Biochemical Pharmacology 77 (2009) 1466–1479

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

The mechanism of cytotoxicity and DNA adduct formation by the anticancer drug ellipticine in human neuroblastoma cells

Jitka Poljaková [a], Tomáš Eckschlager [b], Jan Hraběta [b], Jana Hřebačková [b], Svatopluk Smutný [c], Eva Frei [d], Václav Martínek [a], René Kizek [e], Marie Stiborová [a,*]

[a] Department of Biochemistry, Faculty of Science, Charles University, Albertov 2030, 128 40 Prague 2, Czech Republic
[b] Department of Pediatric Hematology and Oncology, 2nd Medical School, Charles University and University Hospital Motol, V Úvalu 84, 150 06 Prague 5, Czech Republic
[c] 1st Department of Surgery, 2nd Medical School, Charles University and University Hospital Motol, V Úvalu 84, 150 06 Prague 5, Czech Republic
[d] Division of Molecular Toxicology, German Cancer Research Center, In Neuenheimer Feld 280, 69120 Heidelberg, Germany
[e] Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University of Agriculture and Forestry, Zemedelska 1, 613 00 Brno, Czech Republic

ARTICLE INFO

Article history:
Received 6 November 2008
Accepted 26 January 2009

Dedicated to Prof. Dr. Danuše Sofrová.

Keywords:
Ellipticine
Anticancer drug
Neuroblastoma
Cytotoxicity
DNA adduct

ABSTRACT

Ellipticine is an antineoplastic agent, whose mode of action is based mainly on DNA intercalation, inhibition of topoisomerase II and formation of covalent DNA adducts mediated by cytochromes P450 and peroxidases. Here, the molecular mechanism of DNA-mediated ellipticine action in human neuroblastoma IMR-32, UKF-NB-3 and UKF-NB-4 cancer cell lines was investigated. Treatment of neuroblastoma cells with ellipticine resulted in apoptosis induction, which was verified by the appearance of DNA fragmentation, and in inhibition of cell growth. These effects were associated with formation of two covalent ellipticine-derived DNA adducts, identical to those formed by the cytochrome P450- and peroxidase-mediated ellipticine metabolites, 13-hydroxy- and 12-hydroxyellipticine. The expression of these enzymes at mRNA and protein levels and their ability to generate ellipticine-DNA adducts in neuroblastoma cells were proven, using the real-time polymerase chain reaction, Western blotting analyses and by analyzing ellipticine-DNA adducts in incubations of this drug with neuroblastoma S9 fractions, enzyme cofactors and DNA. The levels of DNA adducts correlated with toxicity of ellipticine to IMR-32 and UKF-NB-4 cells, but not with that to UKF-NB-3 cells. In addition, hypoxic cell culture conditions resulted in a decrease in ellipticine toxicity to IMR-32 and UKF-NB-4 cells and this correlated with lower levels of DNA adducts. Both these cell lines accumulated in S phase, suggesting that ellipticine-DNA adducts interfere with DNA replication. The results demonstrate that among the multiple modes of ellipticine antitumor action, formation of covalent DNA adducts by ellipticine is the predominant mechanism of cytotoxicity to IMR-32 and UKF-NB-4 neuroblastoma cells.

1. Introduction

Neuroblastoma, a tumor of the peripheral sympathetic nervous system, is the most frequent solid extra cranial tumor in children and is a major cause of death from neoplasia in infancy [1]. These tumors are biologically heterogeneous, with cell populations differing in their genetic programs, maturation stage and malignant potential [2]. Neuroblastoma may regress spontaneously in infants, mature to benign ganglioneuromas in older children, or grow relentlessly and be rapidly fatal [2]. Prognosis of high risk tumors is poor, because drug resistance arises in the majority of those patients, initially responding to chemotherapy, in spite of intensive therapy including megatherapy with subsequent hematopoietic progenitor cell transplantation, biotherapy and immunotherapy [2]. Little improvement in therapeutic options has been made in the last decade, requiring a need for the development of new therapies.

Ellipticine (5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole, Fig. 1), an alkaloid isolated from *Apocyanacea* plants, and several of its more soluble derivatives (9-hydroxyellipticine, 9-hydroxy-N^2-methyl-ellipticinium, 9-chloro-N^2-methyl-ellipticinium and 9-methoxy-N^2-methyl-ellipticinium) exhibit significant antitumor activities (for a summary see [3]). The main reasons for the interest

* Corresponding author. Tel.: +420 221951285; fax: +420 221951283.
E-mail address: stiborov@natur.cuni.cz (M. Stiborová).

Abbreviations: α-NF, α-naphthoflavone; COX, cyclooxygenase; CYP, cytochrome P450; Δc_T, difference of target minus reference cycle threshold; DMSO, dimethyl sulfoxide; IMDM, Iscove's modified Dulbecco's medium; HPLC, high-performance liquid chromatography; LPO, lactoperoxidase; MDR, multidrug resistance; MPO, myeloperoxidase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazoliumbromide; PBS, phosphate buffered saline; PEI-cellulose, polyethylenimine-cellulose; PVDF, polyvinylidene difluoride; RAL, relative adduct labeling; RT, real-time; PCR, polymerase chain reaction; r.t., retention time; SDS, sodium dodecyl sulphate; TLC, thin layer chromatography.

0006-2952/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2009.01.021

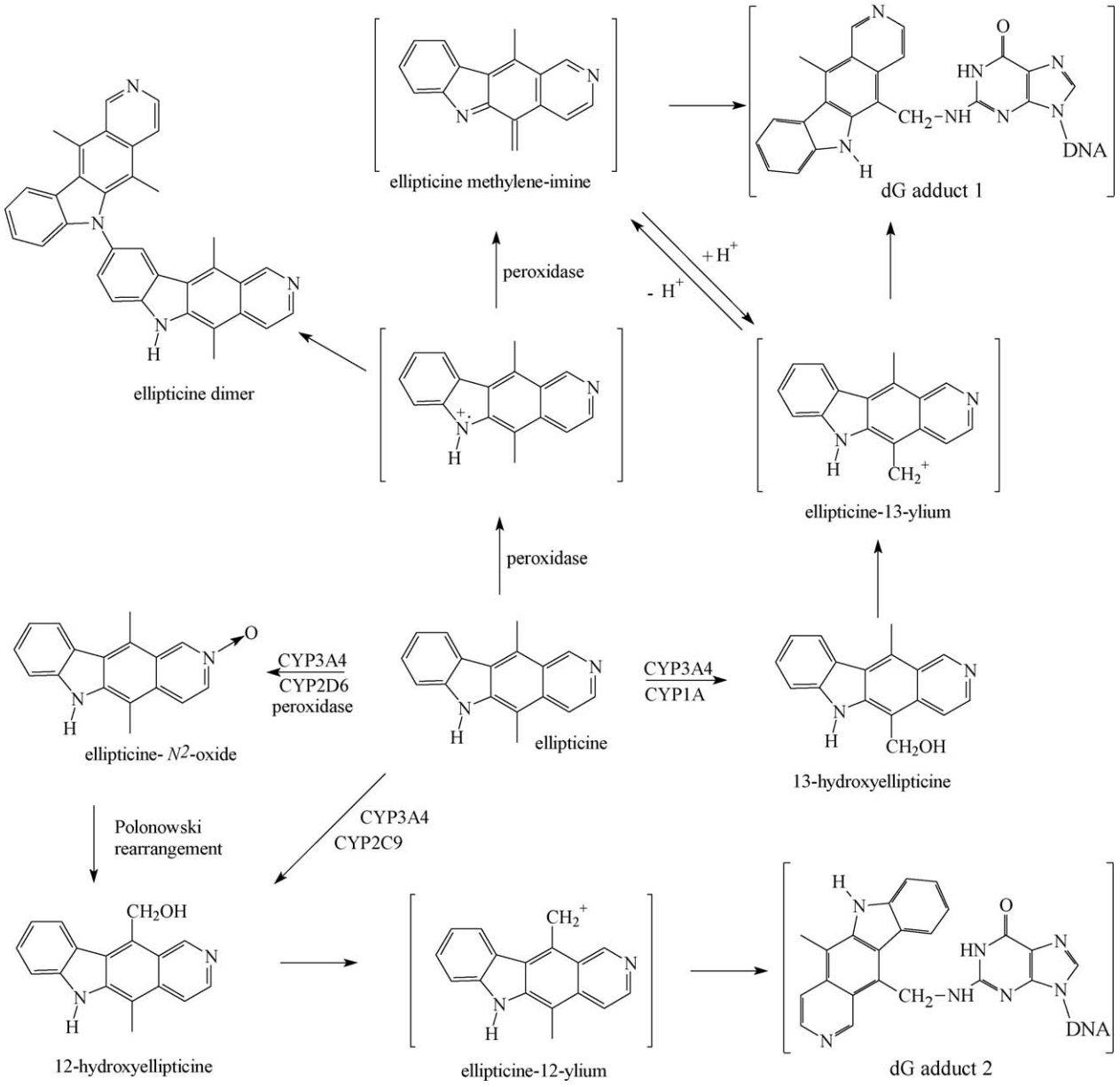

Fig. 1. Metabolism of ellipticine by peroxidases and human CYPs showing the characterized metabolites and those proposed to form DNA adducts. The compounds shown in brackets were not detected under the experimental conditions and are the electrophilic metabolites postulated as ultimate arylating species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

in ellipticine and its derivatives for clinical purposes are their high efficiencies against several types of cancer, their rather limited toxic side effects, and their complete lack of hematological toxicity [4]. Nevertheless, ellipticine is a potent mutagen.

Ellipticine has been reported to arrest cell cycle progression by regulating the expression of cyclinB1 and Cdc2 as well as phosphorylation of Cdc2 [5,6], to induce apoptotic cell death by the generation of cytotoxic free radicals, the activation of Fas/Fas ligand system, the regulation of Bcl-2 family proteins [5–8], an increase of wild-type p53, the rescue of mutant p53 activity and the initiation of the mitochondrial apoptosis pathway [5,6,8,9]. Ellipticine also uncouples mitochondrial oxidative phosphorylation [10] and thereby disrupts the energy balance of cells. Ellipticine and 9-hydroxyellipticine also cause selective inhibition of p53 protein phosphorylation in several human cancer cell lines [11,12], and this correlates with their cytotoxic activity. However, the precise molecular mechanism responsible for these effects has not yet been explained. Chemotherapy-induced cell cycle arrest was shown to result from DNA damages caused by a variety of chemotherapeutics. In the case of ellipticine, it was suggested that the prevalent DNA-mediated mechanisms of their antitumor, mutagenic and cytotoxic activities are (i) intercalation into DNA [4,13] and (ii) inhibition of DNA topoisomerase II activity [4,14–16].

We have demonstrated that ellipticine also covalently binds to DNA *in vitro* and *in vivo* after being enzymatically activated with cytochromes P450 (CYP) or peroxidases [3,17–23], suggesting a third possible mechanism of action. Human and rat CYP1A, 1B1 and 3A are the predominant enzymes catalyzing oxidation of ellipticine *in vitro* either to metabolites that are excreted (7-hydroxy- and 9-hydroxyellipticine) or that form DNA adducts (12-hydroxy- and 13-hydroxyellipticine) [3,17–20]. Of the peroxidases, human cyclooxygenase (COX)-2, ovine COX-1, bovine