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Short communication

Identification of cytochrome P450 1A2 as enzyme involved in the microsomal metabolism of Huperzine A

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Abstract

Huperzine A is a reversible and selective cholinesterase inhibitor approved for the treatment of Alzheimer's disease. To identify which cytochrome P450 (CYP) isoenzymes are involved in the metabolism of Huperzine A, an in vitro study was performed with rat liver microsomes and immunoinhibition and chemical inhibition methods. Huperzine A metabolism was analyzed with high-performance liquid chromatography (HPLC) and expressed as Huperzine A disappearance rate. Result showed that 76.2% of Huperzine A metabolism was inhibited by CYP1A2 antibody and 17.8% by CYP3A1/2 antibody. Inhibitory effects produced by CYP2C11 and 2E1 antibodies were minor. The CYP1A2 substrate phenacetin showed an inhibitory effect of 70.3%. In conclusion, Huperzine A metabolism in rat liver microsomes is mediated primarily by CYP1A2, with a probable secondary contribution of CYP3A1/2. CYP2C11 and 2E1 are likely not involved in Huperzine A metabolism.

Keywords: Huperzine A; Metabolism; Cytochrome P450; Microsome

1. Introduction

Huperzine A, (5*R*,9*R*,11*E*)-5-amino-11-ethylidene-5,6,9,10-tetrahydro-7-methyl-5,9-methano-cycloocctenol [B]pyridin-2 (1*H*)-one, is a potent, reversible and selective cholinesterase inhibitor approved for the treatment of Alzheimer's disease (Bai et al., 2000). Limited data are available on Huperzine A metabolism in humans and other animal species. In young healthy volunteers, the time course of Huperzine A concentrations in plasma follows a one-compartment open model (Qian et al., 1995). In mice, Huperzine A was distributed mainly in kidney and liver. The majority of Huperzine A is excreted in the urine, with only 2.4% being recovered in feces. Paper chromatograms of urine revealed that [^{3}H]Huperzine A was excreted as prototype and its metabolite (Wang et al., 1998). However, the role of cytochrome P450 (CYP) in Huperzine A metabolism remained unknown.

Huperzine A is used by patients with Alzheimer's disease, most of whom are older than 60 years. For old people, combined and successive medication may affect the expression and activity of CYP and elicit drug interactions. It is thus necessary to identify which CYP isoenzymes are involved in the major pathway of Huperzine A metabolism to determine possible drug interactions and to avoid possible adverse drug reactions. In this context, we detected the CYP isoenzymes principally responsible for the metabolism of Huperzine A in rat liver microsomes.

2. Materials and methods

2.1. Chemicals and reagents

Huperzine A was supplied by the Department of Phytochemistry, Shanghai Institute of Materia Medica. Monoclonal antibodies against rat CYP1A2, 2C11, 2E1, 3A1/2 were obtained from Chemcon International (Temecula, CA). All

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other reagents were of the highest quality commercially available.

2.2. Microsomal preparation and incubation conditions

Liver microsomes were obtained from six male Sprague–Dawley rats (about 2 months old, decapitation), prepared by differential centrifugation as described previously (Gibbson and Shett, 1994). The protein content of each microsomal preparation was determined by the method of Lowry et al. (1951). The Huperzine A concentration used for assaying the catalytic activity was 200 nM, which is compatible with its therapeutic plasma concentration. Huperzine A was incubated with microsomes equivalent to 0.025 mg protein and 0.1 M sodium phosphate buffer (pH 7.4). The enzyme reaction was initiated by adding 50 μl NADPH (10 mM) to the mixture and the incubation was continued at 37 °C in a shaking water bath for 30 min.

2.3. Immunoinhibition study

Antibodies against CYP1A2, 2C11, 2E1, and 3A1/2 were used for the immunoinhibition study (Gelboin, 1993). Microsomes were preincubated with incubation buffer containing 0, 1, 2, 5, 10, or 20 μl of different antibodies at 37 °C for 5 min. Then NADPH and Huperzine A were added to the incubation mixture. The respective reaction was carried out under the conditions described above. Huperzine A metabolism was analyzed with high-performance liquid chromatography (HPLC) and expressed as Huperzine A disappearance rate (Svensson and Ashton, 1999).

2.4. Chemical inhibition study

Phenacetin is a classical substrate for CYP1A2 and was used for competitive inhibition experiments in this study (Tassaneeyakul et al., 1993). The substrate used for CYP1A2 was caffeine (Roberts et al., 1994). Huperzine A and caffeine (200 nM) were separately incubated with phenacetin at concentrations of 0, 1, 5, 10, 20 or 50 μM under the conditions described earlier. The metabolism of Huperzine A or caffeine was analyzed with HPLC and expressed as Huperzine A or caffeine disappearance rate. For assessing the inhibitory potency of phenacetin, the concentration associated with 50% inhibition (IC_{50}) of Huperzine A or caffeine metabolism was determined based on the concentration inhibition curves.

2.5. HPLC assays

To each incubation mixture, 4 ml of ethyl acetate was added and the mixture was centrifuged at 10,000 × *g* for 5 min. The organic phase (3 ml supernatant) was drawn into another tube and evaporated under nitrogen gas. The residue was dissolved in 100 μl mobile phases, and 10 μl was injected into the HPLC system (Millipore, USA) consisting of 510 pump, U6 k injector, reverse-phase column (length 30 cm), 486 UV absorbance detector. Column temperature was maintained at 30 °C. The mobile phase used for Huperzine A was water and methanol (10:90, v/v), which was delivered at 0.9 ml/min; detection was 313 nm. The mobile phase used for caffeine was 0.05% acetic acid, acetonitrile and methanol

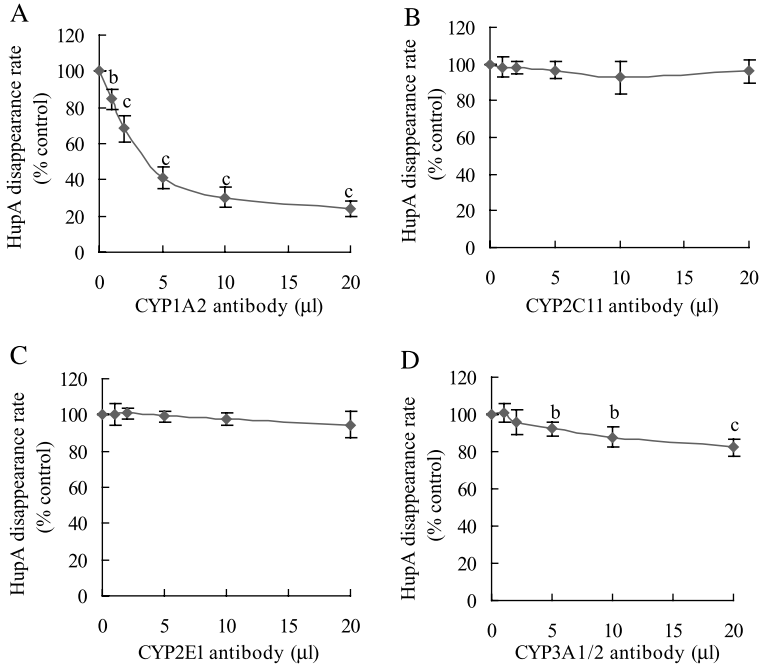

Fig. 1. Immunoinhibition of Huperzine A disappearance rate by antibodies against CYP1A2, 2C11, 2E1 and 3A1/2. Data are expressed as means ± S.E.M. ($n = 6$). $^{b}P < 0.05$, $^{c}P < 0.01$ vs. control. The control value of Huperzine A disappearance rate was 40.9 ± 14.6 pmol/min/mg protein.

(82:8:10, v/v/v), which was delivered at 1 ml/min; detection was 282 nm.

2.6. Data analysis

Data are expressed as means ± S.E.M. and analyzed by one-way analysis of variance (ANOVA). $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. HPLC assays

No peaks interfered with those of Huperzine A and caffeine on chromatography. The retention time for Huperzine A and caffeine was 7.8 min and 9.8 min, respectively. Intra-day and inter-day variations were less than 10%. For Huperzine A and caffeine, the mean recovery from the incubation mixture containing rat liver microsomes was above 90%. When the incubation was carried out without NADPH, no appreciable metabolism of Huperzine A and caffeine was observed.

3.2. Immunoinhibition

Huperzine A disappearance rate decreased significantly with an increase in CYP1A2 antibody (Fig. 1A). The maximum inhibitory effect produced by CYP1A2 antiserum was 76.2% (71.8–80.6%). As for CYP3A1/2 (Fig. 1D), the maximum inhibitory effect on the Huperzine A disappearance rate was 17.8% (13.1–22.5%). CYP2C11 and 2E1 antibodies (Fig. 1B,C) did not produce inhibitory effects equal to or greater than 10%.

3.3. Chemical inhibition

Phenacetin inhibited the disappearance rate of Huperzine A or caffeine in a concentration-dependent manner (Fig. 2A). The IC_{50} was 25.4 and 12.8 μM for Huperzine A and caffeine, respectively. The maximum inhibitory effect elicited by phenacetin was 70.3% (63.0–77.6%) for Huperzine A and 77.3% (71.1–83.5%) for caffeine. A significant correlation ($r = 0.98$) was noted between the phenacetin inhibition of Huperzine A and the caffeine disappearance rate (Fig. 2B). The multinomial logistic regression line of Huperzine A and caffeine disappearance rate was $Y = 0.0162X^2 - 1.1065X + 44.393$.

4. Discussion

CYP isoenzymes collectively catalyze the oxidation of a multitude of xenobiotic and endobiotic substrates (Guengerich, 1994). Identifying the contribution of individual CYP to the metabolism of drugs is important for understanding the regulation of the pathway and the rate of drug metabolism, drug interactions as a result of the CYP inhibition or activation, and the pharmacological, toxicological and therapeutics effects of a drug or multiple drugs.

In the present study, we evaluated the metabolism of Huperzine A in microsomes using immunoinhibition and chemical inhibition methods. The structural homology of CYP varies greatly, and the large differences in substrate and product specificity among CYP isoenzymes make it difficult to determine the precise role of each CYP in the metabolism of individual substrates. The use of CYP antibodies provides the most convincing evidence for the involvement and contribution of a particular CYP in the metabolism of therapeutic agents (Shou et al., 2000). The specificity of antibodies, their interaction with individual epitopes on proteins, homogeneity as defined by chemical reagents, and ease of handling, all contribute to their extraordinary utility. Chemical inhibition of CYP isoenzymes has also proven to be a useful tool in defining the role of individual CYP isoenzymes involved in drug metabolism (Rendic and Di Carlo, 1997). These compounds can be used in vitro and in vivo, thus making it possible to link a particular CYP with a specific toxicological or pharmacological response.

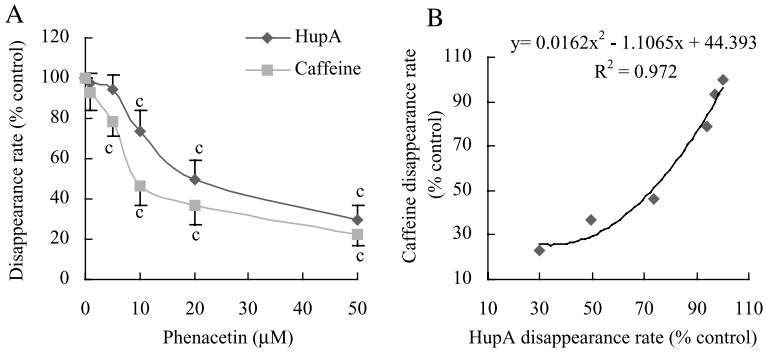

Fig. 2. (A) Inhibition by phenacetin of the disappearance rate of Huperzine A and caffeine. Data are expressed as means ± S.E.M. ($n = 6$). $^cP < 0.01$ vs. control. (B) Multinomial logistic regression line of Huperzine A and caffeine disappearance rate. The control value of Huperzine A disappearance rate was 40.9 ± 14.6 pmol/min/mg protein. The control value of caffeine disappearance rate was 64.7 ± 11.3 pmol/min/mg protein.

Because crude liver microsomal preparations consist of multiple CYP isoenzymes, which have distinct enzyme affinity (Gonzalez, 1990), CYP isoenzymes dominating the overall metabolism of a certain drug may differ depending on substrate concentrations. Pearce et al. (1996) demonstrated that lansoprazole metabolism in liver microsomes appeared to be catalyzed by two kinetically distinct enzymes. They found that the reaction was dominated by CYP2C19 at a therapeutic concentration, but by CYP3A4/5 at super-therapeutic concentrations.

In this context, 200 nM Huperzine A was used in the microsomal metabolism study, a concentration which is largely compatible with its therapeutic plasma concentration. Immunoinhibition study revealed that antibody against CYP1A2 had a potent inhibitory effect on the microsomal metabolism of Huperzine A. An inhibitory effect produced by CYP3A1/2 antibody was also noted, while inhibitory effects produced by CYP2C11 and 2E1 antibodies were minor. In agreement with the immunoinhibition study, the CYP1A2 substrate phenacetin had a significant inhibitory effect on Huperzine A metabolism. Caffeine was used in correlation analysis with Huperzine A in this study. A significant correlation was noted between the inhibition elicited by phenacetin of Huperzine A and caffeine metabolism. All these results suggest that CYP1A2 plays an important role in the metabolism of Huperzine A.

CYP1A2, about 13% of total CYP isoenzymes in liver (Shimada et al., 1994), is an enzyme of both pharmacological and toxicological significance (Curtis et al., 1999). It is responsible for in vivo metabolism of a number of drugs, such as methylxanthines, acetaminophen, theophylline, imipramine and propranolol, and has been implicated in the hepatic metabolic activation of a number of procarcinogens and promutagens, such as food-derived heterocyclic aromatics (Guengerich and Shimada, 1991). CYP1A2 activity can be induced by smoking, caffeinated drinks, and omeprazole, and inhibited by cimetidine, quinolones, furafylline, moclobemide, etc. (Xu and Zhou, 1996). So patients treated with Huperzine A should pay attention to the factors listed above to avoid possible drug interactions and to assure the safety of medication.

In conclusion, Huperzine A metabolism in rat liver microsomes is mediated primarily by CYP1A2, with a probable secondary contribution of CYP3A1/2. CYP2C11 and 2E1 are likely to be not involved in Huperzine A metabolism. Because CYP1A2 is involved in the metabolism of numerous important drugs and chemicals, further studies are required to assess whether Huperzine A has a clinically relevant metabolic interaction with CYP1A2 substrates and/or inhibitors.

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