인체 간 Microsome에서 7, 8-Benzoflavone의 대사

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Metabolism of 7,8-Benzoflavone by Human Liver Microsomes

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Introduction

Cytochrome P450(P450) enzymes play an important role in the metabolism of drugs, steroids and xenobiotics, some of which are chemical carcinogens. 7,8-Benzoflavone(ANF), a synthetic derivative of flavonoid compounds, exerts differential effects on the activities of P450 enzymes. ANF is known to inhibit the genotoxicity induced by benzo(a)pyrene , and 3-methylcholanthrene (1), whereas it stimulates the metabolic activation of aflatoxin B_1 (2). Due to the characterization of P450 enzymes and the intensive investigation of their substrate specificities, the above phenomena may be interpreted as the results of either inhibition of CYP1A2 activity(3) or stimulation of CYP3A4 activity(4) by ANF. These bifunctional activities of ANF may also be partially attributed to the intrinsic metabolism of ANF *per se*. Nesnow(5) studied the inhibitory activities of ANF and its related derivatives on benzo(a)pyrene oxidation, and suggested that ANF itself requires metabolic activation by the P450 enzymes in order to exert its inhibitory action.

Despite the widespread use of ANF as an *in vitro* modulator of metabolism in human liver microsomes, the metabolism of ANF in human liver microsomes has not been characterised. To understand better the *in vitro* modulation of human P450 activities by ANF , studies were undertaken to evaluate the hepatic metabolism of ANF using human liver microsomal fractions and to identify the major cytochrome P450 isozyme(s) involved in ANF metabolism. We also investigated the modulation of human liver microsomal caffeine 3-demethylation (CYP1A2) and testosterone 6β-hydroxylation (CYP3A4) by metabolites of ANF as well as ANF.

Results and Discussion

Identification of ANF metabolites

After incubation of ANF with human liver microsomes in the presence of NADPH-

generating system, five metabolites were resolved by reverse phase HPLC. Each peak was fractionated and subjected to GC-MS after trimethylsilylation. The identification of each metabolite was deduced on the basis of the EI mass spectral data and HPLC elution profile and comparison with the results reported previously. A specific isomerazation of dihydrodiols to corresponding hydroxy derivatives also provided the information for the characterization of metabolites (6). The metabolites identified were 5,6-dihydrodiol, 7,8-dihydrodiol, 7-hydroxyANF, 6-hydroxyANF and AND 5,6-oxide. The stable 5,6-oxide was a major metabolite generated by human liver microsomes and estimated to constitute about 70% of total metabolites. Quantitation of each metabolite was accomplished by counting the radioactivity of each peak in order to evaluate the role of cytochrome P450 enzymes in the metabolism of ANF. The apparent kinetic constants for ANF 5,6-oxide formation, as determined in three different human liver microsomal preparations (HL110, HL114, and HL136) were 17.0 ± 3.6 μM and 2.8 ± 2.0 nmol/min/mg protein for K_m and V_{max}, respectively.

Identification of P450 subtype involved in the Meabolism of 7,8-Benzoflavone.

The role of CYP3A4 in the metabolism of ANF was examined by using a variety approaches, including (1) selective inhibition of ANF metabolism by ketoconazole and gestodene, which are known as a selective inhibitor of CYP 3A4, (2) correlation of rates of ANF 5,6-oxide formation with other enzyme activities in human liver microsomes, (3) immunoinhibition of ANF metabolism in human liver microsomes with rabbit anti-human P450 3A4, and (4) demonstration of ANF metabolism by microsomal fractions expressing specific human P450 enzymes. The results collectively indicate that CYP 3A4 is a major P450 enzyme responsible for the metabolism of ANF. Antibodies specific to the P4503A4 enzyme inhibited the transformation of ANF to oxidized metabolites. In addition, selective inhibitors and a substrate of CYP3A4 inhibited ANF metabolism although the degree of inhibition varied with the individual compound. Ketoconazole, known as a relatively selective inhibitor of CYP3A in humans, both *in vivo* and *in vitro* (7), completely inhibited the metabolism of ANF in a concentration-dependent manner with a Ki value 0.01 μM. Felodipine, a specific substrate for

the CYP3A4 enzyme also inhibited the metabolism of ANF. Microsomal fractions that expressed human P4503A4 catalysed the oxidation of ANF to a greater extent than the control microsome whereas microsomal fractions that expressed P4501A2, 2D6, and 2E1 had very low activities. Additional evidence arises from the high correlation between ANF metabolism and testosterone 6b-hydroxylation catalysed by CYP3A4, whereas no significant correlation was observed with any reactions mediated by other P450 enzymes.

The involvement of the CYP3A enzyme in the metabolism of ANF in human liver microsomes is somewhat unexpected in the light of competitive inhibiton of CYP1A enzyme activities by ANF and the metabolism mediated by P4501A1 in rat liver microsomal fractions. The kinetic data [Ki of <1 μM for inhibition of caffeine N3-demethylation (8) and Km of 17 μM for ANF 5,6-oxide formation] suggest the preferential binding of ANF to a site which does not lead metabolism. A similar case was also seen in quinidine-3-hydroxylation. Quinidine is a strong selective inhibitor of debrisoquine and sparteine oxidation mediated by CYP2D6 (9), whereas CYP3A4 is the principal catalyst of quinidine oxidation (10).

Modulation of P450 activities by ANF and its metabolites

Caffeine 3-demethylation was used as an *in vitro* reaction for the determination of the activity of CYP1A2(3). Inhibition of 1,7-DMX formation by ANF and its metabolites were determined using HL 136 microsomes. ANF was the most potent among all the compounds tested, although almost all of the metabolites were found to be similarly effective as ANF at equimolar concentrations in human liver microsomes. In contrast, 5,6-dihydrodiol ANF did not inhibit caffeine 3-demethylation activities up to 0.7μM, at which concentration ANF inhibited caffeine 3-demethylation activities by 90%. The results of the current investigation suggest that ANF itself exerts inhibitory action on CYP1A2 activity without extensive metabolic activation.

Testosterone 6β-hydroxylation was selected as a marker activity of CYP3A4 in human liver microsomes (8). ANF exhibited a stimulatory effect on testosterone 6β–hydroxylation in a dose-dependent manner. This observation is consistent with previous reports that some activities of CYP3A4 are stimulated by ANF. ANF metabolites with substitutions at positions

5,6 or 7,8 showed stimulatory activities, but dose-dependent enzyme activation was not observed with respect to ANF. Unlike other metabolites, 6- and 7-hydroxy ANF inhibited 30-70% of the testosterone 6β-hydroxylation activities at a concentration of 0.7μM.

Several investigations have demonstrated that the catalytic activities of human CYP3A4 are stimulated by ANF (11). However, the effects of ANF metabolites on the activities of CYP3A4 have not been reported. ANF exhibited a stimulatory effect on testosterone 6β-hydroxylation in a dose-dependent manner. ANF metabolites such as the 5,6-dihydrodiol, 7,8-dihydrodiol, and the 5,6-oxide showed slightly stimulatory effects, though these were not dose-dependent. Hydroxy metabolites were found to inhibit a substantial fraction of the CYP3A4 activity.

At present, the nature of this stimulation is not understood. It has been demonstrated that ANF inhibits the 3α-hydroxylation of aflatoxin B_1(11) and alfentanil oxidation(12). The interaction of ANF with CYP3A4 is complicated and not yet resolved. Raney *et al.* (11) have endeavoured to explain the inhibitory effect of ANF on aflatoxin B_1 3α-hydroxylation and stimulatory effect of ANF on aflatoxin B_1 8,9-epoxidation by an allosteric mechanism. Our results showed that CYP3A4 plays a major role in the oxidation of ANF by human liver microsomes. ANF itself may also be a positive allosteric effector, acting at a second site as well as also fitting into the substrate binding site. During the metabolic activation of ANF, the resultant metabolites may lose some or all of these positive allosteric effects, while their affinities to allosteric site may be greater than the parent compound ANF. 6-Hydroxy- and 7-hydroxy- ANF may act even as negative allosteric effectors. Further investigation is required to test this hypothesis.

In summary, we have shown that ANF exerts an inhibitory or stimulatory activity on P450 enzymes directly. At least, it seems that the biotransformation of ANF to its metabolites does not make a major contribution to the modulation of P450 enzymes by ANF.

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