INTERACTION OF AROMATIC CYTOKININS WITH HUMAN LIVER MICROSOMAL CYTOCHROMES P450

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Aromatic cytokinins (*ortho*-topolin riboside, 6-benzylaminopurine riboside and 6-(2-hydroxy-3-methoxybenzylamino)purine riboside) were tested for their possible interaction with human liver microsomal cytochromes P450 by absorption difference spectroscopy. All three compounds were shown to bind to the CYP enzymes producing a high to low spin shift of the heme iron yielding a Soret absorption band shift to approximately 425 nm. As this type of spectral change means that the substance is able to bind directly to the heme iron, the results obtained open the possibility of an interaction of these compounds with metabolism of other drugs or, in general, with other substrates of cytochromes P450.

INTRODUCTION

Cytokinins are low molecular weight substances (N⁶-substituted adenine derivatives) found in plants. Their most important role is to promote cell division and to stimulate morphogenesis¹. On the contrary, certain cytokinin ribosides cause cell cycle arrest in the animal cell. There are also synthetic cytokinin analogues, acting as inhibitors of cyclin dependent kinases² which are intensively studied as potential antineoplastic drugs³. Information on the interaction of any new drug with the main enzymes of drug metabolism (in this case the cytochromes P450) is essential to understanding the mechanism of drug metabolism⁴. Consequently we have performed a pilot study of the interaction of a new class of cytokinin derivatives with cytochromes P450 (CYP).

CYP enzymes involved in metabolism of foreign compounds (xenobiotics like drugs, food additives, and environmental pollutants) are localized mostly in the endoplasmatic reticulum of hepatocytes; however, they are ubiquitous, being present also in other tissues such as intestine, lung, kidney or brain⁴. There are about eight CYPs responsible for over 95 % of known cases of drug-dependent cytochrome P450 metabolism: CYP3A4, CYP2D6, CYP2C9, CYP2E1, CYP2C19, CYP1A2, CYP2A6 and CYP2B6⁵. In this study, we investigated whether the compounds studied (three cytokinin derivatives with the N⁶-amino group of adenine substituted with aromatic substituents, hence conventionally named “aromatic” cytokinins) are able to interact with CYP enzymes present in human liver microsomes.

MATERIAL AND METHODS

Material

Laboratory chemicals were purchased from Sigma Aldrich CZ (Prague) and were of reagent grade purity. Three cytokinins, *ortho*-topolin riboside (oTR), 6-benzylaminopurine riboside (BAPR) and 6-(2-hydroxy-3-methoxybenzylamino)purine riboside (HMBAPR) were either also purchased from Sigma (BAPR) or synthesized in our laboratory (Laboratory of Growth Regulators, oTR and HMBAPR). Structures of these compounds are shown in Fig. 1. The microsomal fraction of human liver homogenate was purchased from Advancell (Barcelona).

Methods

Difference spectra of interactions of cytokinins with microsomal CYP enzymes were followed according to established procedure⁶. The compounds to be tested were dissolved in an appropriate solvent (70% ethanol). This

Fig. 1. Structures of three aromatic cytokinins (oTR, HMBAPR and BAPR).

stock was further diluted in the respective media used in each assay. The final concentration of ethanol in the media did not exceed 1%, and therefore did not interfere with native CYPs used. An adequate amount of solvent was added to the reference cuvette. Spectra were recorded on a Specord UV VIS M40 spectrophotometer (Carl Zeiss, Jena). The spectral changes (absorbance difference at the peak maximum) generally follow a rectangular hyperbola⁶. The respective values were plotted to obtain the value of spectroscopic binding constant K_s and of the limiting value of the spectral change ($A_{\max}$, with meaning of the CYP binding capacity of the enzymes) using the Sigma Plot v. 8.0 graphing and statistical software (SPSS, Chicago, IL).

RESULTS AND DISCUSSION

Spectroscopic study of interaction of cytokinins with microsomal CYP

Interaction of all three substances with human microsomal fraction resulted in a characteristic difference spectrum with maximum at about 425 nm and minimum at 400 nm. This type of spectral change was formerly named as a Type II difference spectrum. A typical spectrum obtained with oTR is displayed in Fig. 2.

In such a case, the course of the spectra is given by a spin shift of the heme iron atom from the high to the low spin state. As the low spin state of the heme iron is manifested by a Soret absorption maximum at about 420 nm and the high spin state of the heme iron atom is known to be reflected by the position of the Soret band at about 390 nm, a high to low spin shift is expressed in the difference absorption spectrum (in a sample with tested compound + microsomal fraction in the sample cuvette and with the microsomal fraction alone in the reference cuvette) exactly as shown in the Fig. 2. Low spin of the heme iron is, however, typical of the hexacoordinated heme iron with substrate (or, generally, of an interacting compound) bound through its donor atoms to the heme iron. In fact, all three cytokinins possess functional groups or atoms which make this kind of interaction possible, namely, the nitrogen atoms of the adenine skeleton or amino group as well as the hydroxyl groups of oTR and HMBAPR.

Analysis of binding of cytokinins to microsomal CYP

The curve expressing the change of absorbance in the Soret absorption band region with increasing concentration of substrate should be a rectangular hyperbola⁶. The K_s values obtained for substances tested are displayed in Table 1. For illustration, the experimental data are shown in Fig. 3.

Interestingly, the K_s values reflect the structure of the respective molecules: BAPR is the most nonpolar cytokinin with an unsubstituted aromatic ring; HMBAPR possesses two substituents of the aromatic ring: a hydroxyl and a methoxy group. As cytochromes P450 tend to bind

Fig. 2. Difference spectra of interaction of human liver microsomal cytochrome P450 with OTR. CYP concentration 1.41 μ M, OTR 10 μ M (dashed), 16 μ M (dotted), 26 μ M (full line).

Fig. 3. Binding of BAPR to microsomal CYP followed by difference spectroscopy.

Table 1. Spectroscopic binding constants (K_s) and limiting absorbance values ($A_{\max}$) for interaction of cytokinins with human liver microsomal CYP

	BAPR	oTR	HMBAPR
K_s (μ M)	6.1 ± 2.3	7.23 ± 2.0	11.6 ± 2.4
$A_{\max}$	0.0041 ± 0.003	0.0040 ± 0.0003	0.016 ± 0.0001

the nonpolar molecules and convert them to their more polar derivatives, the result seems to be logical.

The results presented here, however, strongly indicate the possibility of direct interaction of these cytokinins with cytochromes P450. As the liver microsomal cytochromes are known to metabolize a variety of substrates including drugs, this interaction might be important for drug-drug interactions. In other words, a detailed study aimed at individual CYP forms is needed to answer the question whether the cytokinins studied may interfere with metabolism of drugs and other substrates of CYP enzymes.

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