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Investigation of sanguinarine and chelerythrine effects on CYP1A1 expression and activity in human hepatoma cells

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Abstract

Quaternary benzo[*c*]phenanthridine alkaloids (QBA) sanguinarine and chelerythrine exhibit a wide spectrum of biological activities whence they are used in dental care products. Recent studies indicated that cytochrome P450 CYP1A attenuates sanguinarine toxicity both in vivo [Williams, M.K., Dalvi, S., Dalvi, R.R., 2000. Influence of 3-methylcholanthrene pretreatment on sanguinarine toxicity in mice. Vet. Hum. Toxicol. 42, 196–198] and in vitro [Vrba, J., Kosina, P., Ulrichová, J., Modrianský, M., 2004. Involvement of cytochrome P450 1A in sanguinarine detoxication. Toxicol. Lett. 151, 375–387]. However, CYP1A converts sanguinarine to the products that form DNA adducts [Stiborová, M., Šimánek, V., Frei, E., Hobza, P., Ulrichová, J., 2002. DNA adduct formation from quaternary benzo[*c*]phenanthridine alkaloids sanguinarine and chelerythrine as revealed by the 32P-postlabeling technique. Chem. Biol. Interact. 140, 231–242]. In our work we examined the effects of sanguinarine and chelerythrine on CYP1A1 expression and catalytic activity in human hepatoma cells—HepG2. Sanguinarine and chelerythrine did not affect basal and dioxin-inducible expression of CYP1A1 mRNA and protein in HepG2 cells. The enzymatic activity of CYP1A1 was assessed by the fluorescent measurement of 7-ethyxoresorufin-*O*-deethylase (EROD) activity. We observed a slight decrease of dioxin-induced EROD activity in HepG2 cells by sanguinarine and chelerythrine. This decrease was attributed to the inhibition of CYP1A1 catalytic activity, as revealed by enzyme kinetic studies on recombinant CYP1A1 protein. The IC50 values for the inhibition of CYP1A1 by sanguinarine and chelerythrine were 2.1 and 1.9 μM, respectively. In conclusion, albeit the CYP1A modulates QBA cytotoxicity and genotoxicity, the QBA themselves do not affect CYP1A1 expression. The data indicate that studied alkaloids do not have specific cellular target and their biological effects are rather pleiotropic.

Keywords: Aryl hydrocarbon receptor; Cytochrome CYP1A1; Sanguinarine; Chelerythrine; HepG2 cells

1. Introduction

Sanguinarine (SA) and chelerythrine (CHE), quaternary benzo[*c*]phenanthridine alkaloids (QBA), are common in the *Papaveraceae*, *Fumariaceae*, and *Rutaceae* families of plants. The main sources of SA and CHE are the plant species *Chelidonium majus*, *Macleaya cordata*, and *Sanguinaria canadensis* (Šimánek, 1985). These alkaloids exert wide spectrum of biological activities, e.g. antimicrobial, anti-inflammatory, adrenolytic, sympatholytic and local anesthetic, including cytotoxicity against various human normal cells and tumor cells

Abbreviations: AhR, aryl hydrocarbon receptor; BNF, beta-naphtoflavon; DMSO, dimethylsulfoxide; EROD, 7-ethoxyresorufine-*O*-deethylase; FCS, foetal calf serum; CHE, chelerythrine; HepG2, human hepatoma cells; 3-MC, 3-methylcholanthrene; NF-κB, nuclear factor kappa beta; PAHs, polyaromatic hydrocarbons; PKC, protein kinase C; QBA, quaternary benzo[*c*]phenanthridine alkaloid; SA, sanguinarine; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

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lines. Owing to their antibacterial, antifungal, and anti-inflammatory activities, QBAs are used in dental care products (Walterová et al., 1995). However, epidemiological studies have shown an association between sanguinarine use and oral premalignant lesions (Damm et al., 1999; Eversole et al., 2000). Sanguinarine was also suggested as an anti-neoplastic and effective against multiple drug resistance of cervical cells (Ding et al., 2002). These activities are tightly bound to the equilibrium between alkanolamine form and iminium ion form of the bases (Colombo and Bosisio, 1996). The conversion of the iminium ion to alkanolamine improves the lipophilicity of SA and CHE and the higher alkanolamine lipophilicity may result in an increase of the alkaloid bioavailability (Slaninova et al., 2001). The alkanolamine forms a complexes with proteins including cytochrome P450 (Peeples and Dalvi, 1982).

Molecular biological studies indicate that sanguinarine has multiple cellular targets (Walterová et al., 1995). Due to its quaternary nitrogen, polycyclic and planar structure it can react with nucleophilic and anionic moieties of amino acids in biomacromolecules (Schmeler et al., 1997). Furthermore, the formation of molecular complex of sanguinarine with DNA by intercalation was described (Maiti et al., 1982). Sanguinarine and chelerythrine are also potent inhibitors of several protein kinases, e.g. protein kinase C, Ca^{2+}-dependent protein kinase C, cyclic AMP-dependent protein kinase, and phospholipid-dependent protein kinase C (Wang et al., 1997). Interestingly, the investigation on the effect of benzo[*c*]phenanthridine alkaloids on nuclear factor kappa beta (NF-κB) activation shown, that sanguinarine but not chelerythrine is a potent inhibitor of NF-κB activation. In the process of NF-κB activation by cytokines, phorbol esters and lipopolysaccharide, sanguinarine blocked the phosphorylation and degradation of IκBα (Chaturvedi et al., 1997). These data revealed a novel mechanism by which sanguinarine exhibits its anti-inflammatory effects. This finding eventually makes sanguinarine a potential candidate for intervening in NF-κB dependent pathological responses.

Cytochrome P450 (CYP), a superfamily of microsomal hemoproteins, play an important role in detoxification, and in the biosynthesis and degradation of endogenous substrates such as steroids, fatty acids or prostaglandins (Gonzalez, 1988). Unfortunately, cytochrome P450 takes a part in the process of chemically induced carcinogenesis, when activating procarcinogens in the ultimate carcinogens (Cooper et al., 1982). An important representative of cytochrome P450 involved in carcinogenesis is CYP1A family. These enzymes are induced by dioxin (TCDD), beta-naphtoflavon (BNF) and polyaromatic hydrocarbons (PAHs), the latter are in turn subjected to the oxidation by CYP1A resulting in the formation of reactive epoxides with carcinogenic potential (Denison and Heath-Pagliuso, 1988). To date, there is only limited information on the interaction between QBAs and CYP1A expression and activity. We can only speculate that the adverse effects of the alkaloids might be caused by their metabolic activation with the involvement of cytochrome P450, taking in account the analogy with known carcinogens, such as PAHs (Peeples and Dalvi, 1982). It was demonstrated that 3 days pretreatment of mice with CYP1A inducer 3-methylcholanthrene (3-MC), attenuates in vivo toxicity of sanguinarine (Williams et al., 2000). Recent in vitro study also claims that CYP1A inducers TCDD and BNF decrease sanguinarine toxicity in primary cultures of rat hepatocytes and human hepatoma cells HepG2 (Vrba et al., 2004). In addition, it was shown that incubation of sanguinarine and chelerythrine with rat hepatic microsomes leads to the production of reactive species able to form DNA adducts (Stiborová et al., 2002). Taken together, it seems that CYP1A determines sanguinarine biological effects, when CYP1A converts sanguinarine to the product with lowered acute toxicity but increased genotoxic potential.

The goal of this study was to examine the effects of sanguinarine and chelerythrine on CYP1A1 in human hepatoma cells—HepG2. The effects of tested compounds were assessed as: (i) CYP1A1 mRNA expression in HepG2 cells; (ii) CYP1A1 protein level in HepG2 cells; (iii) CYP1A1 catalytical activity in HepG2 cells (7-ethoxyresorufine-*O*-deethylase; EROD) and on human recombinant CYP1A1. Submicromolar concentrations of the alkaloids were used in our experiments, taking in account the applicability of these compounds.

2. Materials and methods

2.1. Chemicals

Dulbecco's modified Eagle's medium, minimal essential medium, foetal calf serum, streptomycin, penicillin, L-glutamine, nonessential amino acids, sodium pyruvate, recombinant CYP1A1, NADPH, dicumarol, 7-ethoxyresorufin, and Kodak X-Omat AR photographic film were purchased from Sigma Chemicals (St. Louis, MO). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin from Ultra Scientific (RI, USA). Sanguinarine and chelerythrine were isolated from sanguiritrine (CAMAS Technologies, Inc., Broomfield, USA) using column chromatography on alumina (Dostál et al., 1992). Sanguinarine (SA) in 98.1% purity, M.P. 279–282 °C (Southon and Buckingham, 1989) and chelerythrine (CHE) in 95% purity, M.P. 200–204 °C (Southon and Buckingham, 1989) were obtained. Secondary horseradish peroxidase conjugated antibody, and Western Blotting Luminol Reagent were purchased from Santa Cruz Biotechnology (California, USA). All other chemicals were of the highest grade commercially available.