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Electrochemical Determination of Enzymes Metabolizing Ellipticine in Thyroid Cancer Cells - a Tool to Explain the Mechanism of Ellipticine Toxicity to these Cells

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The antineoplastic alkaloid ellipticine is a prodrug, the pharmacological efficiency of which is dependent on its cytochrome P450 (CYP)- and/or peroxidase-mediated activation to species forming DNA adducts in target tissues. Here, we found that this compound is cytotoxic to human BHT-101, B-CPAP and 8505-C thyroid cancer cells and blocks one or more phases of cell cycle in these cancer cells. Ellipticine toxicity to the thyroid cancer cells corresponded to levels of DNA adducts generated by the CYP- and/or peroxidase-mediated ellipticine metabolites, 12-hydroxy- and 13-hydroxyellipticine, in these cells. Cultivation of all tested cells under hypoxic conditions (1 % oxygen) led to a decrease in ellipticine toxicity. Such a lower sensitivity of cells to ellipticine correlates with a decrease in the formation of ellipticine-derived DNA adducts in these cells. Using Western blotting, the expression of CYP1A1, 1B1, 3A4, thyroid peroxidase (TPO), cyclooxygenase-1 (COX-1) and cytochrome b_5, the enzymes that catalyze, and/or influence ellipticine metabolism, was investigated in the cancer cells. Furthermore, the effects of ellipticine treatment on the expression levels of these proteins in thyroid cancer cells were also examined. The results indicate that the highest expression levels of cytochrome b_5 together with CYP1A1 and 3A4 determine the highest DNA adduct formation and cytotoxicity of ellipticine in B-CPAP cells. They also demonstrate that formation of covalent DNA adducts by ellipticine is the predominant mechanism responsible for its cytotoxicity in studied cells.

Keywords: Ellipticine; Thyroid Cancer Cells; Cytotoxicity; Cytochrome P450; Peroxidase; Protein Expression; Western Blotting; DNA Adducts

1. INTRODUCTION

Ellipticine (5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole) and its derivatives exhibit significant antitumor and anti-HIV activities. Several mechanisms of ellipticine action have been elucidated and indicated a rather complex mode of pharmacological action of this drug (for summary see [1-8]). Ellipticine inhibits AKT kinase [9], restores function of the p53 mutant protein [10] and/or causes uncoupling of mitochondrial oxidative phosphorylation [11]. Ellipticine is also a strong DNA damaging agent acting as DNA intercalator, inhibitor of topoisomerase II and/or a compound generating covalent DNA adducts after enzymatic activation with cytochrome P450 (CYP) and/or peroxidases (for summary see [1-8]). DNA adduct formation by ellipticine is supposed to be responsible for the specificity of this drug to some cancer types. Two major DNA adducts are generated from the CYP- and/or peroxidase-mediated ellipticine metabolites 12/13-hydroxyellipticine (metabolites M2 and M3 in Figure 1) that dissociate to ellipticine-12/13-ylium binding to DNA [3,7,12-14] (Fig. 1). Of the CYP enzymes investigated, human CYP3A4 and rat CYP3A1 are most effective in oxidizing ellipticine to these reactive metabolites, while the human CYP1A enzymes preferentially form the detoxication metabolites, 9-hydroxyellipticine (metabolite M1 in Figure 1) and 7-hydroxyellipticine (metabolite M4 in Figure 1) [7,15].

Recently we have found that cytochrome b_5 alters the ratio of ellipticine metabolites formed by CYP1A1, 1A2 and 3A4. While the amounts of the detoxication metabolites (i.e. 7-hydroxy- and 9-hydroxyellipticine) were either decreased or not changed with added cytochrome b_5, the activation metabolites, 12-hydroxy- and 13-hydroxyellipticine, increased considerably. The change in the amounts of metabolites resulted in an increased formation of ellipticine-derived DNA adducts, one of the DNA-damaging mechanisms of ellipticine's antitumor action [13,15].

The same DNA adducts were also detected in several human cancer cells after exposure to ellipticine such as breast adenocarcinoma MCF-7, the leukemias HL-60 and CCRF-CEM, neuroblastomas IMR-32, UKF-NB-3, UKF-NB-4, and glioblastoma U87MG cells, and in rat mammary adenocarcinoma *in vivo* (for a summary see [3,16-21]).

The aim of this work was to investigate if ellipticine is cytotoxic to another type of cancer cells, thyroid cancer cell lines. Thyroid cancer accounts for more than 1 % of all malignant tumors and its annual incidence was estimated to be more than 200 000 cases worldwide [22]. Most of these tumors originate from thyroid follicular cells; they include well-differentiated papillary carcinoma and follicular carcinoma, as well as poorly differentiated carcinoma and anaplastic carcinoma. A poorly differentiated as well as anaplastic thyroid carcinomas may arise either *de novo* or from the pre-existing papillary or follicular carcinoma. The majority of differentiated thyroid carcinomas progress slowly, and, when detected at a early stage they are cured in majority of patients with surgery and radioactive iodine therapy. Metastatic differentiated thyroid carcinoma that have become refractory to radioactive iodine therapy has poor prognosis, especially anaplastic carcinoma metastasize in up to

half of patients, leading again to poor prognosis. Results of conventional treatment modalities (radiotherapy and/or chemotherapy) are not successful in those patients (5-year overall survival is 100 % in well differentiated, 70 % in poorly differentiated and 0 % in anaplastic thyroid carcinoma) [23]. Therefore, new therapeutic approaches are needed [24].

In this study, we investigated the effect of ellipticine on three different cell lines, the poorly differentiated papillary thyroid cancer cells BHT-101, papillary thyroid cancer cells B-CPAP and anaplastic thyroid cancer cells 8505-C. We also evaluated which of the mechanisms of ellipticine action is responsible for its cytotoxicity to these cancer cells.

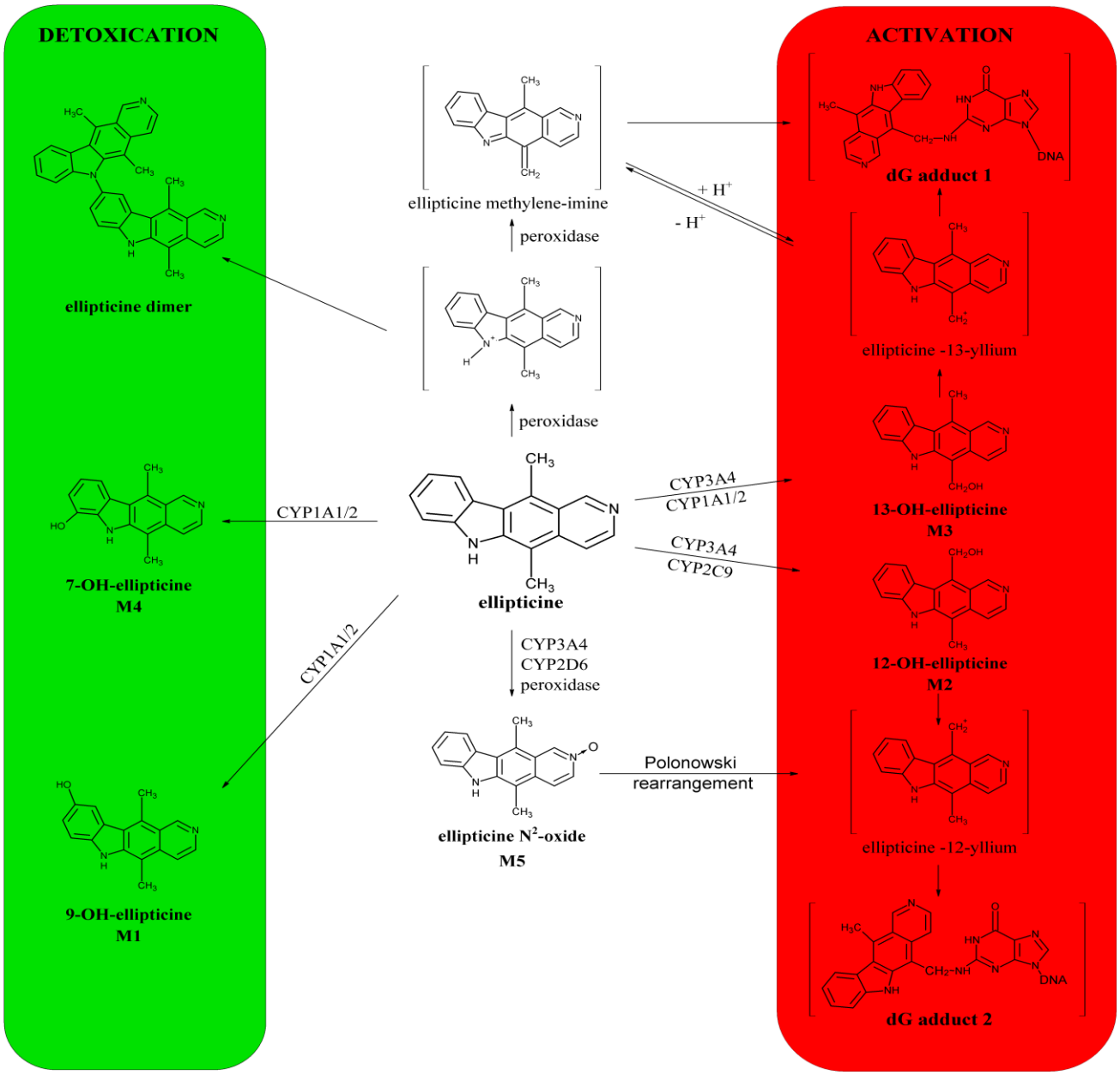

Figure 1. Metabolism of ellipticine by human CYPs and peroxidases showing the characterized metabolites and those proposed to form DNA adducts. The compounds shown in brackets were not detected under the experimental conditions and are the electrophilic metabolites postulated as ultimate arylating species or the postulated *N*²-deoxyguanosine adducts. Adapted from [14]

Because cytotoxicity of ellipticine *in vitro* and *in vivo* depends on amounts and activities of enzymes that activate or detoxicate this drug, their expression levels in thyroid cancer cells were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analyses with specific antibodies raised against such enzymes. In addition, DNA adduct formation by ellipticine was investigated by the ^{32}P-postlabeling method [1-3,7,13]. Because metabolism of ellipticine - both activation and detoxication - are oxidative processes, the influence of hypoxic culture conditions on expression levels of metabolizing enzymes, upon cytotoxicity and on DNA adduct formation was investigated. Hypoxic conditions may result in compensatory over expression of oxidizing enzymes.

2. EXPERIMENTAL PART

2.1. Chemicals and material

Ellipticine was from Sigma Chemical Co. (St. Louis, MO, USA). Enzymes and chemicals for the ^{32}P-postlabeling assay were obtained from sources described [1]. All these and other chemicals used in the experiments were of analytical purity or better. 12-Hydroxy- and 13-hydroxyellipticine were isolated from multiple HPLC runs of ethyl acetate extracts of incubations containing ellipticine and human and/or rat hepatic microsomes as described [7].

2.2. Cell cultures

The human thyroid cancer cell lines BHT-101 and B-CPAP were purchased from the Leibniz Institute DSMZ German Collection of Microorganisms and Cell Cultures (Braunschweigh, Germany) and 8505-C cells from the European Collection of Cell Cultures (ECACC; Salisbury, UK). Cells were grown at 37°C and 5 % CO_2 in Iscove's modified Dulbecco's medium (IMDM) (KlinLab Ltd, Prague, Czech Republic), supplemented with 10 % fetal calf serum, 2 mM L-glutamine, 100 units/ml of penicillin and 100 μg/ml streptomycine (PAA Laboratories, Pasching, Austria). All cells were grown at 37 °C, those under normoxic conditions in an atmosphere of ambient air with 5 % CO_2 (74% N_2, 20 % O_2). For experiments with hypoxia a hypoxic chamber purchased from Billups-Rothenberg (Del Mar, CA, USA) was prepared with an atmosphere containing 1 % O_2, 5 % CO_2, and 94 % N_2.

2.3. MTT assay

The cytotoxicity of ellipticine to thyroid cancer cells in exponential growth was determined in a 96-well plate under the normoxic and hypoxic conditions (1 % oxygen). For a dose-response curve, cells in exponential growth (10^4 cells per well) were seeded in a total volume of 100 μl of medium. Solution of ellipticine in dimethyl sulfoxide (DMSO) (1 μl) in final concentrations of 0.02 - 50 μM was in wells excepting of controls. Cell viability was evaluated by MTT test as previously described [25]. Briefly, after incubation (3 days) at 37^{o}C in 5% CO_2 the MTT solution (2 mg/ml PBS) was added, the plates were incubated for 4 hours and cells lysed in solution containing 20 % of SDS and 50 % *N*,*N*-dimethylformamide pH 4.5. The absorbance at 570 nm was measured for each well by multiwell ELISA reader Versamax (Molecular Devices, CA, USA). The mean absorbance of medium controls was the background and was subtracted. The absorbance of control cells was taken as 100%

viability and the values of treated cells were calculated as a percentage of control. Each value is the average of 8 wells and standard deviation. The IC_{50} values (the half maximal ellipticine concentration inhibiting viability of the cells) were calculated from at least 3 independent experiments using the linear regression of the dose-log response curves by SOFTmaxPro.

2.4. Cell cycle analysis

To determine cell cycle distribution analysis, 5 x 10^5 cells were plated in 60 mm dishes and treated with ellipticine (0, 1 and 10 μM) for 24 h. After treatment, the cells were collected by trypsinization, cells were stained by DNA Prep Reagent Kit (Beckmann Coulter, Fullerton, CA, USA) that contain permeabilisation reagent and propidium iodide solution with RNase, according to manufacturer´s instructions, and analyzed by flow cytometry on a FACSCalibur cytometer (BD, San Jose, CA, USA). The data were analyzed using ModFit LT software (Verity Software House, Topsham, ME, USA).

2.5. Electrochemical estimation of contents of CYPs, peroxidases and cytochrome b_5 in thyroid cancer cell lines

To determine the expression of cytochrome b_5, CYP1A1, 1B1 and 3A4, thyroid peroxidase (TPO) and cyclooxygenase (COX)-1 proteins, cell pellets were resuspended in 25 mM Tris-HCl buffer pH 7.6 containing 150 mM NaCl, 1% detergent NP-40 (Sigma, St. Louis, MO, USA), 1% sodium deoxycholate, 0.1 % SDS and with solution of COMPLETE (protease inhibitor cocktail tablet, Roche, Basel, Swizerland) at concentration described by provider. The samples were incubated for 60 min on ice and centrifuged for 20 min at 14 000 g and 4°C. Supernatant was used for additional analysis. Protein concentrations were assessed using the DC protein assay (Bio-Rad, Hercules, CA, USA) with serum albumin as a standard and 10-75 μg of extracted proteins were subjected to SDS-PAGE on a 11% gel for analysis of CYP1A1, 1B1 and 3A4, TPO and COX-1 protein expression, and a 17% gel for analysis of cytochrome b_5 protein expression [14,19,26,27]. After migration, proteins were transferred to a polyvinylidene fluoride (PVDF) membrane and incubated with 5% non-fat milk to block non-specific binding. The membranes were then exposed to specific rabbit polyclonal anti-cytochrome b_5 (1:750, Abcam, MA, USA), anti-CYP1A1 (1:1000, Millipore, MA, USA), anti-CYP1B1 (1:500, Abcam, MA, USA), anti-CYP3A4 (1:5000, AbD Serotec, Oxford, UK), anti-COX-1 (1:1000, Abcam, MA, USA) antibodies and to specific mouse monoclonal anti-TPO (2.5 μg/ml, Abcam, MA, USA) antibody overnight at 4^o C. Membranes were washed with distilled water and exposed to peroxidase-conjugated anti-IgG secondary antibodies (1:3000, Bio-Rad, Hercules, CA, USA), and the antigen-antibody complex was visualized by enhanced chemiluminiscence's detection system according to the manufacturer's instructions (Immun-Star HRP Substrate, Bio-Rad, Hercules, CA, USA). X-Rays films were from MEDIX XBU (Foma, Hradec Králové, Czech Republic). Antibody against actin (1:1000, Sigma, St. Louis, MO, USA) was used as loading control.

2.6. Treatment of thyroid cancer cells with ellipticine for DNA adduct analyses

Thyroid cancer cell lines were seeded 24 h prior to exposure at a density of 1 x 10^5 cells/ml in two 75 cm^2 culture flasks in a total volume of 20 ml of IMDM and treated with 0, 1 or 10 μM

ellipticine. After 24 h the cells were harvested after trypsinizing by centrifugation at 2000 x g for 3 min and two washing steps with 5 ml of PBS yielded a cell pellet, which was stored at -20ºC until DNA isolation. DNA was isolated and labeled as described in the next section.

2.7. DNA isolation and ^{32}P-postlabeling of DNA adducts

DNA from thyroid cancer cells was isolated by the phenol-chloroform extraction as described [14,19]. The nuclease P1 enrichment version of the ^{32}P-postlabeling methods was used for the detection of ellipticine-derived DNA adducts as nuclease P1 enrichment was previously found to be more suitable than butanol enrichment [1-4,12-15,19,21,28]. Calf thymus DNA incubated with 13-hydroxy- and 12-hydroxyellipticine [7,12], and liver DNA of rats treated with ellipticine [29] were used to compare DNA adduct spot patterns.

3. RESULTS AND DISCUSSION

3.1. Cytotoxicity of ellipticine to human thyroid cancer cell lines

To determine the cytotoxic effect of ellipticine on growth of human poorly differentiated papillary thyroid cancer cells BHT-101, papillary thyroid cancer cells B-CPAP and an anaplastic thyroid cancer cell line 8505-C, we used the MTT assay. As shown in Figure 2, all three thyroid cancer cell lines were sensitive to ellipticine having the IC_{50} values of 4.8 ± 2.5, 2.8 ± 1.0 and 3.6 ± 1.2 μM in BHT-101, B-CPAP and 8505-C cells after 72 h, respectively. However, culture conditions (1 % O_2) reduced ellipticine toxicity in all thyroid cancer cell lines, increasing the IC_{50} values of ellipticine to 7.7 ± 4.2, 5.6 ± 1.9 and 8.3 ± 2.7 μM for BHT-101, B-CPAP and 8505-C cells after 72 h, respectively.

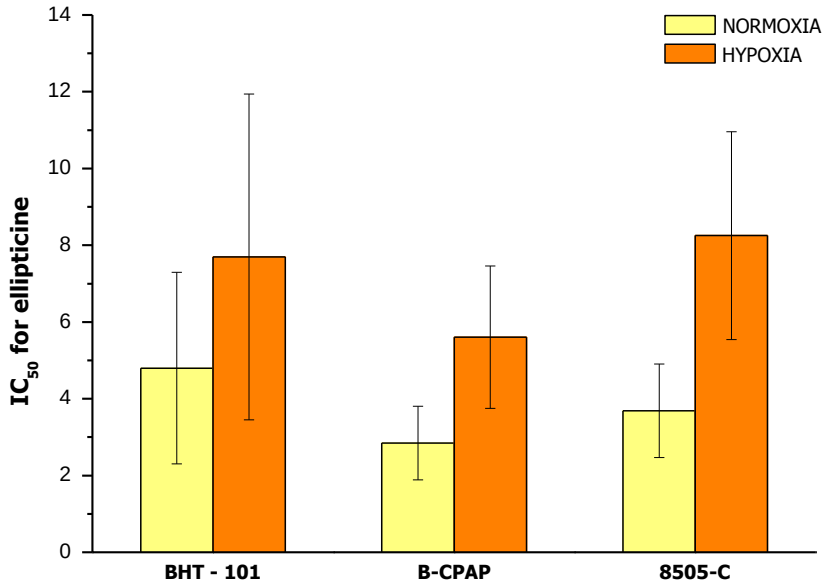

Figure 2. Cytotoxicity (expressed in IC_{50}) of ellipticine to human thyroid cancer cell lines BHT-101, B-CPAP and 8505-C after 72 h exposure to the compound, determined by the MTT assays. Cell cultivation was performed under the standard and hypoxic (1 % of oxygen) conditions.

As ellipticine acts, besides other mechanisms, *via* covalent modification of DNA which is mediated by oxygen-dependent ellipticine bioactivation through CYPs, we speculate that a decrease in ellipticine cytotoxicity under the hypoxic conditions might be caused by reduced CYP-catalyzed bioactivation due to the oxygen deficiency. Therefore, formation of ellipticine-derived DNA adducts and expressions levels of CYP enzymes in thyroid cancer cells were investigated (see 3.3. and 3.4.).

3.2. Ellipticine induced cell cycle arrest in human thyroid cancer cell lines

As many cancer drugs act by arresting cells in the cell cycle, we investigated the effect of ellipticine treatment on the cell cycle distribution of thyroid cancer cells cultivated under the standard (aerobic) and hypoxic conditions. Flow cytometric analysis was used for such a study (Fig. 3).

Compared to controls, treatment of cells with ellipticine for 24 h resulted in an appreciable arrest of BHT-101, B-CPAP and 8505-C cells in S and/or G2/M phases of cell cycle with a concomitant decrease in G0/G1 phase. Hypoxic conditions had no effect on the cell cycle distribution nor influenced the effect of ellipticine exposure upon the cell cycle distribution. Treatment of BHT-101 and B-CPAP thyroid cancer cells with 10 μM ellipticine caused an arrest of around 40 % cells in S phase of cell cycle. Even stronger effects of ellipticine on S and G2/M phases of the cell cycle were found in the anaplastic thyroid cancer cells 8505-C (Fig. 3).

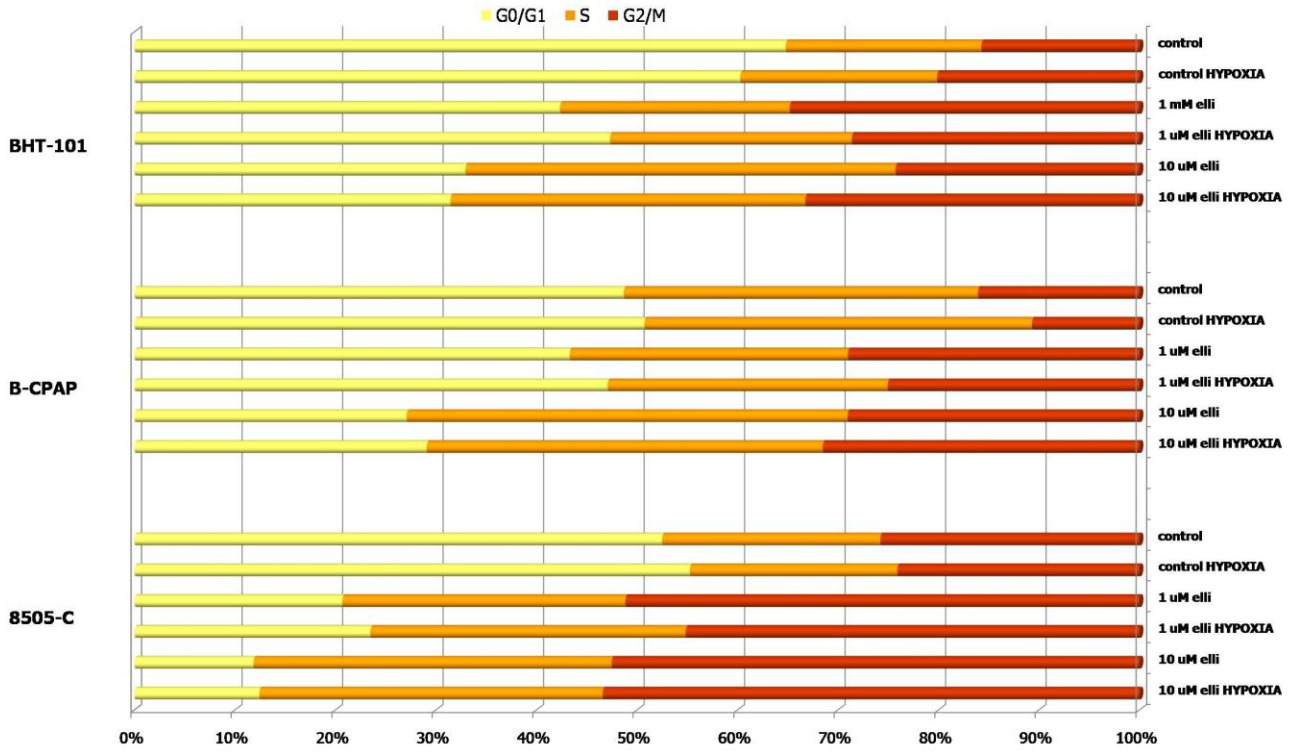

Figure 3. Effect of ellipticine treatment on cell cycle distribution in human thyroid cancer cells after 24 h ellipticine treatment under the standard or hypoxic cell culture cultivation conditions assessed by flow cytometry.

3.3. DNA adduct formation by ellipticine in thyroid cancer cells

The human thyroid cancer cell lines were treated with 1 and 10 μM ellipticine for 24 h both under the normoxic and the hypoxic cell culture conditions. Using the nuclease P1 version of ^{32}P-postlabeling assay as described previously [1,7,19-21,28-31], ellipticine-derived adducts were detected in DNA of all tested cells (see Fig. 4A-C). Two major ellipticine-DNA adducts (spots 1 and 2 in Figure 4) were detected in all cells, while no adducts were found in DNA of control thyroid cancer cells treated with solvent only (data not shown). Both adducts were identified as deoxyguanosine adducts formed by 12-hydroxy- and 13-hydroxyellipticine in DNA as described in our former studies [7,12] (compare Fig. 4E and F). Besides these major adducts, two additional adducts (compare spots 6 and 7 in Fig. 4D) were detected at 10 μM ellipticine at low levels (Table 1). Both these minor adducts are known to be generated in DNA *in vivo* (Fig. 4D) and *in vitro,* when DNA reacted with metabolites formed mainly by peroxidase-mediated oxidation of ellipticine [12,31]. However, the low levels of these adducts prevented their further characterization. The electrochemical techniques [32,33] will be tested in our laboratory in future to determine whether they might be suitable for their characterization

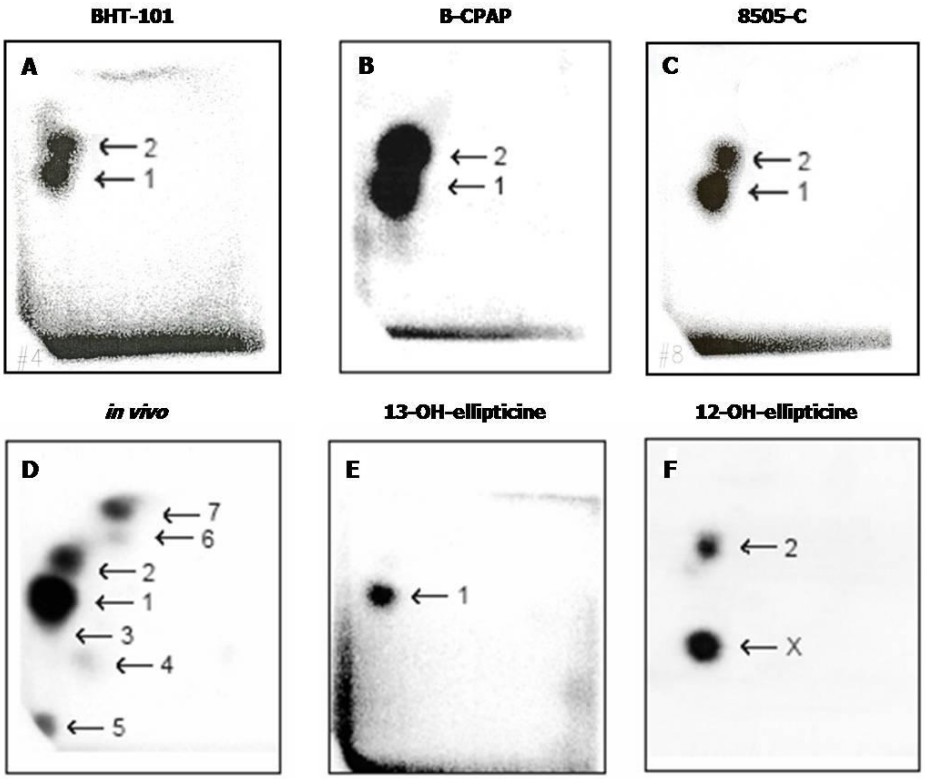

Figure 4. Autoradiographs of PEI-cellulose TLC maps of ^{32}P-labeled digests of DNA isolated from thyroid cancer cells BHT-101 (A), B-CPAP (B) and 8505-C (C) exposed to 10 μM ellipticine for 24 h, of liver DNA of rats treated with 40 mg ellipticine per kilogram body weight (D), from calf thymus DNA reacted with 13-hydroxyellipticine (E) and 12-hydroxyelipticine (F). Besides adduct 2 formed by 12-hydroxyellipticine, another strong adduct (spot X in panel F), which was not found in any other activation systems or *in vivo* [1-4,7,12,28,29] was generated. Analyses were performed by the nuclease P1 version of the ^{32}P-postlabeling assay. (A,B,D) Origins, located at the bottom left corners, were cut off before autoradiography.

Quantitative analyses showed that DNA adduct formation by ellipticine in human thyroid cancer cell lines was dose-dependent with an over-proportional increase between 1 and 10 μM ellipticine, and influenced by the cell culture conditions (Table 1, Fig. 5). The highest amounts of ellipticine-derived DNA adducts were found in B-CPAP cells, followed by BHT-101 and 8505-C cells (Table 1, Fig. 5). A decrease in DNA adduct formation was found in thyroid cancer cell lines treated with 10 μM ellipticine cultured under hypoxic conditions (Table 1, Fig. 5). The decrease in DNA adduct level correlated with a decrease in cytotoxicity of ellipticine to these cells (see Fig. 2). This was not the case when thyroid cancer cells were treated with 1 μM ellipticine under the lower oxygen concentration; practically the same DNA adduct levels were found both under the standard and hypoxic conditions. Therefore, low concentrations of oxygen seem to be sufficient for the oxidative bioactivation of ellipticine at low concentrations (1 μM).

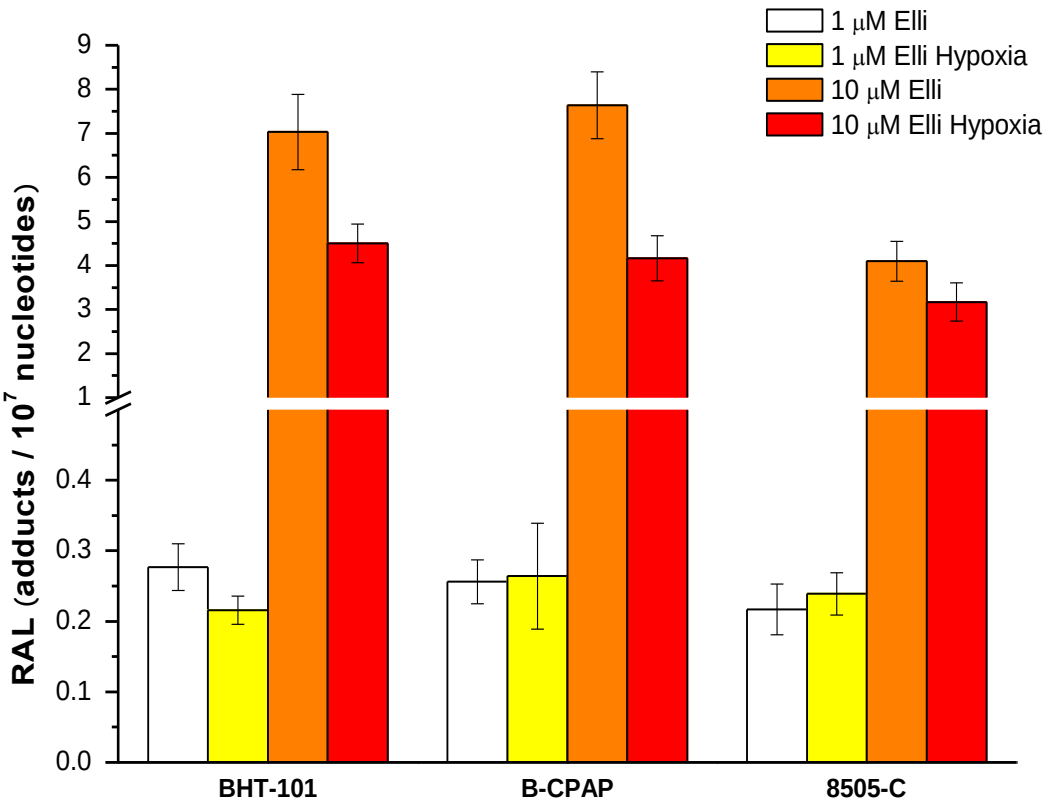

Figure 5. DNA adduct formation by ellipticine in human thyroid cancer cell lines cultured under normoxic and hypoxic conditions (total amounts of ellipticine-derived DNA adducts expressed as RAL, relative adduct labeling). Thyroid cancer cells were exposed to ellipticine for 24 h. DNA adduct formation was analyzed by the nuclease P1 version of the ^{32}P-postlabeling assay [1]. Data are expressed as averages ± S.D. of three experiments.

Table 1. Amounts of individual DNA adducts formed by ellipticine in human thyroid cancer cell lines cultured under normoxic and hypoxic conditions

Cells	Levels of DNA adducts (RAL x 10^{-7})[a]				
	Adduct 1	Adduct 2	Adduct 6	Adduct 7	Total
BHT-101					
Normoxia					
1 μM ellipticine	0.15 ± 0.03	0.13 ± 0.01	n.d.	n.d.	0.28 ± 0.03
10 μM ellipticine	3.9 ± 0.28	3.0 ± 0.50	0.1 ± 0.01	0.04 ± 0.01	7.04 ± 0.86
Hypoxia					
1 μM ellipticine	0.12 ± 0.07	0.10 ± 0.01	n.d.	n.d.	0.22 ± 0.02
10 μM ellipticine	2.35 ± 0.48	2.46 ± 0.17	0.05 ± 0.01	0.04 ± 0.01	4.9 ± 0.44
B-CPAP					
Normoxia					
1 μM ellipticine	0.12 ± 0.01	0.14 ± 0.02	n.d.	n.d.	0.26 ± 0.03
10 μM ellipticine	3.33± 0.31	4.18 ± 0.42	0.09 ± 0.01	0.03 ± 0.01	7.63 ± 0.76
Hypoxia					
1 μM ellipticine	0.11 ± 0.06	0.15 ± 0.09	n.d.	n.d.	0.26 ± 0.07
10 μM ellipticine	1.67 ± 0.20	2.43 ± 0.22	0.05 ± 0.01	0.01 ± 0.01	4.16 ± 0.51
8505-C					
Normoxia					
1 μM ellipticine	0.13 ± 0.05	0.08 ± 0.01	n.d.	n.d.	0.21 ± 0.04
10 μM ellipticine	2.22 ± 0.56	1.81 ± 0.28	0.05 ± 0.01	0.01 ± 0.01	4.09 ± 0.45
Hypoxia					
1 μM ellipticine	0.07 ± 0.01	0.16 ± 0.02	n.d.	n.d.	0.23 ± 0.03
10 μM ellipticine	1.39 ± 0.22	1.69 ± 0.20	0.06 ± 0.01	0.02 ± 0.02	3.16 ± 0.44

Thyroid cancer cells were exposed to ellipticine for 24 h. DNA adduct formation was analyzed by the nuclease P1 version of the ^{32}P-postlabeling assay [1]. [a]RAL, relative adduct labeling; data are expressed as averages ± S.D. of three experiments. N.d. - not detected (the detection limit of RAL was $1/10^{10}$ nucleotides).

3.4. Determination of CYP1A1, CYP1B1, CYP3A4, COX-1, TPO and cytochrome b_5 protein levels in thyroid cancer cells

Since a decrease in toxicity of ellipticine in the thyroid cancer cell lines was found under hypoxic conditions that corresponded to a decrease in DNA adduct formation, the expression levels of enzymes activating or detoxicating ellipticine (i.e. CYP1A1, 1B1, 3A4, COX-1 and TPO) were analyzed by Western blotting. Furthermore, as cytochrome b_5, a component of the CYP-dependent

enzymatic system, influences ellipticine oxidation by the CYP1A1 and 3A4 enzymes [13,15], expression levels of this protein were also evaluated.

As shown in Figure 6, expression patterns of metabolizing enzymes under different conditions are quite complex. Except for the expression of CYP1A1, the levels of which were high in all cells under all conditions, and TPO the levels of which were so low that the influence of culture conditions, cell lineage or ellipticine exposure cannot be assessed, expression levels of all other enzymes were different in the three cell lines. Most conspicuous was the near complete absence of cytochrome b_5 in 8505-C cells.

B-CPAP cells seem to be the thyroid cell line most sensitive to ellipticine and are also the cells with the highest ellipticine-DNA adduct levels. These cells also show consistent expression of CYP3A4, the enzyme responsible for formation of the active intermediates 13-hydroxy- and 12-hydroxyellipticine which lead to the two major ellipticine-derived DNA adducts [3,7,12,13,15]. The line 8505-C showed the lowest DNA adduct levels, was a slightly less sensitive to ellipticine in the cytotoxicity assay but the biggest effect of ellipticine on cell cycle distribution was observed, despite a near complete lack of cytochrome b_5 expression and a low CYP3A4 expression under normoxic conditions. In the BHT-101 line cytotoxicity, DNA-adduct levels and expression of activating enzymes such as the high levels of COX-1 are congruent. From the data on the three cell lines it seems as if effects on cell cycle are not entirely dependent on activating enzymes while DNA adduct levels and cytotoxicity as determined by the MTT assay are more dependent on ellipticine activation.

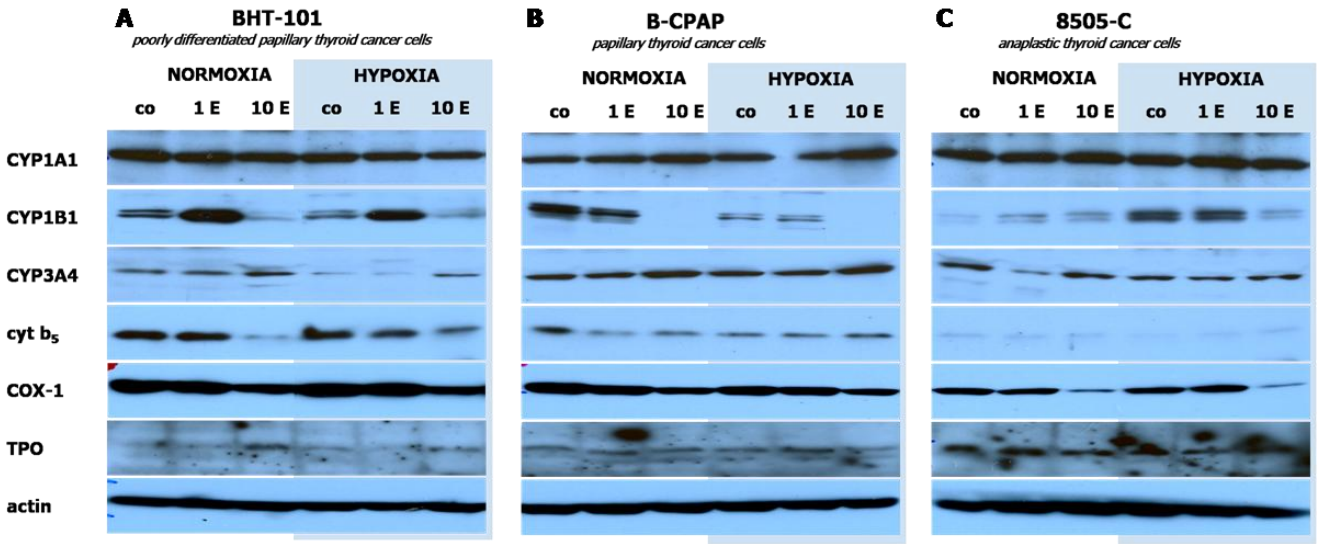

Figure 6. Immunoblots of cytochrome P450 (CYP) 1A1, 1B1, 3A4, cytochrome b_5, cyclooxygenase-1 (COX-1) and thyroid peroxidase (TPO) in BHT-101 (A), B-CPAP (B) and 8505-C (C) thyroid cancer cell lines. Actin was used as loading control. Control cells (CO) were either grown under normoxic or hypoxic conditions and exposed to 1 μM (1E) or 10 μM (10E) ellipticine for 24 h, harvested and proteins of the cells were separated by gel electrophoresis (SDS-PAGE), electro-blotted onto PVDF membranes and probed with the appropriate antibodies against the individual proteins as described in the methods section.

4. CONCLUSIONS

This study showed for the first time that ellipticine is toxic to BHT-101, B-CPAP and 8505-C thyroid cancer cell lines and that this toxic effect corresponded to ellipticine-derived DNA adduct formation in these cells. Our results indicate that expression of cytochrome b_5, CYP1A1, 3A4 and COX-1 influences the cytotoxicity and genotoxicity in the studied cell lines. Therefore, monitoring of the expression levels of enzymes metabolizing the anticancer drug ellipticine (activation and detoxication) by the Western blotting together with the ^{32}P-postlabeling technique utilized for detection and quantitation of DNA adducts are appropriate tools for evaluating the mechanism(s) of ellipticine toxicity in thyroid cancer cells. Future investigations will show whether ellipticine might be useful for the treatment of anaplastic thyroid carcinoma.

ACKNOWLEDGEMENTS
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