DNA ADDUCT FORMATION BY THE ANTICANCER DRUG ELLIPTICINE AND ITS HYDROXY DERIVATIVES IN HUMAN BREAST ADENOCARCINOMA MCF-7 CELLS

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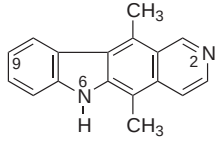
Dedicated to the 50th anniversary of the foundation of the Department of Biochemistry, the first biochemical department in Czechoslovakia.

The cytotoxicity of the antineoplastic agent ellipticine and its 9- and 7-hydroxylated metabolites to human breast adenocarcinoma MCF-7 cells and their ability to generate DNA adducts in these cancer cells were investigated. Ellipticine and its 9-hydroxylated metabolite were found to be toxic to MCF-7 cells with IC_{50} values of 1.25 and 3.25 μmol l^{-1} for ellipticine and 9-hydroxyellipticine, respectively. In contrast, no toxicity to these cancer cells was detectable for 7-hydroxyellipticine. The nuclease P1 version of the ^{32}P-postlabeling assay yielded a pattern of ellipticine–DNA adducts with two major and one minor adducts in MCF-7 cells, similar to the pattern of adducts detected in DNA reacted with ellipticine and the reconstituted cytochrome P450 enzyme system *in vitro* and in DNA *in vivo*. The identity of two major adducts formed in DNA of MCF-7 cells with those formed by cytochrome P450-mediated ellipticine activation *in vitro* was confirmed by HPLC of the isolated adducts. 9-Hydroxyellipticine was also capable of inducing DNA adducts in MCF-7 cells, but to a lesser extent. In addition, the adducts generated by 9-hydroxyellipticine were different from those generated by ellipticine. Negligible levels of DNA adducts were detectable in DNA of MCF-7 cells exposed to 7-hydroxyellipticine. The results presented here are the first report showing the formation of covalent DNA adducts with ellipticine in human breast cancer cells in culture, and suggest the formation of covalent DNA adducts as a new mode of antitumor action of ellipticine in breast cancer.

Keywords: Ellipticine; Anticancer drug; Human breast cancer MCF-7 cells; Cytotoxicity; Human cytochrome P450; Activation; DNA adducts; ^{32}P-Postlabeling; Antineoplastic activity.

Ellipticine (5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole, **I**), an alkaloid isolated from *Apocyanacea* plants, and several of its more soluble derivatives (9-hydroxyellipticine, 9-hydroxy-N^2-methylellipticinium, 9-chloro-N^2-methylellipticinium and 9-methoxy-N^2-methylellipticinium) exhibit significant antitumor and anti-HIV activities (for a summary, see literature[1]). The main reason for the interest in ellipticine and its derivatives for clinical purposes

is their high efficiencies against several types of cancer, their rather limited toxic side effects, and their complete lack of hematological toxicity[2]. Nevertheless, ellipticine is a potent mutagen.



I

9-Hydroxyellipticine and 7-hydroxyellipticine were found to be ellipticine metabolites in rats, as demonstrated *in vivo* and *in vitro* with microsomal preparations[1,3]. Cytochrome P450 (CYP)+ enzymes are believed to be the major enzymes responsible for formation of these metabolites[1–3].

Ellipticines are anticancer drugs, whose precise mechanisms of action have not yet been explained. It was suggested that the prevalent mechanisms of their antitumor, mutagenic and cytotoxic activities are (i) intercalation into DNA [4,5] and (ii) inhibition of DNA topoisomerase II activity[2,6–8]. Ellipticine and 9-hydroxyellipticine also cause selective inhibition of p53 protein phosphorylation in several human cancer cell lines[9], and this correlates with their cytotoxic activity. Ellipticines also uncouple mitochondrial oxidative phosphorylation[10] and thereby disrupt the energy balance of cells.

Recently, we found another mode of the ellipticine action[1,11,12]. We demonstrated that ellipticine covalently binds to DNA after being enzymatically activated. CYP3A4, 1A1 and 1B1 were found to be the most efficient enzymes activating ellipticine to form covalent DNA adducts *in vitro*[1]. The formation of these CYP-mediated covalent DNA adducts by ellipticine was also detected in V79 Chinese hamster lung fibroblast cells transfected with human CYP3A4, 1A1 and 1A2 [11] and *in vivo* in rats exposed to this anticancer drug[13]. On the basis of these data, ellipticine might be considered a drug, whose pharmacological eficiency and/or genotoxic side effects are dependent on its enzymatic activation in target tissues.

While CYP-mediated ellipticine–DNA adducts were detected in animal models (rats *in vivo*[13], cell lines of Chinese hamsters[11]), their formation in

+ *Abbreviations used*: CYP, cytochrome P450; DMEM, Dulbecco's modified Eagle's medium; HPLC, high-performance liquid chromatography; MDR, multidrug resistance; PBS, phosphate buffered saline; PEI-cellulose, polyethylenimine-cellulose; RAL, relative adduct labeling; r.t., retention time; SRB, sulforhodamine B; TLC, thin layer chromatography.

human tissues or cells has not yet been evaluated. We here examine whether DNA adducts are formed in human cell lines. Because breast cancer is one of the targets for ellipticine pharmacological action in humans, the breast adenocarcinoma cell line MCF-7 was used as model. The ^{32}P-post-labeling method to determine DNA adduct formation by ellipticine was used and cytotoxicity of ellipticine was determined with the sulforhodamine B (SRB) assay[14]. Besides ellipticine, its metabolites, 9- and 7-hydroxyellipticine, were evaluated for their potential to generate DNA adducts in MCF-7 cells and for their cytoxicity to these human cancer cells.

MATERIAL AND METHODS

Chemicals

Ellipticine, NADPH, and sulforhodamine B were obtained from Sigma (St. Louis, MO, U.S.A.). 9-Hydroxyellipticine was purchased from Calbiochem (San Diego, CA, U.S.A.). All these and other chemicals used in the experiments were of analytical purity or better. 7-Hydroxyellipticine was synthesized according to literature[15] by J. Kučka (Charles University, Prague); its purity was 99.7% as estimated by HPLC.

Cell Culture

The MCF-7 cell lines were from the collection of cell lines of the German Cancer Research Center (Heidelberg, Germany). MCF-7 cells were cultivated in Dulbecco's modified Eagle's medium (DMEM, Biochrom AG, Berlin, Germany), high-glucose type (DMEM with 4.5 g D-glucose/l), supplemented with 4 mM L-glutamine, 25 mM HEPES (Sigma, St. Louis, MO, U.S.A.), 5% fetal calf serum (Biochrom AG, Berlin, Germany) at 37 °C, 5% CO_2 and 95% atmospheric humidity.

Preparation of Microsomes and Assays

Microsomes were isolated from MCF-7 cells by the procedure described earlier[1,11,12]. Protein concentrations in the microsomal fractions were assessed using the bicinchoninic acid protein assay (Pierce Rockford, IL, U.S.A.) with serum albumin as a standard[16]. Supersomes™, microsomes isolated from insect cells transfected with baculovirus constructs containing cDNA of human CYP1A1 and 1A2 and expressing NADPH:CYP reductase were obtained from Gentest Corp. (Woburn, MA, U.S.A.). The concentration of CYP was estimated according to Omura and Sato[17] on the basis of the absorption of the complex of reduced CYP with carbon monoxide.

Isolation of CYP Enzymes and Preparation of Anti-CYP Antibodies

Recombinant rat CYP1A1 protein was purified to homogeneity by the procedure described previously[18] for membranes of *Escherichia coli* transfected with a modified CYP1A1 cDNA, in the laboratory of H. W. Strobel (University of Texas, Medical School of Houston, TX, U.S.A.)

by P. Hodek (Charles University, Prague). Human recombinant CYP3A4 was a gift of P. Anzenbacher (Palacký University, Olomouc, Czech Republic).

Leghorn chickens were immunized subcutaneously three times a week with the isolated CYP proteins (rat recombinant CYP1A1, human recombinant CYP3A4) (0.1 mg/animal) emulsified in complete Freund's adjuvant for the first injection and in incomplete one for boosters. Immunoglobulin fraction was purified from pooled egg yolks as described by Polson et al.[19,20] using fractionation by poly(ethylene glycol) 6000.

Estimation of CYP Content in Microsomes of MCF-7 Cells

Immunoquantitation of microsomal CYP1A and 3A was performed by Western blot. Samples containing 10–50 μg microsomal proteins were solubilized and subjected to electrophoresis on SDS/10% polyacrylamide gels[21–23]. After migration, proteins were transferred onto poly-(vinylidene difluoride) membranes. Microsomal CYP1A1 protein was probed with a chicken polyclonal antibody raised against rat recombinant CYP1A1 as reported elsewhere[24,25]. This antibody recognized human recombinant CYP1A1 and 1A2 expressed in Supersomes™ (Gentest, Woburn, MA, U.S.A.) as two distinct bands, exhibiting very similar mobilities[26] as well as CYP1A1 in microsomes of MCF-7 cells. The CYP3A4 protein of microsomes from MCF-7 cells was probed with chicken polyclonal antibody raised against human CYP3A4. The antigen–antibody complex was visualized with an alkaline phosphatase-conjugated anti-chicken IgG rabbit antibody and 5-bromo-4-chloroindol-3-yl phosphate/Nitro Blue Tetrazolium as dye. Membranes were scanned with a computerized image-analyzing system (Imstar). The detection limit was 0.005 pmol CYP1A per lane (see also literature[26]) and 0.01 pmol CYP3A4 per lane.

Sulforhodamine B (SRB) Assay

The cytotoxicity of ellipticine and its 9- and 7-hydroxy derivatives was determined in a 96-well plate by the SRB assay[14]. For a dose-response curve, cells in exponential growth were seeded in 200 μl of medium with 10^4 cells per well. After 24 h, 5 μl of methanolic solution of elipticine, 9- or 7-hydroxyellipticine in final concentrations of 0.5, 1, 1.5, 2, 3, 5, 10, 15 or 20 μmol l^{-1} was added. Control cells and medium controls without cells received 5 μl of methanol. After an incubation period of 48 h, cells were fixed with 5% acetic acid and 95% ethanol, proteins were stained with 0.4% of SRB in 1% acetic acid. An amount of 100 μl of 10 mM Tris (pH 10.5) was added to dissolve the protein-bound dye. Spectral measurement was performed at 546 nm on a multiplate reader with a control wavelength of 680 nm (μQuant, Biotec Instruments Inc., Winooski, Vermont, U.S.A.). The mean absorbance of medium controls was the background and was substracted. 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 mean of 8 wells with standard deviations. The IC_{50} values were calculated from the linear regression of the dose–log response curves.

Treatment of MCF-7 Cells with Ellipticine, 9-Hydroxyellipticine and 7-Hydroxyellipticine for DNA Adduct Analyses

MCF-7 cells were seeded 24 h prior to treatment at a density of 1×10^5 cells/ml in 75 cm^3 culture flasks in a total volume of 30 ml of DMEM. Ellipticine and its hydroxy derivatives were dissolved in 30 μl of methanol and the solvent was added to a final concentration of

10 μmol l^{-1}. After 24 h the medium was removed and the cells were washed twice with 5 ml of PBS. During this step all dead cells were washed away. Cells were harvested by trypsinization with 2 ml PBS containing 0.025% of trypsin and 0.01% of EDTA (Biochrom AG, Berlin, Germany). Trypsinization was stopped by addition of 2 × 4 ml of medium. Subsequently, centrifugation at 2000 rpm for 10 min and one washing step with 10 ml of PBS yielded a cell pellet, which was stored at –20 °C till the DNA isolation. DNA was isolated and labeled as described in the next section.

DNA Isolation and ^{32}P-Postlabeling of DNA Adducts

DNA from cells was isolated by the phenol extraction version as described[11]. ^{32}P-Postlabeling analyses were performed using nuclease P1 enrichment as described previously[1,11–13]. Separation was carried out on PEI-cellulose thin layer plates (Macherey and Nagel, Düren, Germany). Chromatographic conditions used were: D1, 1.0 M sodium phosphate, pH 6.8; D2 was omitted; D3, 3.5 M Li formiate, 8.5 M urea, pH 4.0; D4, 0.8 M LiCl, 0.5 M Tris, 8.5 M urea, pH 9.0; D5, 1.7 M sodium phosphate, pH 6.0. Normal nucleotides were separated in 280 M $(NH_4)_2SO_4$ and 50 M NaH_2PO_4, pH 6.5. Quantitative analysis was performed using a Canberra Packard instant imager. Adduct levels were calculated in units of relative adduct labeling (RAL) which is the ratio of cpm of adducted nucleotides to cpm of total nucleotides in the assay.

HPLC Analysis of ^{32}P-Labeled Adducts

HPLC analysis was performed essentially as described previously[11,12,27–29]. Individual spots detected by ^{32}P-postlabeling were excised from the thin layer and extracted[27–29]. Cut-outs were extracted with two 800 μl portions of 6 M ammonium hydroxide/isopropanol (1:1) for 40 min. The eluent was evaporated in a Speed-Vac centrifuge. The dried extracts were dissolved in 100 μl of methanol/phosphate buffer (pH 3.5) 1:1 (v/v). Aliquots (50 μl) were analyzed on a phenyl-modified reversed-phase column (250 mm × 4.6 mm, 5 μm Zorbax Phenyl; Säulentechnik Knauer) with a linear gradient of methanol (from 40 to 80% in 45 min) in aqueous 0.5 M sodium phosphate and 0.5 M phosphoric acid (pH 3.5) at a flow rate of 0.9 ml/min. Radioactivity eluting from column was measured by monitoring Cerenkov radiation with a Berthold LB 506 C-I flow-through radioactivity monitor (500 μl cell, dwell time 6 s).

RESULTS

Determination of DNA Adduct Formation by Ellipticine, 9-Hydroxyellipticine and 7-Hydroxyellipticine in MCF-7 Cells

Human breast adenocarcinoma MCF-7 cells in culture were treated with 10 μM ellipticine for 24 h. Using the nuclease P1 version of ^{32}P-postlabeling assay, which was found to be suitable to detect and quantify ellipticine–DNA adducts[1,11–13], ellipticine-derived adducts were detected in the DNA of these cells (Fig. 1b). The pattern of ellipticine–DNA adducts consisted of two major (spots 1 and 2) and one minor (spot 3) adducts (Fig. 1b). No ad-

ducts were detected in DNA of control MCF-7 cells treated with solvent (methanol) only (Fig. 1a).

Chromatographic properties of two major adduct spots on PEI-cellulose TLC plates (spots 1 and 2) were similar to those of ellipticine-derived DNA adducts found previously after *in vitro* incubation of calf thymus DNA with ellipticine and isolated CYP [1,12] or after treatment of V79 cells with this anticancer drug[11] or *in vivo*, in several organs of rats exposed to this agent[13,28,29]. For comparison, Fig. 1e shows adducts in DNA reacted with ellipticine and human CYP3A4 reconstituted with NADPH:CYP reductase[1,12]. Both adducts were identified as adducts of ellipticine bound on deoxyguanosine in DNA [12].

The adduct spots 1 and 2 obtained from DNA of MCF-7 cells and those from experiments with human recombinant CYP3A4 *in vitro* were excised, extracted and analyzed by co-chromatography on reversed-phase HPLC.

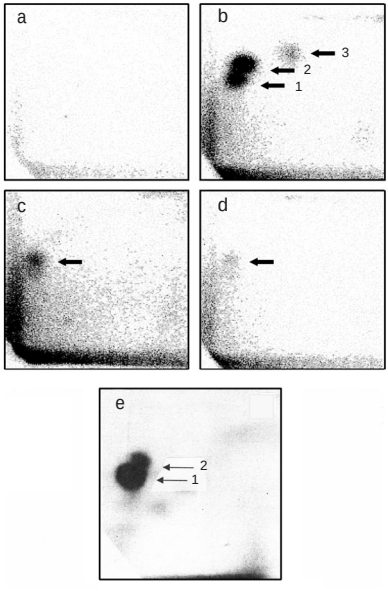


FIG. 1

Autotoradiographs of PEI-cellulose TLC maps of ^{32}P-labeled digests of DNA isolated from MCF-7 cells a treated with 5 μl of methanol (control); b exposed to 10 μM ellipticine; c 9-hydroxyellipticine; d 7-hydroxyellipticine for 24 h; e calf thymus DNA reacted with ellipticine, NADPH and recombinant human CYP3A4 reconstituted with NADPH:CYP reductase *in vitro*[1]. Analyses were performed by the nuclease P1 version of the ^{32}P-postlabeling assay. a–d Scans of the plates for 25 min from the imager; e an autoradiograph of a film exposed at –80 °C for 2 h. Origins are located at the bottom left corners (D3 from bottom to top and D4 from left to right)

These experiments showed that the ^{32}P-labeled ellipticine adducts were stable under the alkaline extraction conditions used and that both major adducts formed in DNA of MCF-7 cells were indistinguishable from those obtained in the *in vitro* experiments. The major adduct formed in DNA of MCF-7 cells (spot 1 in Fig. 1b) eluted with retention time (r.t.) of 11.78 min (Fig. 2b), corresponding to the r.t. of 11.77 min of deoxyguanosine adduct spot 1 in DNA exposed to ellipticine activated with purified human CYP3A4 *in vitro* (Fig. 2a)[1,12]. When equal amounts of radioactivity of these adduct spots were mixed prior to analysis, a single peak was found (data not shown). Adduct spot 2 of Fig. 1b produced a major radioactive peak (r.t. 9.0 min) (Fig. 2d) corresponding to adduct spot 2 formed by ellipticine activated with purified CYP3A4 *in vitro* (Fig. 2c). A minor peak of radioactivity was eluted at 14.23 min, showing that a minor adduct is present both in

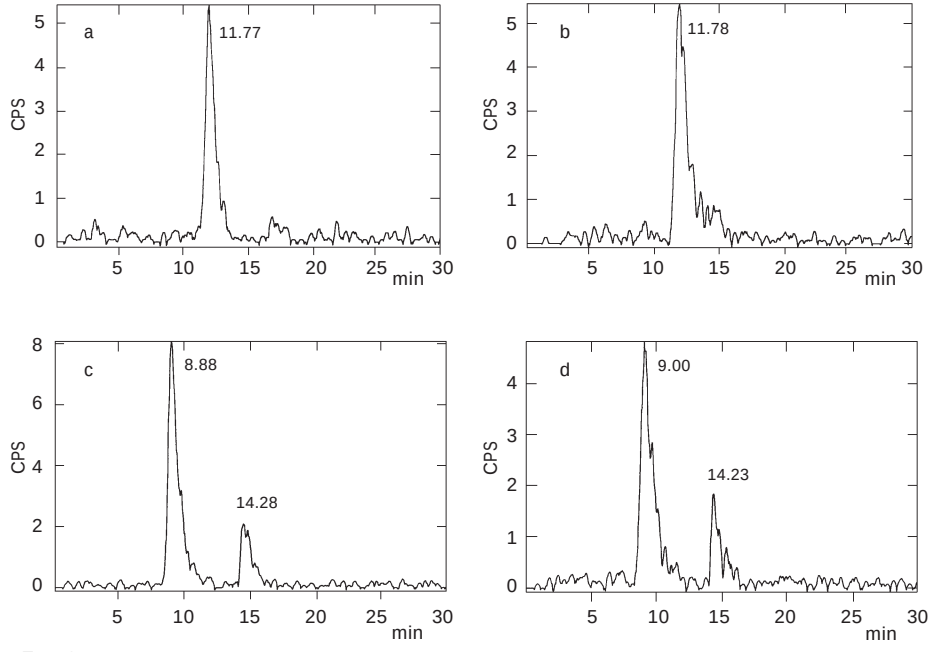


FIG. 2

Separation of ^{32}P-labeled nucleoside 3′,5′-bisphosphate adducts on a phenyl-modified reversed-phase column to compare adducts obtained *in vitro* (Fig. 1e) with those isolated from MCF-7 cells (Fig. 1b). Adduct spots were excised and extracted from PEI-cellulose TLC plates, dissolved and injected into the HPLC system. a Spot 1 from Fig. 1e; b spot 1 from Fig. 1b; c spot 2 from Fig. 1e; d spot 2 from Fig. 1b. The abcissa shows the retention time, the ordinate radioactivity in arbitrary units

DNA of MCF-7 cells and in DNA after ellipticine activation with human CYP3A4 *in vitro*, comigrating with adduct spot 2 on TLC (Fig. 2c, 2d). The minor adduct spot 3 found in DNA of MCF-7 cells seems to migrate similarly to the adduct detected in DNA of V79 cells exposed for more than 24 h [11], in human hepatic microsomes[1,12] and in several organs of rats treated with ellipticine *in vivo*[13], but the low adduct levels prevented HPLC co-chromatographic analysis or its further characterization.

Recently, we found that the formation of ellipticine–DNA adduct spot 1 *in vitro* and *in vivo* is dependent on its activation by CYP enzymes of 3A and 1A subfamilies[1,11–13,28,29]. As the same DNA adduct was identified in DNA of MCF-7 cells, these enzymes should be expressed in MCF-7 cells. Indeed, expression of CYP1A1 and CYP3A4 proteins in microsomes isolated from MCF-7 cells was proved with Western blot analysis (data not shown).

9-Hydroxyellipticine was also capable of inducing DNA adducts in MCF-7 cells (Fig. 1c). Even though their migration on PEI-cellulose TLC plates seemed to be similar to that of adducts formed by ellipticine, co-chromatography of these adducts on HPLC showed that the 9-hydroxyellipticine adducts are different from adducts 1 and 2 generated by ellipticine itself (not shown). Quantitative analyses revealed that levels of DNA adducts generated with 9-hydroellipticine were ≈20-fold lower than those formed in DNA by ellipticine (Table I). The potential of 7-hydroxyellipticine to form DNA adducts was even lower (Fig. 1d). Negligible levels of DNA adducts were detectable in DNA of MCF-7 cells exposed to this hydroxylated metabolite of ellipticine (Table I).

TABLE I
DNA adduct formation by ellipticine and its 9- and 7-hydroxylated metabolites in MCF-7 cells and their cytotoxicity to these cells

Compound	Total DNA adduct formation RAL × 10^{-7}	IC_{50} [a] μmol l^{-1}
Ellipticine	9.3	1.25
9-Hydroxyellipticine	0.4	3.25
7-Hydroxyellipticine	0.01	–[b]

[a] IC_{50} values were calculated from the linear regression of the dose–log response curves. MCF-7 cells were exposed to the compounds for 48 h. DNA adducts were analyzed by the nuclease P1 version of the ^{32}P-postlabeling assay. [b] No toxic effects of 7-hydroxyellipticine were observable.

Cytotoxicity of Ellipticine, 9-Hydroxyellipticine and 7-Hydroxyellipticine in Human Breast Cancer MCF-7 Cells

To determine the cytotoxicity of ellipticine and its 9- and 7-hydroxy-metabolites to human breast cancer MCF-7 cells, these cells were treated with increasing concentrations of ellipticine and its derivatives. While 7-hydroxyellipticine did not exhibit any cytotoxic effect to MCF-7 cells up to 20 μmol l^{-1}, toxicity of ellipticine and its 9-hydroxylated metabolite to these cells was clearly detectable. Their toxicity was dose-dependent, being stronger for ellipticine (Fig. 3). The IC_{50} value for ellipticine calculated from the dose–log response curves was less than half of that for 9-hydroxy-ellipticine (Table I).

DISCUSSION

In previous studies[1,12] we used two direct independent methods, namely ^{32}P-postlabeling and tritium-labeled ellipticine to show that ellipticine binds covalently to DNA *in vitro* after CYP-catalyzed activation. The ^{32}P-postlabeling technique was also employed to detect ellipticine-derived DNA adducts in Chinese hamster fibroblast V79 cells[11], in rats *in vivo*[13,28,29] and, here, also in human breast cancer MCF-7 cells. The results of our present work clearly demonstrate that human breast cancer MCF-7 cells mediate the bioactivation of the anticancer drug ellipticine to DNA-binding species.

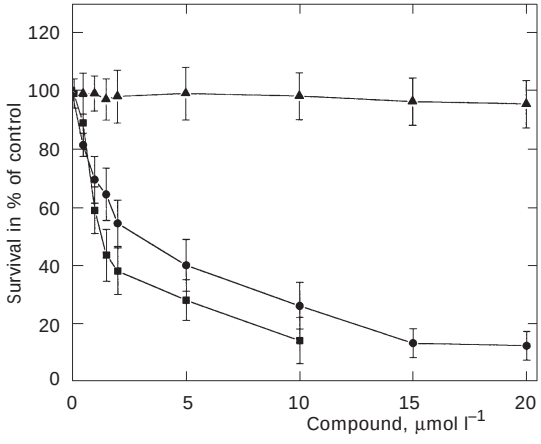


FIG. 3
Cytotoxicity (viable cells as percentage of control) of ellipticine (■), 9-hydroxyellipticine (●) and 7-hydroxyellipticine (▲) to MCF-7 cells after 48 h exposure to the compounds, determined by the SRB assay. N = 8, values are means ± SD

The active derivatives generated in the cells, which bind to DNA, seem to be the same as those formed from ellipticine by human CYPs *in vitro*[1,12], because the HPLC profiles of the two major DNA adducts isolated from cells and from *in vitro* incubations are the same. These adducts were found to be the adducts of ellipticine bound on deoxyguanosine in DNA *in vitro*[1,11,12] and *in vivo*[13,28,29]. Besides these two adducts, an additional minor adduct (spot 3) was detectable in MCF-7 cells, migrating similarly to the adduct formed in enzymatic systems containing high levels of CYP3A enzymes (V79 cells expressing this enzyme, human hepatic microsomes containing CYP3A4 as the major CYP enzyme, rat organs expressing CYP3A1, an orthologous form of human CYP3A4)[1,11–13]. Low levels of this adduct precluded its further characterization.

In addition to the two known mechanisms of DNA damage (intercalation of ellipticine into DNA and generation of DNA strand breaks by inhibiting mammalian topoisomerase II), we have now fully confirmed a third one, covalent binding of activated ellipticine. Because here we clearly show that the ellipticine–DNA adducts were detectable in human breast cancer cells, this new mode of ellipticine action might be possible also in humans. Nevertheless, formation of ellipticine–DNA adducts in humans *in vivo* has to be confirmed by further studies. This would be feasible, because ellipticine is used in tumor therapy[2].

9-Hydroxyellipticine, a metabolite formed in rats *in vivo*, and in human hepatic microsomes *in vitro*[2], is also capable of forming adducts in DNA of MCF-7 cells, but these adducts are different from those formed in DNA by ellipticine. Another ellipticine metabolite, 7-hydroxyellipticine, generates only traces of DNA adducts. Lower levels of DNA adducts generated by 9-hydroxyellipticine than by ellipticine itself or negligible amounts of the DNA adducts formed by 7-hydroxyellipticine correspond to the toxicity of these compounds to MCF-7 cells. No toxic effect of 7-hydroxyellipticine on the cancer cells was detected up to 20 μmol l^{-1}.

The ellipticine species, which reacts with deoxyguanosine in DNA *in vitro* and *in vivo* remains to be elucidated as well as the position in guanine where this species is bound. The present study indicates that the C-hydroxylated ellipticine metabolite, 9-hydroxyellipticine is not responsible for formation of ellipticine-derived DNA adducts. Preliminary results[28,29] indicate that the major adduct 1 is generated from an ellipticine metabolite produced by CYP3A4 (and/or CYP1A), containing one atom of oxygen in its molecule, whose structure is speculated to be 5-hydroxyellipticine, while the adduct 2 is formed from the N^2-oxide of ellipticine. Confirmation of these results is under way in our laboratory.

A covalent binding of metabolically activated ellipticine to DNA, as one type of the multiple genotoxic effects of ellipticine, has already been suggested by DeMarini and coworkers[30]. Because almost all of the complex mutations induced by ellipticine in *Salmonella* strain TA98 occur at sites containing deoxyguanosine within or near a repeated sequence[30], this deoxynucleoside seems to be crucial for the mutagenic effects of ellipticine. In view of our finding that deoxyguanosine is also the target in DNA of human MCF-7 cells and for the CYP-catalyzed covalent binding of ellipticine to DNA *in vivo*, further studies into the involvement of such ellipticine–deoxyguanosine adducts in the genotoxic or antitumor effects of ellipticine in mammals including humans seem warranted.

At the present time, it is not possible to demonstrate whether the antitumor, cytostatic and/or genotoxic activities of ellipticine are related to only one or several of the DNA damaging effects. Recently, we demonstrated that the cytotoxicity for Chinese hamster fibroblast V79 cells shown by ellipticine, was not dependent on CYP expression[11]. Acute toxicity in these cells could be caused by the parent compound itself for instance by uncoupling mitochondrial oxidative phosphorylation and disrupting the cell energy balance[10], or by CYP-independent metabolites. For ellipticine antitumour activity against cancer cells, however, other properties such as mutagenicity caused by DNA adducts might be relevant. Indeed, Rekha and Sladek[31] demonstrated that the cytotoxic activity of ellipticine to MCF-7 cells depends on the levels of CYP enzymes activating ellipticine to DNA-binding species. These authors showed that MCF-7 cells treated with 3-methylcholanthrene transiently expressed elevated levels of CYP1A and were transiently much more sensitive to ellipticine. The DNA adducts we have observed might be responsible for the observed higher sensitivity.

Another important feature related to the expression of CYP and ellipticine was detected in MCF-7 cells selected for resistance to adriamycin (Adr^R MCF-7), exhibiting the phenotype of multidrug resistance[32] (MDR). Ivy et al.[32] postulated that the resistance of Adr^R MCF-7 cells involves several biochemical and genetic changes besides MDR. One of them is a regulatory defect at the level of CYP1A1 mRNA [32] resulting in lower CYP1A1-mediated metabolism of xenobiotics in these cells. Adr^R MCF-7 cells are cross-resistant to elipticine[32], which we would explain by a decrease in the CYP1A1-dependent activation of ellipticine. Taken together, the activities and expression levels of CYP enzymes, which effectively activate ellipticine to metabolites forming DNA adducts, may be important factors in the specificity of ellipticine for breast cancer.

CONCLUSIONS

The results presented in this paper are the first report demonstrating the formation of ellipticine–DNA adducts in a cell line of human breast adenocarcinoma. One of the most important results of this study is the finding that activation of ellipticine to species binding to DNA in these human cancer cells is analogous to that observed with human and rat enzymatic systems *in vitro*[1,11,12] and in rats *in vivo*[28,29]. These results suggest that this mode of ellipticine action found previously as a new mechanism for its anticancer efficiency in animal models seems to be probable also in humans. The similarities in DNA adduct formation and the fact that MCF-7 cells express both CYP 1A and CYP3A4 makes these cells an appropriate model to evaluate the fate of ellipticine in a target tissue of its pharmacological action, namely, human breast cancer.

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