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Original research article

Imidazoacridinone antitumor agent C-1311 as a selective mechanism-based inactivator of human cytochrome P450 1A2 and 3A4 isoenzymes

Agnieszka Potęga *, Barbara Fedejko-Kap, Zofia Mazerska

Department of Pharmaceutical Technology and Biochemistry, Chemical Faculty, Gdańsk University of Technology, Gdańsk, Poland

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ABSTRACT

Background: 5-Diethylaminoethylamino-8-hydroxyimidazoacridinone (C-1311), a promising antitumor agent that is also active against autoimmune diseases, was determined to be a selective inhibitor of the cytochrome P450 (CYP) 1A2 and 3A4 isoenzymes. Therefore, C-1311 might modulate the effectiveness of other drugs used in multidrug therapy. The present work aimed to identify the mechanism of the observed C-1311-mediated inactivation of CYP1A2 and CYP3A4.

Methods: The inactivation experiments were performed *in vitro* using the human recombinant CYP1A2 and CYP3A4 (Bactosomes). CYP isoenzyme activities were determined using the CYP-specific reactions, 7-ethoxycoumarin O-deethylation (CYP1A2) and testosterone 6β-hydroxylation (CYP3A4). The concentrations of CYP-specific substrates and their metabolites formed by CYP isoenzymes were measured by RP-HPLC with UV-Vis detection.

Results: The inhibition of CYPs by C-1311 was time-, concentration- and NADPH-dependent, which suggested a mechanism-based mode of action. Using a 10-fold dilution method and potassium ferricyanide we demonstrated the irreversible nature of the inhibition. In addition, the inhibition was attenuated by the presence of alternate substrates (alternative active site ligands) but not by a nucleophilic trapping agent (glutathione) or a reactive oxygen scavenger (catalase), which further supported a mechanism-based action. Substrate depletion partition ratios of 299 and 985 were calculated for the inactivation of CYP1A2 and CYP3A4, respectively.

Conclusions: Our results indicated that C-1311 is a potent mechanism-based inactivator of CYP1A2 and CYP3A4. This finding provided new insights into the mechanism of C-1311 antitumor action, particularly in relation to potential pharmacokinetic drug–drug interactions between C-1311 and/or its derivatives and the substrates of CYP isoforms.

Introduction

Imidazoacridinones are a novel class of antitumor agents developed in our department [1,2]. 5-Diethylaminoethylamino-8-hydroxyimidazoacridinone (C-1311) (Fig. 1) is the most active compound in this group. As an inhibitor of both topoisomerases and certain receptor kinases, including FMS-like tyrosine kinase FLT3 [3,4], C-1311 has shown activity against experimental models of murine and colorectal cancer *in vitro* and in animals [5]. In phase I and II of clinical trials, C-1311 exhibited activity against advanced solid tumors, and it was effective in women with metastatic breast cancer [6,7]. It was also tested for the treatment of autoimmune diseases. Moreover, C-1311 in combination with paclitaxel was shown to be efficacious against human bladder cancer in the *in vivo* hollow fiber assay [8]. Unlike other antitumor agents, C-1311 expresses only limited mutagenic potential and has a low potency to generate oxygen free radicals, which suggests that it has reduced cardiotoxic properties [9]. Cellular uptake of this agent occurs rapidly, and most of the drug accumulates in the nucleus, which is believed to enable its myeloperoxidase-mediated metabolism and fast interaction with DNA [10].

Multiple studies on the molecular mode of C-1311 antitumor action revealed that the metabolic activation of this drug by intracellular enzymes might be a prerequisite step in the biochemical mechanism of its action [10]. Activation under enzymatic oxidative conditions resulted in the intercalation and the following covalent binding of the drug to DNA and other cellular macromolecules [11]. Thus, studies on the molecular mechanism of the enzymatic oxidative activation of C-1311 with

* Corresponding author.
E-mail addresses: agnieszka.potega@pg.gda.pl (A. Potęga), basia_look@wp.pl (B. Fedejko-Kap), zofia.mazerska@pg.gda.pl (Z. Mazerska).

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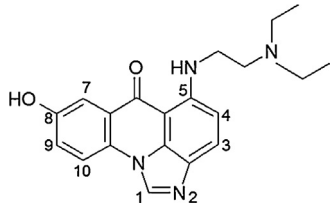

Fig. 1. Chemical structure of antitumor imidazoacridinone C-1311.

different liver drug-metabolizing enzymes were investigated. We showed that under *in vitro* conditions C-1311 underwent metabolic transformations in the presence of rat and human liver microsomes [12]. Thus, C-1311 was a good substrate for microsomal and the selected human recombinant flavin-containing monooxygenases [13] and UDP-glucuronosyltransferases [14]. In contrast, no products of C-1311 were observed with any tested human recombinant cytochrome P450 (CYP) isoenzymes, which should be emphasized. However, it was demonstrated that CYP1A2 and CYP3A4 were significantly inhibited by C-1311, but no inhibition was observed in the case of CYP2C19 and CYP2D6 [13]. Nevertheless, these studies were rather limited in scope, and no attempt was made to elucidate the type and the mechanism of the observed inhibition.

Many drugs that are substrates of CYP1A2 and CYP3A4 can be the modulators of their catalytic activity and consequently might have a strong potential to influence the metabolic transformation of other therapeutics. Thus, undesirable drug–drug interactions may result in problems of clinical significance during multidrug therapy. A number of substances with structural similarities to C-1311 (*i.e.*, compounds containing an imidazole and dialkylaminoalkylamino functional groups) were shown to be irreversible inhibitors of CYPs [15–17]. It was believed that they formed a reactive intermediate (RI) that could irreversibly modify critical active site moieties and thus inactivate the enzyme in a process characterized as mechanism-based inactivation (MBI) [18]. Thus, it was reasonable to expect that such interactions between C-1311 and CYPs may also occur.

Considering the above, the aim of the present study was to evaluate the interactions between C-1311 and human CYPs. We intended to identify the mechanism(s) of C-1311-mediated CYP1A2 and CYP3A4 enzyme inhibition, and we particularly intended to know whether C-1311-CYP interaction is MBI. To verify this hypothesis, we used a two-step incubation scheme to measure the effects of C-1311 on the following CYP-specific reactions: 7-ethoxycoumarin O-deethylation (CYP1A2) and testosterone 6β-hydroxylation (CYP3A4) [19,20]. The elucidation of the mechanism of CYPs' inhibition by C-1311 may help to evaluate the role of C-1311-CYP interactions in the antitumor action of this drug, particularly considering that the metabolism of C-1311 by CYPs was not observed.

Materials and methods

Chemicals and enzymes

An imidazoacridinone derivative, C-1311, was synthesized at the Gdańsk University of Technology [1]. Catalase (CAT; from bovine liver), 7-ethoxycoumarin (7EC), L-glutathione reduced (GSH), potassium ferricyanide ($K_3[Fe(CN)_6]$), and testosterone (TT) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Methanol (gradient grade for liquid chromatography) and NADPH were obtained from Merck KGaA (Darmstadt, Germany). Ammonium formate was from Fisher Scientific (Loughborough, UK). All other chemicals and solvents were of the highest purity available. The human recombinant CYP1A2 and CYP3A4 isoenzymes, co-expressed with human NADPH-cytochrome P450 oxidoreductase in *Escherichia coli* cells (Bactosomes), were purchased from Tebubio (Le Perray-En-Yvelines, France).

Enzyme inactivation assays

The activities of CYPs were studied by measuring the rate of the CYP-specific reactions, 7-ethoxycoumarin O-deethylation (CYP1A2) and testosterone 6β-hydroxylation (CYP3A4).

The inactivation experiments involved two steps: a primary (preincubation) reaction with the inhibitor and a secondary (incubation) reaction with the substrate (Fig. 2). The preincubation mixtures consisted of CYP (1 μM) and increasing concentrations of C-1311 (0.05, 0.1, 0.2, 0.5, and 1 mM) in 0.1 M potassium phosphate buffer, pH 7.4 (assay buffer). These primary reaction mixtures were preincubated at 37 °C for 3 min and were initiated by adding NADPH (1 mM). Negative control incubations lacked NADPH. After incubation at 37 °C for the times indicated, the secondary reactions were started by transferring 10 μl of the primary reaction mixtures to 90 μl of assay buffer containing 7-ethoxycoumarin or testosterone (0.02 mM) and NADPH (0.5 mM). Incubations were conducted at 37 °C for 30 min, and the reactions were terminated by adding ice-cold methanol (1:1, v/v). The incubation mixtures were placed in ice for 10 min and centrifuged for 5 min at 10,000 × *g*.

Concentrations of CYP-specific substrates and their metabolites, formed in Bactosomes, were assessed by the HPLC method with UV-Vis detection, as described previously [13].

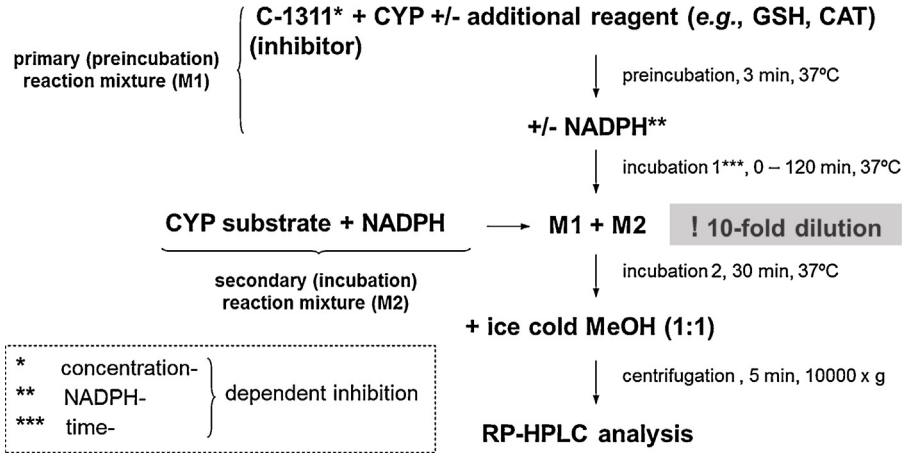

Fig. 2. Scheme of inactivation experiments.