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The mechanism of the rat liver cytochrome P_{450}2E1 inhibition by the synthetic prostanoids of A-type

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ABSTRACT

Aim: The elucidation of mechanism of A-type synthetic prostanoids inhibitory action on microsomal cytochrome P_{450}2E1 (CYP2E1) from rat liver activity was carried out.
Results: Analogs U-34 and U-26 in a final concentration of 1×10^{-5} M inhibited CYP2E1 activity by 93% and 46%, respectively; however natural prostaglandins had no effect. These synthetic prostanoids of A-type (5×10^{-8} to 10^{-4} M) inhibited chlorzoxazone hydroxylation in a dose-dependent manner while $IC_{50} = 7.1 \times 10^{-7}$ M and 8.0×10^{-7} M for U-26 and U-34, respectively. The curves of CYP2E1 activity in the presence of different concentrations of chlorzoxazone and varying concentration of prostanoids were hyperbolic. Double-reciprocal plots of these results $1/V = f(1/S)$ indicated that prostanoids inhibit CYP2E1 through a competitive mechanism with particular effect.
Conclusion: CYP2E1 is a target for A-type prostanoids, possessing 2-oxo-4-amino-oct-3(E)-enyl in α- or ω-chain, which are able to inhibit its action through a competitive mechanism.
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1. Introduction

Prostaglandins (PGs) are biologically active molecules arising from arachidonic acid via the cyclooxygenase pathway and regulating different cellular function under physiological and pathological conditions [1]. In accordance to their chemical structure and molecular mechanisms of action prostaglandins are divided into two groups: cyclopentanone (E, F, and D) and cyclopentenone (A, B, and C) [1]. Prostaglandins of the A-series contain an α,β-unsaturated carbonyl group at the five-member ring and are derived from E-type prostaglandins by dehydration in plasma or aqueous solution [2]. Biological action of PGAs includes the modulation of stress reaction, cell cycle inhibition, virus replication suppression, regulation of cellular differentiation, induction of thermotolerance, cell immunity, and neuroprotective action [3–7].

It is established that some of PGAs effects (e.g. hypotension) are mediated by low affinity binding to the known G-protein-coupled prostanoid receptors of EP-, DP- and FP-type [5]. However, the involvement of "classic" PGs receptors in anti-inflammatory, antitumor, and antiviral effects of PGA was not determined [5,8]. The majority of PGAs effects are provided with plasma membrane receptor-independent regulation of stress-inducible gene expression (e.g. genes encoding with heat shock proteins, γ-glutamyl-cysteine synthetase, collagen, and heme oxygenase) [5,6]. Several biological effects of PGA may be realized at least in part through reaction with cysteine residues of many cellular proteins [5,8]. Studies have also revealed the existence of specific receptor for PGA on rat intestine plasma membranes [9].

Nevertheless, the list of protective effects, possible mechanisms and cellular targets for their realization for PGAs and their synthetic derivatives is not complete and is under investigation nowadays.

Earlier we have analyzed the cytoprotective effects of novel collection of synthetic PGA_2 analogs against CCl_4 on the cellular model of a liver damage [10]. It was determined that two prostanoids, possessing 2-oxo-4-amino-oct-3(E)-enyl in α-chain, as well as natural PGA_2 and PGI_2 are able to decrease a toxic action of this agent. The extent of prostanoids protective activity correlated with a decrease of triene conjugate formation in cellular membranes and a stabilization of quantity of intracellular SH-groups in isolated hepatocytes after CCl_4 treatment [10]. It is known that CCl_4 metabolism to its free radical species by microsomal CYP2E1 is the main reason for CCl_4-induced liver injury [11,17,18]. So, it was suggested that cytoprotective action of prostanoids given was realized by their inhibitory effect on this cytochrome [19]. Despite to the fact that natural PGAs are not very good inhibitors for xenobiotic oxidation catalysed by recombinant P_{450} enzymes as well as their derivatives [19], the protective properties of the novel prostanoids were closely connected with their ability to suppress CYP2E1 activity in rat liver microsomes in presence and absence of CCl_4 [10]. However the biochemical mechanism of this phenomenon was not studied.

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The present work was undertaken to study the mechanism of inhibitory action of prostanoids with modifications in α- and ω-chains on CYP2E1 activity in rat liver microsomes.

2. Materials and methods

2.1. Materials

PGE_1, PGE_2 and PGA_2, HEPES, NADPH, chlorzoxazone, 6-hydroxychlorzoxazone and arachidonic acid were obtained from Sigma Chemical; BSA and EDTA were obtained from DiaM. Ordinary salts (analytical grade) were obtained from various sources. All reagents were prepared with bidistilled water.

Six synthetic prostanoids of A-group were synthesized in the Laboratory of Prostaglandins Chemistry (National Academy of Sciences, Minsk, Belarus). The structure of the prostanoids was confirmed by the methods of nuclear magnetic resonance, mass-spectrometric identification and infrared spectroscopy [12,13]. Prostanoids' structural formulas and systematic names are shown in Table 1. Stock solutions of prostanoids were prepared using 96% ethanol. The necessary concentrations of analogs were obtained from their initial solutions by diluting with 25 mM Hepes–NaOH (pH 7.4).

2.2. Methods

2.2.1. Isolation of rat liver microsomal fraction

Isolation of microsomal fraction was performed as described by Niwa et al. [14]. Briefly, rat livers (10 g) were homogenized using a motor-driven Teflon glass homogenizator for 1.5 min in a buffer containing 25 mM Tris–HCl (pH 7.5), 1 mM EDTA and 0.25 M sucrose. The homogenate was centrifuged at 18,000 × *g* for 20 min. The supernatant was then centrifuged at 100,000 × *g* for 60 min. The resulting pellet was resuspended in 0.1 M sodium phosphate buffer (pH 7.5) containing 20% glycerol and stored at −86 °C until used.

Protein concentration was determined by the method of Peterson [15] using BSA as a standard.

2.2.2. Determination of CYP2E1 activity

Determination of CYP2E1 activity was performed by the method of Lucas et al [16]. Briefly, samples (0.1 mL) containing 200 μM of chlorzoxazone (specific cytochrome P4502E1 substrate), 100 μg of microsomal protein, appropriate concentration of prostanoids solution, 500 μM of NADPH and 0.5% of CCl_4 and 50 mM of Hepes NaOH (pH 7.4) were incubated for 30 min at 37 °C. Proteins were then precipitated with 0.4 mL of 0.6 M perchloric acid. After centrifugation for 10 min at 3500 × *g*, chlorzoxazone and 6-hydroxychlorzoxazone were extracted from the supernatant with two 0.4 mL volumes of ethyl acetate by shaking for 10 min. Following centrifugation for 10 min at 4 °C, the organic phases were evaporated to dryness under a stream of nitrogen. The residues were dissolved in 250 μL of acetonitrile–0.5% acetic acid (mobile phase) and 20 μL samples were used for the HPLC determination of chlorzoxazone and 6-hydroxychlorzoxazone on liquid chromatography mass-spectrometer (Shimadzu LCMS-QP 8000x; Japan).

The HPLC column C18 (150 mm × 4.6 mm) was used, and the flow-rate was 0.75 mL/min. Chlorzoxazone and 6-hydroxychlorzoxazone were detected at 287 nm. Peak area measurements were calculated for quantification and compared with standard solutions (0.5–20 μg/mL) of chlorzoxazone and 6-hydroxychlorzoxazone.

2.2.3. Statistical analysis

Statistical analysis was performed by Stadia 6.0 Software. The results were presented as means ± S.E.M. The significance of differences between groups was established by the Student's *t*-test. The value of $P < 0.05$ was considered significant.

3. Results and discussion

At the first stage of current investigation we have compared effect of natural prostaglandins E_1, E_2, A_2, I_2 and arachidonic acid with the action of synthetic prostanoids of A-type (U-26 and U-34) on CYP2E1 activity in rat liver microsomes (Fig. 1). Each of compounds was used in a final concentration of 10 μM. It was found that arachidonic acid inhibited 6-hydroxylation of chlorzoxazone by 47%. The effects of natural prostaglandins were unreliable that comes to a good agreement with the data of other authors [19]. However, PGAs analogs U-26 and U-34 were observed to be the strong inhibitors of cytochrome activity. Then, in presence of analog U-34 a suppression of enzyme activity reached 93%; prostanoid U-26 decreased 6-hydroxychlorzoxazone formation by 46% (Fig. 1).

To establish the quantitative characteristic of inhibition the IC_{50} value (inhibition constant) for analogs tested was determined. So, we investigated the dependence of cytochrome activity from prostanoid's concentration (10^{-10} to 10^{-4} M) (Fig. 2). It was found that prostanoids suppressed 6-hydroxychlorzoxazone formation in a concentration-dependent manner. The maximal effective concentration for prostanoids tested was 1×10^{-4} M. The value of half maximal concentration (IC_{50}) was 7.1×10^{-7} M for U-26 and 8.3×10^{-7} M for U-34 (Table 2).

It was recognized that CYP2E1 action could be regulated by direct interaction with different compounds [19,20]. There are at least four important mechanisms underlying P_{450} direct inhibition, namely competitive inhibition, reversible inhibition by nitrogen heterocycles, inhibition through formation of metabolite intermediate complexes and mechanism-based inhibition [19,20]. To clarify the mechanism of the prostanoids tested action, we have performed kinetic analysis. It was shown that curves of cytochrome activity in the presence of different concentration of chlorzoxazone and varying concentrations of U-34 were hyperbolic (Fig. 3). Double-reciprocal plots of these results ($1/V = f(1/S)$) have demonstrated that the prostanoids changed Michael's constant of the reaction but did not influence on V_{max} value (Fig. 4). However the inhibition was not complete even when extremely high doses of these substances

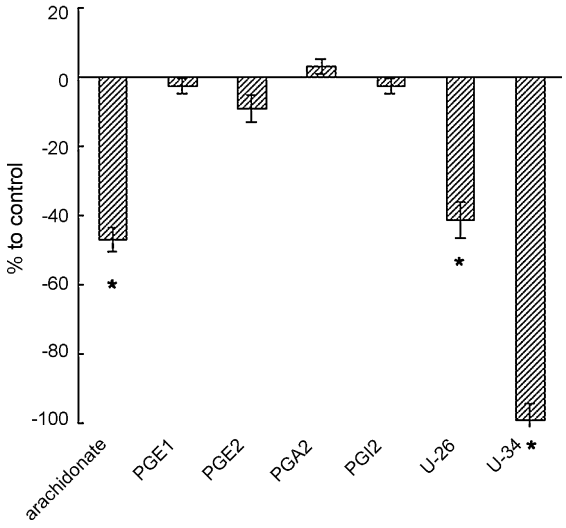

Fig. 1. The effect of arachidonic acid, natural prostaglandins and synthetic prostanoids of A-type on microsomal cytochrome P_{450}2E1 activity. CYP2E1 basal activity was measured in the medium containing 25 mM Hepes–NaOH (pH 7.4) with 0.96% ethanol. The final concentration of all substances tested was 10 μM, ethanol—0.96%. $^*P \leq 0.05$, compared to basal cytochrome activity ($n = 3$).

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