THE ANTICANCER DRUG ELLIPTICINE IS AN INDUCER OF RAT NAD(P)H:QUINONE OXIDOREDUCTASE

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The antineoplastic agent ellipticine was investigated for its ability to induce the biotransformation enzyme NAD(P)H:quinone oxidoreductase (DT-diaphorase, EC 1.6.99.2) in male Wistar rats. Using the real-time polymerase chain reaction, the levels of NAD(P)H:quinone oxidoreductase mRNA were determined in livers, kidneys and lungs of rats treated intraperitoneally with ellipticine (40 mg/kg body weight) and of control (untreated) rats. Cytosolic fractions were isolated from the same tissues of control and ellipticine-treated rats and tested for NAD(P)H:quinone oxidoreductase protein expression and its enzymatic activity. The results demonstrate that ellipticine is a potent inducer of NAD(P)H:quinone oxidoreductase in rat livers and kidneys, while no induction of this enzyme was detectable in rat lungs. The increase in levels of NAD(P)H:quinone oxidoreductase mRNA correlates with the increase in expression of its protein and enzymatic activity, measured with menadione and 3-nitrobenzanthrone as substrates. The results, the identification of the potential of ellipticine to induce NAD(P)H:quinone oxidoreductase, suggest that this drug is capable of modulating biological efficiencies of the toxicants and/or drugs that are reductively metabolized by this enzyme.

Keywords: NAD(P)H:quinone oxidoreductase; Enzyme induction; Anticancer drugs; Carcinogens; Metabolic activation; Ellipticine.

Pharmacological efficiencies of many drugs and genotoxic effects of most carcinogens are dependent on their metabolic activation. Although a majority of such xenobiotics is activated by oxidative reactions, participation of reductive metabolism in activation of xenobiotics is unquestionable. Knowledge of enzymes participating in such reductive activations is crucial for many reasons. For example, it is important for elucidation of the fate of

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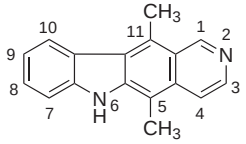
protoxicants and procarcinogens, which become toxic after their reductive activation in organisms. Furthermore, it is essential for the development of an ideal cancer chemotherapeutic prodrug, which would be fully inactive until reductively metabolized by tumor-specific enzymes, or by an enzyme that is specific only for the prodrug under physiological conditions and is unique for the tumor. An enzyme system that fulfils one or both of these criteria might be the cytosolic enzyme, NAD(P)H:quinone oxidoreductase (DT-diaphorase, NQO1; EC 1.6.99.2.). In general, NQO1 activity is higher in tumors than in the surrounding normal tissues[1]. Schlager and Powis[2] showed that enhanced levels of this enzyme have been found in primary colonic, breast and lung carcinoma as well as human hepatoma, whilst gastric adenocarcinomas had low NQO1 activities compared with adjacent normal tissues[2]. The enzyme is efficient both under aerobic and hypoxic (anaerobic) conditions, the latter being typical features of tumor cells[3,4]. Cytostatic agents have been designed to become activated by the NOQ1 enzyme[5], the prototype compound for bioreductive activation being mitomycin C [6]. Conversely, resistance to mitomycin C was associated with a decreased activity of NQO1 [7].

The obligatory two-electron reduction of quinones catalyzed by NQO1 circumvents the semiquinone stage and thereby prevents redox cycling and alkylation by these highly reactive compounds[8]. This is well documented for many quinones. However, some hydroquinones are also autoxidizable or can act as alkylation agents. Such compounds are activated by NQO1 to their ultimately toxic form[9]. Likewise, reductive activation of numerous other compounds such as toxic chemicals (azo dyes and nitroso- or nitroaromatics) or anticancer drugs (e.g. prodrugs mitomycin C and indoloquinone EO9) was discovered as a function of NQO1 [3,10–21]. In many cases, a mixture of several anticancer drugs is utilized in cancer treatment. Therefore, drug–drug interactions and the effects of each of the drugs on expression and activities of NQO1 and/or other biotransformation enzymes (induction and/or repression) might be essential for their fate in organisms as well as for their pharmacological efficiencies.

The NQO1 enzyme is inducible by a variety of agents[10,22,23]. Two distinct regulatory elements in the 5′ flanking region of the *NQO1* gene that have been studied extensively are the antioxidant response element (ARE), also called the electrophile response element (EpRE), and the xenobiotic response element (XRE), also called aryl hydrocarbon response element (AhRE). The ARE and the XRE have been shown to mediate NQO1 induction as well as repression, in many cellular systems[10]. Induction through the XRE involves the liganded aromatic hydrocarbon receptor (AhR). The

human NQO1 XRE shares significant homology with the human CYP1A1 XRE [24]. Both *NQO1* and *CYP1A1* genes can be induced by 2,3,7,8-tetrachlorodibenzo[1,4]dioxine (TCDD) and polycyclic aromatic hydrocarbons[25], while DeLong et al.[26] have suggested that the induction of NQO1 is largely dependent on the ability of bifuctional inducers such as azo dye, Sudan I, Sudan III and flavonoid β-naphthoflavone to first undergo conversion to oxidative labile metabolites through a fuctional CYP-dependent mechanism. ARE-mediated *NQO1* gene expression is increased by a variety of antioxidants such as butylated hydroquinone and butylated hydroxyanisole, tumor promoters and hydrogen peroxide[27–29]. Nuclear factor-erythroid 2 (NF-E2) related factor 2 (Nrf2) is a basic leucine zipper transcriptional factor that plays a key role in ARE-mediated *NQO1* gene expression[30]. Antiestrogens tamoxifen and hydroxytamoxifen are also inducers of NQO1; they stimulate expression of NQO1 by activation of a receptor specific for estrogens (the ER receptor), which is different from the *Ah* locus[31,32]. In contrast to livers, an induction of the enzyme in other tissues has not been studied in details as yet.

The aim of the present study was to evaluate whether NQO1 is induced not only in liver, but also in other tissues, and to investigate the potential of one of the anticancer agents, an alkaloid ellipticine (5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole (**1**)), known for its significant antitumor and anti-HIV activities (for a review, see refs[33,34]), to induce the expression and activities of NQO1 enzyme. Ellipticine was found to bind to the ligand-activated transcription factor, AhR [35,36], suggesting the potential of this drug to induce expression of biotransformation enzymes that are regulated by this receptor (CYP1A, NQO1 and/or glutathione-*S*-transferases)[20,36–38]. Indeed, recently we demonstrated that ellipticine is capable of inducing two of such enzymes, CYP1A1 and 1A2, in rats[39]. However, the question whether this drug induces NQO1 remains to be answered. Therefore rats, the animals found to be suitable models mimicking the fate of ellipticine in humans[40–43], were treated with ellipticine, and expression of NQO1 in livers, kidneys and lungs was monitored.



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EXPERIMENTAL

Abbreviations Used

AAI, aristolochic acid I; AAII, aristolochic acid II; AhR, aromatic hydroxarbon receptor; ARNT, AhR nuclear translocator; ARE, antioxidant response element; c_T, cycle threshold; dA, deoxyadenosine; dG, deoxyguanosine; dA-N^6-ABA, 3-amino-2-(2′-deoxyadenosin-N^6-yl)-benzanthrone 3′-phosphate; dG-N^2-ABA, 3-amino-*N*-(2′-deoxyguanosin-N^2-yl)benzanthrone 3′-phosphate; dG-C8-*N*-ABA, 3-amino-*N*-(2′-deoxyguanosin-8-yl)benzanthrone 3′-phosphate; DMSO, dimethyl sulfoxide; EpRE, electrophile response element; ER, estrogen receptor; *N*-Aco-ABA, *N*-acetoxy-3-aminobenzanthrone; NQO1, NAD(P)H:quinone oxidoreductase; 3-NBA, 3-nitrobenzanthrone; NF-E2, nuclear factor-erythroid 2; Nrf2, nuclear factor-erythroid 2 related factor; PVDF, poly(vinylidene difluoride); CYP, cytochrome P450; SDS, sodium dodecyl sulfate; TCDD, 2,3,7,8-tetrachlorodibenzo[1,4]dioxine; TLC, thin-layer chromatography; HPLC, high-performance liquid chromatography; RT, real-time; PCR, polymerase chain reaction; XRE, xenobitoic response element.

Chemicals and Reagents

NADH, NADPH, ellipticine, menadione (2-methyl-1,4-naphthoquinone), calf thymus DNA and dimethyl sulfoxide (DMSO) were obtained from Sigma Chemical Co., St. Louis (MO), U.S.A. 9-Hydroxyellipticine (5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazol-9-ol) was from Calbiochem, San Diego (CA), U.S.A. All these and other chemicals from commercial sources used in the experiments were reagent grade or better. 3-NBA was synthesized as described recently[44] and its authenticity was confirmed by UV spectroscopy, electrospray mass spectra and high-field ^{1}H NMR spectroscopy.

Animal Experiments

The study was conducted in accordance with the Regulations for the Care and Use of Laboratory Animals (311/1997, Ministry of Agriculture, Czech Republic), which is in compliance with Declaration of Helsinki. Male Wistar rats (~100 g) were treated with a single dose of 40 mg/kg body weight (n = 3) of ellipticine by intraperitoneal injection by the procedure as described[39]. The animals were killed 48 h after treatment by cervical dislocation. Livers, lungs and kidneys were removed immediately after death and used for isolation of DNA, mRNA and for preparation of cytosolic fractions.

Preparation of Cytosolic Fractions

Cytosolic fractions were isolated from the livers of rats, either non-induced or pretreated with ellipticine (see above) as described[11,12]. Protein concentrations in the cytosolic fractions were assessed using the bicinchoninic acid protein assay with serum albumin as a standard[45]. Hepatic, renal and pulmonary cytosolic preparations from rats that had been pretreated with ellipticine were analyzed for the presence of ellipticine and its metabolites using the HPLC as described[42]. None of the chemicals was detectable in cytosolic fractions from tissues of rats treated with ellipticine.

NQO1 mRNA Content in Rat Livers, Kidneys and Lungs

Total RNA was isolated from frozen livers, kidneys and lungs of three untreated rats and three rats pretreated with 40 mg/kg body weight of ellipticine, using Trizol Reagent (Invitrogen, Carlsbad (CA), U.S.A.) according to the manufacturer. The quality of isolated RNA was verified by horizontal agarose gel electrophoresis, RNA quantity was assessed by UV-VIS spectrophotometry on a Carry 300 spectrophotometer (Varian, Palo Alto (CA), U.S.A.). RNA samples (1 μg) were reversely transcribed using 200 U of reverse transcriptase per sample with random hexamer primers utilizing RevertAid™ First Strand cDNA Synthesis Kit (MBI Fermentas, Vilnius, Lithuania) according to the manufacturer's instructions. The prepared cDNA was used for real-time (RT) polymerase chain reaction (PCR), which was performed as described[46]. Data were analyzed by the program RotorGene v6 (Corbett Research, Sydney, Australia) and evaluated by comparative cycle threshold (c_T) method for relative quantitation of gene expression. Cycle thresholds, at which a significant increase in fluorescence signal was detected, were measured for each sample. Then $\Delta\Delta c_T$ was evaluated according to the following equations: $\Delta c_T = c_T$ (target) – c_T (internal standard), $\Delta\Delta c_T = \Delta c_{T\text{treated}} - \Delta c_{T\text{control}}$, where $\Delta c_{T\text{treated}}$ is Δc_T for treated rats and $\Delta c_{T\text{control}}$ is Δc_T for untreated rats. Δc_T is positive if the target is expressed at a lower level than the internal standard (β-actin), and negative if expressed at a higher level. The induction of mRNA expression of the studied target genes (fold change) in pretreated animals was evaluated as $2^{-(\Delta\Delta c_T)}$.

Preparation of Antibodies

Leghorn chickens were immunized subcutaneously three times (with a week interval) with human recombinant NQO1 (Sigma, St. Louis (MO), U.S.A.) and immunoglobulin fraction isolated from pooled egg yolks as described[46].

Estimation of NQO1 Protein Content in Cytosols

Immunoquantitation of rat liver, kidney and lung cytosolic NQO1 was done as reported elsewhere[46]. Human recombinant NQO1 were used as positive control to identify the band of NQO1 in cytosols. The antigen–antibody complex was visualized with an alkaline phosphatase-conjugated rabbit anti-chicken IgG antibody and 5-bromo-4-chloroindol-3-yl phosphate/NitroBlueTetrazolium dye[46].

NQO1 Enzyme Activity Assays

The cytosolic samples were characterized by the NQO1 activity, using menadione (2-methyl-1,4-naphthoquinone) as a substrate[11–13]. The NQO1 activity was determined by following the oxidation of NADH (or NADPH) spectrophotometrically at 340 nm on a Hewlett–Packard 8453 diode array spectrophotometer for 1 min.

The cytosolic samples were also characterized by the NQO1 activity, using another NQO1 substrate, 3-NBA, measuring formation of 3-NBA-derived DNA adducts[14,46]. DNA was isolated from incubations by the phenol/chloroform extraction as described[14,46].

^{32}P-Postlabeling Analysis and HPLC Analysis of DNA Adducts

^{32}P-Postlabeling analysis using butanol extraction, thin layer chromatography (TLC) and HPLC were performed as described[46,47]. Enrichment by butanol extraction has been shown

to yield more adduct spots and a better recovery of 3-NBA-derived DNA adducts than using enrichment by nuclease P1 digestion[49]. DNA adduct spots were numbered as reported[14]. DNA adduct standard samples of 3-NBA, 3-amino-2-(2′-deoxyadenosin-N^6-yl)benzanthrone 3′-phosphate (dA-N^6-ABA), 3-amino-*N*-(2′-deoxyguanosin-N^2-yl)benzanthrone 3′-phosphate (dG-N^2-ABA) and 3-amino-*N*-(2′-deoxyguanosin-8-yl)benzanthrone 3′-phosphate (dG-C8-*N*-ABA), were prepared by reacting *N*-acetoxy-3-aminobenzanthrone (*N*-Aco-ABA) with deoxyadenosine (dAp) and deoxyguanosine (dGp) 3′-monophosphates and analyzed as described[50].

RESULTS

The effect of ellipticine on induction of liver, kidney and lung NQO1 of male Wistar rats was investigated evaluating levels of expression of its mRNA, protein and enzymatic activities.

The Effect of Ellipticine on NQO1 mRNA and Protein Expression

Modulation of NQO1 mRNA expression was investigated utilizing the RT-PCR analysis. As shown in Table I, treatment of rats with ellipticine induced a 2.8- and 1.6-fold increase in mRNA expression levels of NQO1 in livers and kidneys, respectively, while no increase in NQO1 mRNA expression was found in lungs (Table I).

As NQO1 is a cytosolic enzyme, cytosolic fractions were isolated from livers, kidneys and lungs of rats, either control (non-induced) or pretreated with ellipticine and analyzed for the expression of NQO1 protein and its enzymatic activity. Western blots with chicken polyclonal antibodies raised against NQO1 showed that the expression of liver and kidney NQO1 protein was induced in rats treated with ellipticine, while its expression in lungs was essentially not altered by treating rats with this compound

TABLE I
Expression of NQO1 mRNA in livers, kidneys and lungs of control (untreated) rats and rats treated with 40 mg/kg of body weight of ellipticine

Parameter	$\Delta c_{\mathrm{Tcontrol}}$	$\Delta c_{\mathrm{Ttreated}}$	Fold change
Livers	4.77 ± 0.40	3.28 ± 0.83	2.82*
Kidneys	9.37 ± 0.67	8.68 ± 0.14	1.61*
Lungs	5.44 ± 0.34	5.70 ± 0.59	0.84

Means and standard deviations are shown from data found for three rats. The induction of mRNA expression of studied target genes was calculated as described in Experimental. The asterisks denote statistically significant results (* $P < 0.05$).

(Fig. 1). An 8.1-, 4.2- and 1.2-fold increase in NQO1 expression was found in cytosolic fractions isolated from livers, kidneys and lungs of rats treated with ellipticine, respectively (Fig. 1).

The Effect of Ellipticine on the NQO1 Enzymatic Activities

Using menadione as the substrate, the NQO1 activity was found in cytosolic fractions of all the tissues tested in this study, but it was about ten-times lower in kidneys and lungs than in livers (Table II). The increase in the expression of NQO1 protein by ellipticine resulted in the increase in NQO1 activity in hepatic and renal cytosolic fractions. A more than 4.4- and 2.2-fold increase in NQO1 activity was found in liver and kidney cytosols of rats treated with ellipticine, respectively. However, no increase in NQO1 enzyme activity was detectable in lungs (Table II).

Besides menadione, 3-NBA was used as another substrate of this enzyme. In the case of 3-NBA, we evaluated the NQO1 potential of reductive activation of this carcinogen, measuring the formation of adducts in calf thymus DNA by 3-NBA incubated with rat cytosolic fractions. Cytosolic samples

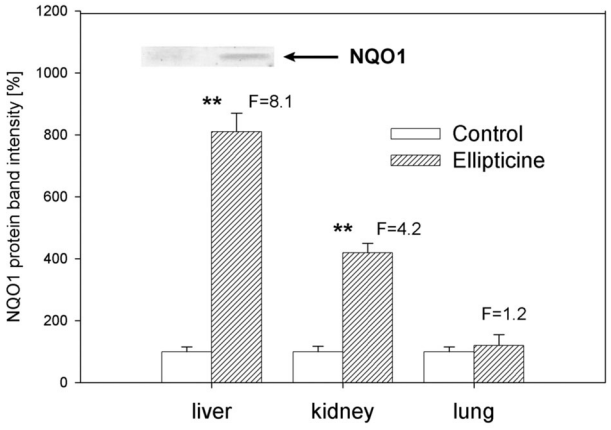

FIG. 1

Induction of NQO1 protein in livers, kidneys and lungs of rats treated with 40 mg/kg of body weight of ellipticine. Mean values ± S.D. shown in the figure are the results obtained from organs of three rats (n = 3). Inset: immunoblots of hepatic cytosolic NQO1 untreated and ellipticine-treated rats, stained with antibody against human NQO1. Cytosolic samples were subjected to SDS-PAGE, proteins were transferred to PVDF membranes and probed with antibody as described in Experimental. ** Values significantly different from control (P < 0.001)

TABLE II
NQO1 specific activity in rat hepatic, renal and pulmonary cytosol

Parameter	NADH			NADPH		
	Control	Ellipticine	Relative induction	Control	Ellipticine	Relative induction
Livers	0.84 ± 0.06	3.51 ± 0.24	4.44**	0.74 ± 0.05	3.45 ± 0.24	4.66**
Kidneys	0.07 ± 0.01	0.16 ± 0.02	2.28*	0.08 ± 0.01	0.17 ± 0.02	2.13*
Lungs	0.05 ± 0.01	0.06 ± 0.01	1.20	0.08 ± 0.01	0.08 ± 0.01	1.00

The results (units mg^{-1}) are averages and standard deviations of five parallel measurements. Enzyme activities with menadione as a substrate and protein content were assayed as described in Experimental. The asterisks denote statistically significant results (* $P < 0.05$, ** $P < 0.01$).

from livers of non-induced[14] and ellipticine-treated rats (Fig. 2) were capable of activating reductively 3-NBA to form DNA adducts. Likewise, cytosolic fractions from rat kidneys and lungs activate 3-NBA to form DNA adducts, but were almost ten-fold less efficient than the cytosolic samples of rat livers (Table III). Hepatic and renal cytosolic samples from rats pretreated with ellipticine were more effective in the formation of 3-NBA-DNA adducts than the cytosolic fractions of untreated (control) rats. Formation of 3-NBA-DNA adducts was 5.1- and 2.2-fold higher in hepatic and renal cytosols of rats treated with ellipticine than in those of control rats, respectively. No increase in the formation of these adducts was generated by pulmonary cytosolic fractions of rats treated with ellipticine (Table III). The DNA adduct pattern generated by 3-NBA consisted of a cluster of up to five adducts (spots 1–5 in Fig. 2), essentially identical to that observed *in vivo* in rats and mice treated with 3-NBA [14,48,50,51], and *in vitro* incubations using human and rat hepatic cytosols[14] or microsomes[52]. Chromatographic analysis of individual spots on HPLC confirmed that the adduct spots 1–5 formed with rat hepatic cytosolic fractions are derived from 3-NBA by reduction of nitro group (data not shown). Three of these adducts were identified previously by us[46,50] as 3-amino-2-(2′-deoxyadenosin-N^6-yl)benzanthrone 3′-phosphate (dA-N^6-ABA; spot 1), 3-amino-*N*-(2′-deoxyguanosin-N^2-yl)benzanthrone 3′-phosphate (dG-N^2-ABA; spot 3) and 3-amino-*N*-

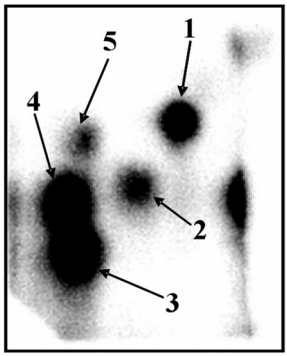

FIG. 2

Autoradiographic profile of DNA adducts generated in calf thymus DNA by 3-nitrobenzanthrone after its activation with cytosol isolated from livers of rats treated with 40 mg/kg body weight of ellipticine by using the butan-1-ol enrichment version of the ^{32}P-postlabeling assay. The exposure time of autoradiographs was 20 min. Spot 1, dA-N^6-ABA; spot 2, unknown; spot 3, dG-N^2-ABA; spots 4/5, dG-C8-*N*-ABA

TABLE III
DNA adduct formation from 3-nitrobenzanthrone activated by rat hepatic, renal and pulmonary cytosol

Parameter		Spot 1	Spot 2	Spot 3	Spot 4	Spot 5[a]	Total
Livers	control	157 ± 6	29 ± 6	140 ± 25	73 ± 32	29 ± 8	428 ± 61
	induced	785 ± 75	148 ± 15	718 ± 68	409 ± 51	118 ± 12	2178 ± 203
Kidneys	control	3.7 ± 0.4	12.8 ± 2.2	26.3 ± 2.7	2.6 ± 0.3	1.3 ± 0.1	46.7 ± 5.2
	induced	7.9 ± 0.9	27.5 ± 2.4	58.9 ± 5.9	6.0 ± 0.5	2.8 ± 0.2	103.1 ± 15.2
Lungs	control	18.3 ± 2.1	3.0 ± 0.4	12.1 ± 1.7	8.8 ± 0.9	1.1 ± 0.1	43.3 ± 5.3
	induced	18.5 ± 1.9	4.1 ± 0.4	15.2 ± 1.6	9.2 ± 1.1	1.2 ± 0.2	48.2 ± 5.5
Control 1		24.3 ± 1.9	15.2 ± 1.6	30.2 ± 3.3	4.5 ± 3.9	1.1 ± 0.1	75.3 ± 7.3
Control 2		3.5 ± 0.3	3.2 ± 0.3	3.7 ± 0.4	0.8 ± 0.1	n.d.	11.2 ± 1.2
Control 3		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

[a] See Fig. 2.

The results (relative adduct labeling, RAL/10^9 nucleotides) are averages and standard deviations of four parallel determinations (duplicate analyses of two independent *in vitro* incubations). Spot 1, dA-N^6-ABA; spot 2, unknown; spot 3, dG-N^2-ABA; spots 4/5, dG-C8-*N*-ABA; n.d., non-detectable (the detection limit of RAL was $1/10^{10}$ nucleotides). Control 1 – hepatic cytosol, rats treated with ellipticine, NADPH omitted; control 2 – hepatic cytosol, untreated rats, NADPH omitted; control 3 – NADPH added, no cytosol.

(2′-deoxyguanosin-8-yl)benzanthrone 3′-phosphate (dG-C8-*N*-ABA; spots 4/5). Thin-layer chromatograms of ^{32}P-labeled DNA from control incubations carried out in parallel without cytosol, without DNA, or without 3-NBA, were devoid of adduct spots in the region of interest. However, low levels of 3-NBA-DNA adducts were detected in incubations containing cytosolic samples, DNA and 3-NBA without NADPH, due to the presence of endogenous NADPH in cytosolic fractions[14] (Table III).

DISCUSSION

The results presented in this paper extend our knowledge of the induction potential of anticancer drug ellipticine for the enzymes metabolizing xenobiotics. Here we show that ellipticine is an effective inducer of NQO1, the important enzyme participating in bioreduction of many drugs and toxicants[10]. The expression of NQO1 protein was significantly induced by ellipticine in livers and kidneys, but not in lungs of rats treated intraperitoneally with the 40 mg/kg body weight dose of ellipticine. The NQO1 protein induction by ellipticine in rat livers and kidneys resulted in an increase in its enzymatic activities measured with two model substrates, menadione and carcinogenic environmental pollutant 3-NBA. As shown previously[39], ellipticine is able to induce two CYP enzymes, namely rat CYP1A1 and 1A2, whose expression is regulated by analogous mechanisms as NQO1 [20,35,36,38]. The induction might result from the ellipticine-mediated AhR activation described for this compound by several authors[35,36,38]. Ellipticine activation allows the cytosolic AhR to translocate into the nucleus and to dimerize with AhR nuclear translocator (ARNT). The AhR–ARNT complex acts as a transcriptional activator by binding to Ah responsive element in the regulatory domains of numerous genes[36]. Genes transcriptionally regulated by AhR–ARNT complexes encode several foreign chemical-metabolizing enzymes including CYP enzymes CYP1A1 and 1A2 [36,38] and NQO1 [10,20]. Therefore, their activation stimulates transcription of CYP1A1/2 (ref.[39]) and NQO1 (this work).

The increase in expressions of NQO1 protein and enzymatic activities induced by ellipticine corresponded to elevated mRNA levels of this enzyme. However, an increase in NQO1 mRNA levels was more than twice lower than that of NQO1 protein. Similar discrepancies between induction of mRNAs and protein levels of several enzymes were observed also by others[53,54]. It has been reported that some inducers might prolong half-lives of mRNAs, while others increase transcription. Moreover, half-lives of mRNAs are usually much shorter than those of proteins[53–56]. Detailed

analyses of the time dependence of the expression levels of mRNA and protein of the tested enzyme were not performed in this study; they might answer the questions whether the transient induction of the NQO1 mRNA, or the different half-lives for mRNA and protein, and/or the effects of ellipticine on the stability of mRNA and this enzyme are the rationale for our observation.

The potential of ellipticine to induce NQO1 expression and activities in rat livers was similar to that of other NQO1 inducers such as Sudan I, Sudan III, β-naphthoflavone and two of NQO1 own substrates, aristolochic acid I (AAI) and 3-NBA [11,12,46]. In the case of AAI and 3-NBA, by inducing hepatic NQO1 both compounds increase their own enzymatic activation to reactive DNA adduct-forming species, thereby enhancing their genotoxic potential[11,12,46]. This is also the case for the ellipticine-mediated induction of CYP1A1 and 1A2 found by us previously[39]. The CYP1A1 enzyme is induced by ellipticine in livers and kidneys more efficiently than NQO1 (more than four-times); it is induced even in rat lung. The efficiency of ellipticine to induce CYP1A2 in the rat liver is analogous to that to increase NQO1 content in this tissue[39]. The induction of these CYPs by ellipticine leads to an increase in its enzymatic metabolism causing both activation of this drug to reactive DNA adduct-forming species (13-hydroxy- and 12-hydroxyellipticine)[57,58] and its elimination from organism (formation of 9-hydroxy- and 7-hydroxyellipticine metabolites)[41]. The formation of ellipticine-derived DNA adducts was found to be one of the mechanisms responsible to its antitumour and/or genotoxic effects[42,57–59]. On the contrary, the induction of NQO1 by ellipticine does not influence such biological effects because ellipticine is not metabolized by this enzyme. However, the induction potential of ellipticine for this enzyme might have a great significance for NQO1-mediated metabolism of other drugs (e.g. mitomycin C, EO9)[5,20] and of several toxic environmental pollutants (mutagenic and carcinogenic nitroaromatics 3-NBA, 1-nitropyrene, 2,6-dinitropyrene)[46,60,61]. Due to the NQO1 induction effect, ellipticine might influence the susceptibility of organisms to these chemicals. Nevertheless, the question whether induction of NQO1 and CYP1A by ellipticine occurs in cancer patients and healthy individuals remains to be answered.

CONCLUSIONS

The discussed results demonstrate for the first time the ability of the anticancer drug ellipticine to induce rat NQO1, the enzyme that plays a crucial role in biotransformation, being responsible for reductive metabolism of

several drugs and toxicants. The potential of this compound to induce NQO1 differs in three rat tissues tested in this study (livers, kidneys and lungs). Although the NQO1 induction has been produced by ellipticine in rat livers and kidneys, essentially no increase in levels of NQO1 mRNA, protein and its enzyme activities was detected in lungs. The mechanism of the different NQO1 induction by ellipticine in various rat tissues and organs of cancer patients as well as the biological significance of the induction remains to be solved. Therefore, a study concerning these aspects is the aim of our future work.

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