tamoxifen. As shown in Fig. 1, MCF-7Tam cells responded to treatment and cell proliferation decreased to 38% after 100 μg/ml EGCG and 10^{-7} M 4-OH-tamoxifen treatment for 72 h. The cell viability was comparably reduced (34%) in MCF-7Tam cells only treated with EGCG (data not shown).

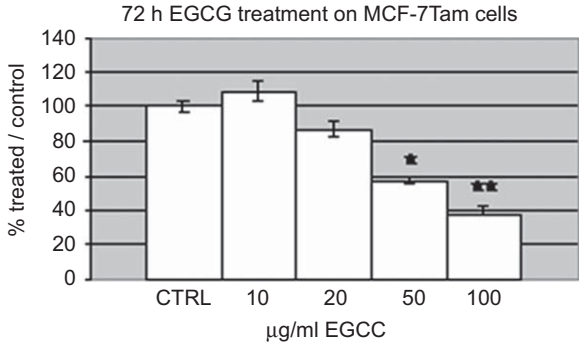

Fig. 1. EGCG and 10^{-7}M 4-OH-tamoxifen (TAM) treatment on MCF-7Tam cells. MTT assay was performed in quadruplicate on 96 well plates. Experiments were repeated three times. Cells were grown in α-MEM medium without phenol red, 10% charcoal-stripped Foetal Bovine Serum (FBS), 100 U/ml penicillin/streptomycin, 2 mM L-glutamine, 1 mM Na pyruvate. Dose-dependent cytotoxicity was detected in MCF-7Tam cells treated with 10-100 μg/ml EGCG and 10^{-7} M 4-OH-tamoxifen for 72 h. The results are expressed as a percentage of treated samples with respect to control (100%). Asterisks indicate significant difference with respect to control: $^*p < 0.05$; $^{**}p < 0.001$.

Apoptosis

Annexin V FITC-conjugated staining and Western Blot analysis of Bax and Bcl2 expression were used after EGCG treatment in order to detect apoptosis. Annexin V detects externalized phosphatidylserine on the cell membrane, which is one characteristic of apoptosis, whereas viable cells are unable to bind the molecule. As AnnexinV is conjugated to a fluorocrome, the cells having externalized phosphatidylserine show an intense fluorescence labelling on the membrane whereas viable cells are unstained. The number of fluorescence labelled cells significantly increased in 10-100 μg/ml EGCG treated cells (Fig. 2a). We confirmed that 100 μg/ml EGCG treatment for 24 h induced apoptosis in MCF-7Tam cells (Fig. 2b, c ,d) as we also found a significant increase of Bax expression (Fig. 2b) and a parallel Bcl2 decrease (Fig. 2c) by Western Blot. Altogether the data suggested that EGCG induced growth arrest and cell death in MCF-7Tam cells.

MRP1, P-gp and BCRP expression in MCF-7Tam cells as detected by Western blot and RT-PCR

Breast carcinoma cells develop drug resistance by several mechanisms, including the expression MRP1, P-gp and BCRP. These proteins act as energy-dependent efflux pumps that reduce the intracellular concentration of cytotoxic compounds. We first used Western blot analysis to detect presence of MRP1, P-gp and BCRP in MCF-7Tam cells after EGCG treatment. As shown in Fig. 3a, MRP1 and BCRP were detectable in MCF-7 wild type cells, whereas P-gp expression was hardly detectable. In contrast, MRP1, P-gp and BCRP were all expressed in control MCF-7Tam cells. After EGCG treatment, MRP1 (c) quantity did not change significantly (7%), whereas P-gp (d) dramatically decreased (53%, $p < 0.0001$) and BCRP (b) showed a modest decrease (20%).

Since MRP1 protein was not significantly affected by EGCG treatment, we only focused our attention to P-gp and BCRP and we performed RT-PCR to clarify whether downregulation of P-gp and BCRP gene expression occurred after EGCG treatment. As shown in Fig. 4, P-gp (a) and BCRP (b) decrease was not due to down-regulation of gene expression, as the densitometry analysis of the bands corresponding to P-gp and BCRP proteins showed very little changes after 50 and 100 μg/ml EGCG administration for 24 h with respect to the β-actin band. We did not treat cells for 48 h as cell death could have altered nucleic acids before performing RNA extraction and the results could have been questionable.

The dramatic P-gp decrease we found after EGCG treatment, was not related with gene transcription downregulation, as shown by RT-PCR. To investigate whether EGCG could influence P-gp level by accelerating protein degradation, we treated MCF-7Tam cells with 5 μM MG132, a proteasome inhibitor which blocks the degradation of many proteins. P-gp level remarkably increased after MG132 treatment and it reduced when EGCG treatment followed MG132 administration. But when EGCG was given together with MG132, P-gp accumulation and decrease balanced, since P-gp quantity was as high as the untreated control cells (Fig. 5).

The effects of EGCG on P-gp and BCRP efflux pump activity

Functional test on MDR1 and BCRP ability to efflux drugs was carried out on MCF-7Tam cells treated with 100 μg/ml EGCG for 24 and 48 h by rhodamine 123 and mitoxantrone. Rhodamine 123 assayed the functional activity of P-gp protein, whereas mitoxantrone is considered a reliable assay for BCRP.

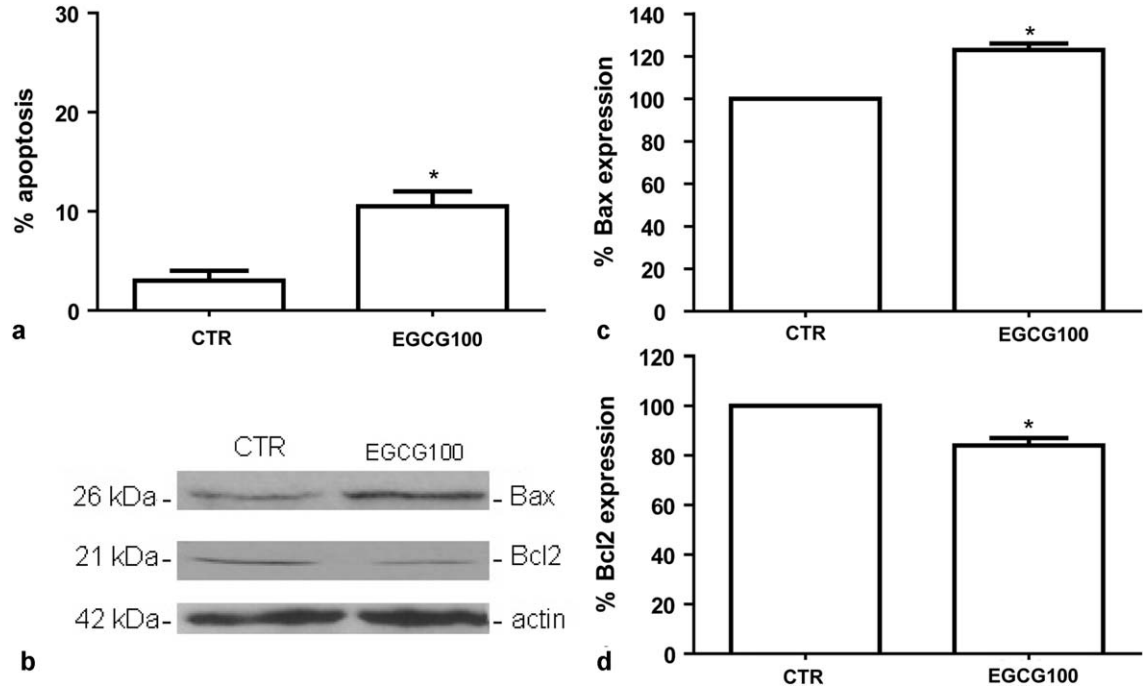

Fig. 2. AnnexinV apoptosis assay in MCF-7Tam cells after 100 μg/ml EGCG treatment for 24 h (a). FITC labelled cells, visualized by a fluorescence microscope, were counted in at least five random fields and expressed as a percentage of labelled on total cells. CTR: untreated cells. Each bar represents the mean of four experiments, performed in duplicate (± SE). * $p < 0.05$. Western blot analysis (b): Bax and Bcl2 protein level was assessed in MCF-7Tam cells after 100 μg/ml EGCG treatment for 24 h. Densitometry data are expressed as a percentage of treated samples with respect to control (CTR). Bax (c), Bcl2 (d). Each bar represents the mean (± SE) of three independent experiments.* $p < 0.05$.

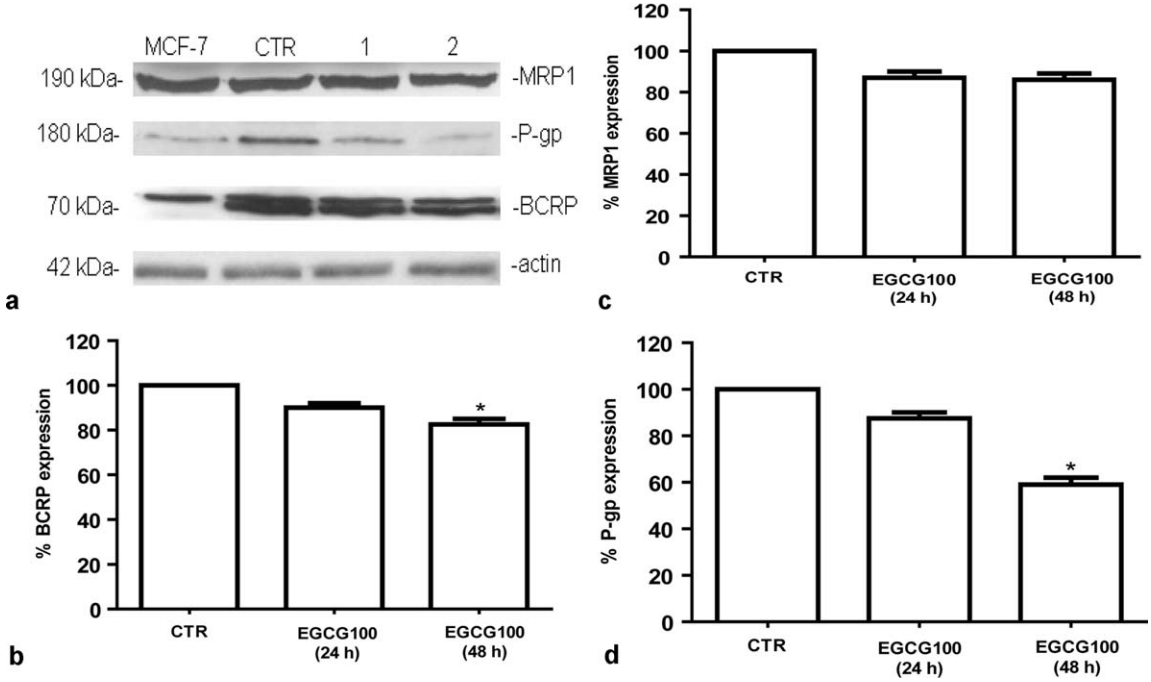

Fig. 3. MRP1, P-gp and BCRP protein expression (a) as assessed by Western blot in cell lysates of MCF-7Tam, after 24 (1) and 48 h (2) 100 μg/ml EGCG treatment. MRP1 protein did not change after EGCG treatment (c) whereas P-gp decrease was statistically highly significant (d). BCRP was detectable in MCF-7 cells, and its expression increased in MCF-7Tam cells. After EGCG treatment and it was slightly reduced (b). Densitometry data after immunodetection of P-gp in MCF-7Tam, cells are expressed as percentage of treated samples with respect to control (CTR). Bars show mean with SEM and asterisks indicate significant difference with respect to control: *$p < 0.05$.

Higher mitoxantrone uptake was found in MCF-7 wild type cells with respect to MCF-7Tam cells ($p < 0.001$). After 100 μg/ml EGCG treatment for 24 h the level of mitoxantrone uptake was similar to MCF-7 wild type cells (Fig. 6). In contrast, P-gp functional activity, as investigated by Rhodamine 123, was scarcely affected by EGCG administration and the decrease in activity in treated with respect to the control samples was not statistically significant.

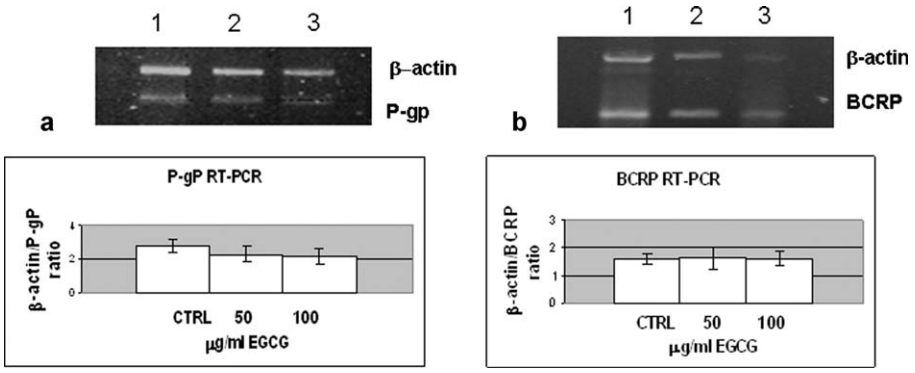

Fig. 4. RT-PCR of P-gp and BCRP expression in MCF-7Tam cells treated with EGCG. RNA from control (**1**) and from cells treated with 50 (**2**) and 100 (**3**) **μ**g/ml EGCG for 24 h, was used in a one-step procedure for detecting P-gp and BCRP expression. **β**-actin, corresponding to the top bands, was used as a standard in both reactions. Ten **μ**l PCR product was loaded onto a 1% agarose gel and stained with ethidium bromide. P-gp (**a**) and BCRP (**b**) were detectable in all samples. Cells treated with 100 **μ**g/ml EGCG for 24 h showed faint bands corresponding to **β**-actin and BCRP, possibly because of the poor quality of samples, in which many cells might be damaged or dead. Band densitometry analysis was performed by Kodak EDAS 290. The histograms show the **β**-actin/Pg-p and **β**-actin/BCRP ratio in control and treated samples. No significant difference in Pg-p and BCRP expression between control and EGCG treated samples was detected. Bars show mean with SEM.

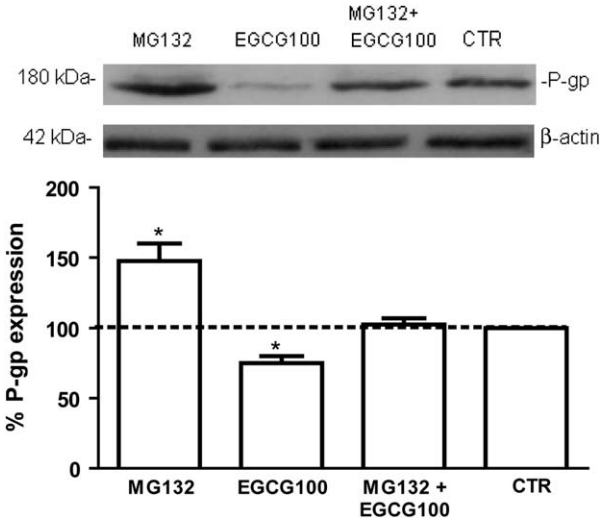

Fig. 5. Effect of 5 μM MG132, 100 μg/ml EGCG and in combination on P-gp protein levels, as assessed by Western blot in cell lysates of MCF-7Tam. Densitometry data are expressed as a percentage of treated samples with respect to control (100%). Bar shows mean with SEM and asterisks indicate significant difference with respect to the control: *$p < 0.05$.

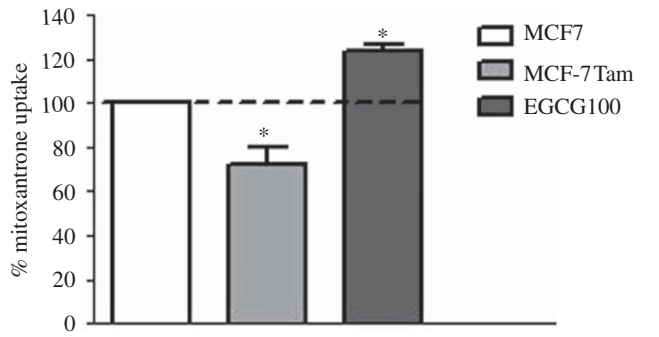

Fig. 6. Analysis of mitoxantrone uptake in MCF-7Tam and MCF-7 cells. Cells were cultured in basal medium in presence or not of 100 μg/ml EGCG for 24 h. At the end of treatment 5 μM mitoxantrone was added for 30 min and fluorescence was measured by FACSCalibur. Data are expressed as a percentage of mitoxantrone uptake in MCF-7Tam with respect to MCF-7 cells (100%). Bar shows mean with SEM and asterisks indicate significant difference with respect to mitoxantrone uptake in MCF-7 cells: *$p < 0.05$.

Discussion

Clinical resistance to chemotherapeutic drugs is still a major problem in cancer treatment and it has been associated with the MDR phenotype. Cancer cells develop the resistant phenotype by many different mechanisms, which have not been completely elucidated, and the drug-resistance can be extended to a broad range of structurally and functionally distinct drugs. We previously found that EGCG, the most abundant and active catechin present in green tea, was able to kill MCF-7 breast carcinoma cells resistant to 4-OH-tamoxifen, at a concentration greater than in MCF-7 wild type cells (Farabegoli et al., 2007). Resistance to tamoxifen is an event occurring in more than 30% patients affected by hormone responsive breast cancer that are treated with tamoxifen and it is associated with poor outcome (Nicholson and Johnston, 2005). As EGCG was demonstrated to kill resistant cancer cells by different mechanisms (Eddy et al., 2007; Sadava et al., 2007; Mei et al. 2005) in the present study we investigated the EGCG effects in MCF-7Tam cells, particularly in relation to MRP1, P-gp and BCRP.

We found that EGCG could downregulate P-gp and BCRP, two proteins that efflux drugs and concur to the development of the MDR phenotype. MRP1, a third protein frequently involved in resistant disease, was scarcely affected. Similar results were reported by Katayama et al. (2007), who investigated the effect of 32 flavonoids on a cancer cell line and found that none was effective on MRP1, a few compounds had a low effect on P-gp and 20 flavonoids specifically inhibited BCRP activity. Interestingly, EGCG down-regulated P-gp and BCRP by two different mechanisms of action.

EGCG treatment was able to reduce the quantity of P-gp dramatically. When EGCG was given together with MG132 to MCF-7Tam cells, it was able to contrast P-gp accumulation, which did not occur. We concluded that EGCG induced P-gp decrease only in part by proteasome degradation. If the proteasome degradation was the unique way by which P-gp was reduced, we should have found P-gp accumulation even after EGCG and MG132 were administrated together. This finding also showed that EGCG did not behave as a proteasome inhibitor with respect to P-gp decrease. This is apparently in contrast with many reports, which showed that EGCG is a strong proteasome inhibitor (Dou et al., 2008). We considered that we investigated EGCG and MG132 effect on a specific protein and not a generic effect on the proteasome function. When EGCG and MG132 were given

together to multiple myeloma and glioblastoma cell lines they were neither synergic nor antagonist with respect to the cytotoxicy. In contrast, EGCG clearly antagonized the effects of boronic acid-based proteasome inhibitors (BZM, MG-262, PS-IX) (Golden et al., 2009). So, the effects of EGCG on proteasome can be very different and need further studies. P-gp decrease after EGCG treatment was also reported by Jodoin et al. (2002), which also demonstrated a binding between the two molecules. The structural model of the complex EGCG-P-gp indicated that EGCG was tightly bound to the ATP-binding site (Qian et al. 2005). We speculated that the binding between P-gp and EGCG could occur early in the protein synthesis, and the molecule could not be recognized as a functionally active and eliminated.

Since P-gp and BCRP had different molecular structure, the interaction between EGCG and the two molecules might induce different effects. BCRP is a half-ABC transporter and contains only six transmembrane domains and one ATP binding site, whereas P-gp has 12 transmembrane domains and two ATP binding sites (Litman et al., 2001; Allikmets et al., 1998). This difference might be critical for triggering different effects of EGCG treatment.

P-gp was not the mainly implicated molecule in tamoxifen resistance, since Rhodamine 123 test failed to show significant intracellular accumulation, but the important effect demonstrated after EGCG treatment on P-gp has to be considered in other models of MDR disease (Mei et al., 2005). BCRP seemed to be a noteworthy molecular target of EGCG action in MCF-7-Tam cells, since the mitoxantrone test demonstrated a strong inhibitory effect after EGCG treatment. Significant and variable expressions of BCRP have been detected in myeloid leukaemia and breast cancer (Ross et al., 2000; Kanzaki et al., 2001). The contribution of BCRP to clinic MDR was subject of investigation in myeloid leukaemia: patients who expressed high levels of BCRP had a worse prognosis (Steinbach et al., 2002). In addition, BCRP, like P-gp, is also expressed in normal tissues such as liver hepatocytes, the apical membrane of the epithelium in the intestine, the ducts and lobules of the breast, the luminary surface of brain capillaries, and the human placenta (Maliepaard et al., 2001) and it plays an important role in the disposition of endobiotic and xenobiotic compounds, protecting the body or certain tissues from exposure to toxic substrates (Jonker et al., 2002). It was the first time that the relation between EGCG and BCRP was investigated in tamoxifen resistant cells: the present results demonstrated that BCRP increased in resistant cells and EGCG was able to downregulate its activity. BCRP might be a useful molecular target in resistant disease and in course of treatment: inhibitors of BCRP could be used not only to reverse MDR mediated by this transporter but also to alter the pharmacokinetics of BCRP substrate drugs (intestinal absorption, biliary excretion, and brain penetration) increasing the efficacy of therapy. So far, only the green tea component (−)-epicatechin (50 μM) was found to decrease BCRP expression (Ebert et al., 2007). As both P-gp and BCRP are involved together in MDR disease, the double effect of EGCG represents a potentially useful tool to revert MDR.

In conclusion, we think there are several reasons to suggest EGCG and green tea catechins as a subject of targeted studies in MDR disease. First, EGCG can modulate P-gp and BCRP, two of the most important proteins involved in drug efflux, and this effect may increase the efficacy of drugs used in chemotherapy. Second, EGCG showed the ability to kill resistant cancer cells by affecting various molecular signalling pathways. Third, green tea is healthy, cheap and safe: side effects are modest and well tolerated. Intravenous administration, not used in humans but experimented in animals (Isbrucker et al., 2006), suggested that high concentrations might be reached, and, hopefully, might be used as a complement of therapy.

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