Metabolism-dependent inhibition of CYP3A4 and CYP2D6 by extracts from 26 herbal medicines

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A total of 26 herbal medicines were examined for their inhibitory effects on cytochrome P450 3A4 (CYP3A4) and 2D6 (CYP2D6). A methanol extract of each herbal medicine was prepared and then preincubated with human liver microsomes in the presence of an NADPH-generating system. Residual microsomal CYP3A4 and CYP2D6 activity was then determined by measuring the *N*-demethylation of erythromycin and the *O*-demethylation of dextromethorphan, respectively. Of the 26 herbal medicines tested, 16 were found to decrease the residual CYP3A4 activity in a preincubation time-dependent manner. The extract of Evodia Fruit caused the most dramatic decrease in residual CYP3A4 activity (i.e. 22.3% residual activity after 30 min preincubation). A substantial decrease in residual CYP3A4 activity was also observed from extracts of Sappan Wood, Incised Notopterygium Rhizome, Schisandra Fruit, Great Burdock Achene, Angelica Dahurica Root and Rhubarb (residual activity of 40.6, 41.2, 53.4, 47.1, 53.4 and 59.2% after 30 min preincubation, respectively). These results are comparable to those using troleandomycin, a known irreversible inhibitor of CYP3A4, which gave a residual activity of 49.4% under identical conditions. We found 5 herbal medicines that showed a preincubation time-dependent inhibition of CYP2D6. The extract of Incised Notopterygium Rhizome caused the most dramatic decrease in residual CYP2D6 activity (i.e. 61.9% residual activity after 30 min preincubation). These results suggest that extracts of herbal medicines contain metabolism-dependent inhibitors of CYP, especially CYP3A4.

Key words herb, human liver microsomes, inhibition, CYP3A4, CYP2D6.

Introduction

Cytochrome P450 (CYP) enzymes are generally considered to be involved in 95% of drug-drug interactions associated with the metabolism of drugs. Furthermore, about 70% of drug interactions associated with CYP-mediated metabolism are the result of enzyme inhibition.[1] Some CYP inhibitors show more potent effects in a manner dependent on the incubation time. Such inhibitors are called metabolism-dependent inhibitors. Since the inhibitory effect is dependent on the preincubation time, it is highly likely that a metabolism-dependent inhibitor alters the pharmacokinetics of other drugs. Metabolism-dependent inhibitors are classified as reversible, quasi-irreversible or irreversible.[2] Quasi-irreversible and irreversible metabolism-dependent inhibitors are referred to as suicidal or mechanism-based inhibitors. As the inhibition is irreversible, these compounds often cause serious adverse effects, especially in a clinical setting, since their effect can persist even after withdrawal of the inhibitor. Recovery of enzyme activity after irreversible inhibition can be accomplished by biosynthesis of the enzyme molecule to a sufficient level. The concomitant use of sorivudine and an anticancer fluorouracil derivative, such as 5-fluorouracil, is a well-known example of a drug interaction caused by an irreversible metabolism-dependent inhibition.[3] Other examples include macrolide antibiotics such as erythromycin and troleandomycin (CYP3A4 inhibitors),[4] furafylline (CYP1A2 inhibitor)[5] and orphenadrine (CYP2B1 inhibitor).[6] It is therefore important to clarify the mechanism of enzyme inhibition in order to better understand possible drug interactions. This is particularly important for herbal medicines because of their complex composition.

We are currently carrying out a survey of possible interactions of herbal medicines with synthetic drugs. In total we have studied crude extracts of 78 herbal medicines for their inhibitory effects on CYP3A4 and CYP2D6, which are involved in the metabolism of numerous synthetic drugs.[7] In a previous study, several herbal medicines showed potent inhibition for CYP3A4 and/or CYP2D6. However, their mechanism of inhibition remains unclear. In the present study, 26 out of the 78 herbal medicines, which showed relatively potent inhibition for CYP3A4 and/or CYP2D6, were examined as potential metabolism-dependent inhibitors by determining their residual enzyme activity after preincubation.

Materials and Methods

Chemicals. Powders of the 26 herbal extracts listed in

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Table 1 List of the herbs used in this study

No.	English name[a]	Latin name[b]	Japanese name		Family name	Lot No.
1	Angelica Dahurica Root	Angelicae Dahuricae Radix	白芷	Byakushi	Umbelliferae	231076010
2	Atractylodes Lancea Rhizome	Atractylodis Lanceae Rhizoma	蒼朮	Sojutsu	Compositae	281005010
3	Cassia Bark	Cinnamomi Cortex	桂皮	Keihi	Lauraceae	271003020
4	Clove	Caryophylli Flos	丁子	Choji	Myrtaceae	2991017010
5	Coptis Rhizome	Coptidis Rhizoma	黄連	Ouren	Ranunculaceae	2001014010
6	Corydalis Tuber	Corydalis Tuber	延胡索	Engosaku	Papaveraceae	251019010
7	Dried Ginger	Zingiberis Siccatum Rhizoma	乾姜	Kankyou	Zingiberaceae	2001067010
8	Ephedra Herb	Ephedrae Herba	麻黄	Mao	Ephedraceae	2991037010
9	Evodia Fruit	Evodiae Fructus	呉茱萸	Gosyuyu	Rutaceae	2991042010
10	Forsythia Fruit	Forsythiae Fructus	連翹	Rengyo	Oleaceae	271097010
11	Gambir Plant	Uncariae Uncis Cum Ramulus	釣藤鈎	Chotoko	Rubiaceae	291089010
12	Great Burdock Achene	Arctii Fructus	牛蒡子	Goboshi	Compositae	281047010
13	Incised Notopterygium Rhizome	Notopterygii Rhizoma	羌活	Kyokatsu	Umbelliferae	251136010
14	Licorice Root	Glycyrrhizae Radix	甘草	Kanzo	Leguminosae	281013010
15	Loquat Leaf	Eriobotryae Folium	枇杷葉	Biwayo	Rosaceae	271142010
16	Magnolia Bark	Magnoliae Cortex	厚朴	Koboku	Magnoliaceae	241035010
17	Moutan Bark	Moutan Cortex	牡丹皮	Botanpi	Paeoniaceae	251006010
18	Phellodendron Bark	Phellodendri Cortex	黄柏	Obaku	Rutaceae	2001034010
19	Rhubarb	Rhei Rhizoma	大黄	Daio	Polygonaceae	2991028010
20	Sappan Wood	Sappan Lignum	蘇木	Soboku	Leguminosae	271134010
21	Schisandra Fruit	Schisandrae Fructus	五味子	Gomishi	Schisandraceae	291043010
22	Scutellaria Root	Scutellariae Radix	黄芩	Ogon	Labiatae	281024010
23	Sinomenium Stem	Sinomeni Caulis et Rhizoma	防已	Boi	Menispermaceae	261029010
24	Sophora Flower	Magnoliae Flos	辛夷	Shin-i	Magnoliaceae	2991054010
25	Whiteflower Hogfennel Root	Peucedani Radix	前胡	Zenko	Umbelliferae	2011068010
26	Zanthoxylum Fruit	Zanthoxyli Fructus	山椒	Sansho	Rutaceae	251094010

a) Herbs are listed in the alphabetical order of their English names. b) Origin plant name.

Table I were kindly provided by Tsumura Ltd. (Tokyo, Japan). Each herbal extract powder was prepared by immersing the herb in distilled water and heating it to 95-100 ℃ for 60 min. The resultant extract was then filtered. The filtrate was evaporated under reduced pressure and then spray-dried to make the powder.

[*N*-methyl-^{14}C]Erythromycin (2.035 GBq/mmol; radiochemical purity >99%) and [*O*-methyl-^{14}C]dextromethorphan (2.035 GBq/mmol; radiochemical purity >99%) were purchased from American Radiochemicals Inc. (St. Louis, MO, USA). Erythromycin, dextromethorphan hydrobromide monohydrate, ketoconazole and quinidine were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The oxidized form of nicotinamide adenine dinucleotide phosphate ($NADP^+$), glucose 6-phosphate (G-6-P), and G-6-P dehydrogenase were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). All other reagents and solvents were analytical grade and were supplied by Wako Pure Chemical Industries, Ltd.

Human liver microsomes. Human liver microsomes which were prepared from 16 individuals (male, 11; female, 5) were obtained from Xeno Tech LLC (Kansas City, KS, USA) and stored at -80℃ until use.

Preparation of methanol soluble fractions from water extracts of herbs. Powder for each herbal extract (1 g) was extracted with methanol, as described previously.[7] One microliter of the methanol soluble fraction was equivalent to 0.33 mg of powdered herbal extract. These methanol soluble fractions were diluted with methanol and used in the inhibitory studies.

Assay of CYP3A4 and CYP2D6 specific activities. The *N*-demethylation of erythromycin and the *O*-demethylation of dextromethorphan, specific reactions mediated by CYP3A4 and CYP2D6 respectively, were determined by quantifying the amount of formaldehyde produced as described previously.[7] The reaction mixture (0.5 mL) contained 100 μM [*N*-methyl-^{14}C]erythromycin or 10 μM [*O*-methyl-^{14}C]dextromethorphan, 100 mM potassium phosphate, pH 7.4, 50 μM EDTA, 0.4 mg/mL human liver microsomal protein and an NADPH-generating system (0.5 mM $NADP^+$, 5 mM $MgCl_2$, 5 mM G-6-P, and 1 U/mL G-6-P dehydrogenase). The mixture was incubated at 37 ℃ for 10 min for the erythromycin *N*-demethylation and 20 min for the dextromethorphan *O*-demethylation. All incubations were performed in duplicate.

Preincubation time-dependent CYP3A4 and CYP2D6 inhibition. Human liver microsomes were preincubated at 37 ℃ with herbal extracts in the presence of an NADPH-

generating system. The preincubation time was 0, 10, 20 and 30 min. After preincubation, ^{14}C-labeled erythromycin and dextromethorphan were added and the erythromycin *N*-demethylation and dextromethorphan *O*-demethylation activities were measured according to the method described in the previous section. Troleandomycin and paroxetine, which are known mechanism-based inhibitors of CYP3A4 and CYP2D6, respectively, were used as the positive control. Ketoconazole and quinidine, which are known competitive inhibitors of CYP3A4 and CYP2D6, respectively, were used as the negative control.

Results

Preincubation time-dependent CYP3A4 inhibition. Figure 1 shows the effects of 26 herbal medicines on CYP3A4 activity in human liver microsomes upon preincubation for various time periods. Troleandomycin, a known mechanism-based inhibitor of CYP3A4,[2] caused a preincubation time-dependent decrease in CYP3A4 activity. The residual activity after 30 min preincubation was 49.4% of that for the 0 min sample. In contrast, ketoconazole, a known competitive inhibitor of CYP3A4,[2] caused no significant preincubation time-dependent decrease in the CYP3A4 activity. Indeed, the level of inhibition of CYP3A4 decreased as the length of the preincubation period increased.

Preincubation time-dependent decreases in the CYP3A4 activity were observed in the following 16 out of the 26 herbal medicines: Angelica Dahurica Root, Cassia Bark, Coptis Rhizome, Dried Ginger, Evodia Fruit, Forsythia Fruit, Great Burdock Achene, Incised Notopterygium Rhizome, Licorice Root, Magnolia Bark, Moutan Bark, Phellodendron Bark, Rhubarb, Sappan Wood, Schisandra Fruit and Sophora Flower. Among these herbal medicines, Evodia Fruit showed the most effective inhibition for CYP3A4. The residual activity in microsomes after 30 min preincubation with an extract of Evodia Fruit was 22.3%. A substantial decrease in enzyme activity upon preincubation was also observed for the following extracts: Sappan Wood, Incised Notopterygium Rhizome, Schisandra Fruit, Great Burdock Achene, Angelica Dahurica Root and Rhubarb (residual activity after 30 min preincubation of 40.6, 41.2, 53.4, 47.1, 53.4 and 59.2%, respectively). The other 10 herbal medicines did not cause a significant preincubation time-dependent decrease in CYP3A4 activity.

Preincubation time-dependent CYP2D6 inhibition. Figure 2 shows the effects of 26 herbal medicines on the CYP2D6 activity in human liver microsomes upon preincubation for various time periods. Paroxetine, a known mechanism-based inhibitor of CYP2D6,[8] caused a preincubation time-dependent decrease in CYP2D6 activity. The residual activity after 30 min preincubation was 38.7% of that for the 0 min sample. However, quinidine, a known competitive inhibitor of CYP2D6,[2] caused no significant preincubation time-dependent decrease in CYP2D6 activity.

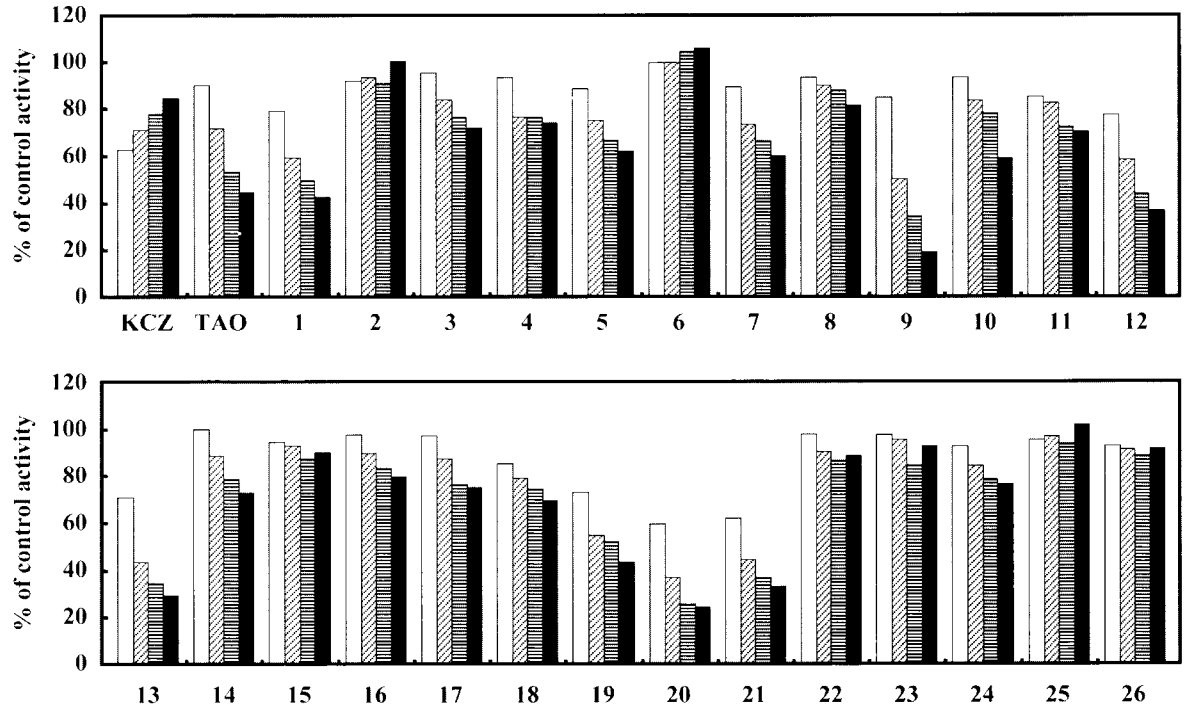

Fig. 1 Preincubation time-dependent inhibition of human microsomal erythromycin *N*-demethylation activity by herbal extracts. Human liver microsomes were first preincubated with each herbal extract or 1% methanol (control) for 0 (□), 10 (▨), 20 (▤), and 30 (■) min at 37°C in the presence of an NADPH-generating system. Each herbal extract concentration was 55 μg/mL. KCZ, ketoconazole (0.1 μM); TAO, troleandomycin (10 μM). Data are expressed as means of duplicate experiments.

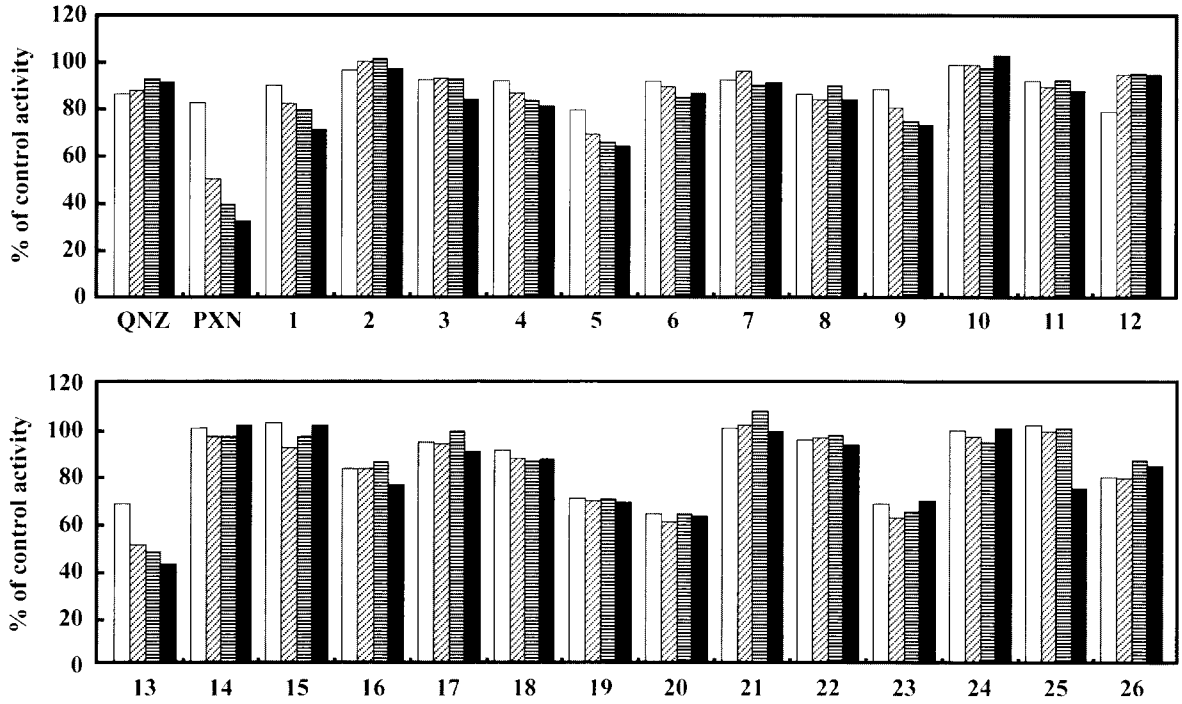

Fig. 2 Preincubation time-dependent inhibition of human microsomal dextromethorphan *O*-demethylation activity by herbal extracts. Human liver microsomes were first preincubated with each herbal extract or 1% methanol (control) for 0 (□), 10 (▨), 20 (▤), and 30 (■) min at 37°C in the presence of an NADPH-generating system. Each herbal extract concentration was 55 μg/mL (Coptis Rhizome; 3.3 μg/mL, Phellodendron Bark; 0.33 μg/mL, Sinomenium Stem; 16.5 μg/mL). QNZ, quinidine (10 nM); PXN, paroxetine (1 μM). Data are expressed as means of duplicate experiments.

Indeed, the level of CYP2D6 inhibition decreased as the length of the preincubation period increased.

Preincubation time-dependent decreases in the CYP2D6 activity were observed in the following 5 out of the 26 herbal medicines examined: Angelica Dahurica Root, Clove, Coptis Rhizome, Evodia Fruit and Incised Notopterygium Rhizome. Among these herbal medicines, Incised Notopterygium Rhizome showed the most effective inhibition for CYP2D6. The residual activity in microsomes after 30 min preincubation with an extract of Incised Notopterygium Rhizome was 61.9% of that for the 0 min sample. The other 21 herbal medicines did not cause any significant preincubation time-dependent decrease in activity.

Discussion

Troleandomycin and paroxetine, model mechanism-based inhibitors of CYP3A4 and CYP2D6, were used as positive controls in this study, respectively. As expected, preincubation of microsomes with these agents caused inhibition of the respective CYP activity in a preincubation time-dependent manner. Ketoconazole and quinidine were used as negative control substances of mechanism-based CYP3A4 and CYP2D6 inhibitors, respectively. No significant preincubation time-dependent decrease in CYP3A4 or CYP2D6 activity was observed using these agents. Indeed, preincubation of the microsomes with ketoconazole and quinidine appeared to lower the observed level of CYP3A4 and CYP2D6 inhibition, respectively. This result suggests that these compounds are metabolized by the microsomes during the preincubation period, thereby lowering the level of inhibitor prior to the assay.

In the present study we found 16 herbal medicines that decreased the residual CYP3A4 activity in a preincubation time-dependent manner. Indeed 7 of these (Evodia Fruit, Sappan Wood, Incised Notopterygium Rhizome, Schisandra Fruit, Great Burdock Achene, Angelica Dahurica Root and Rhubarb) showed a comparable or even greater inhibitory effect than that of troleandomycin, a known irreversible inhibitor. Among these herbal medicines, Evodia Fruit showed the greatest inhibitory activity. The inhibitory effects against CYP3A4 activity in a preincubation time-dependent manner in above 7 herbal medicines were not observed in the absence of NADPH-generating system (data not shown). There is quite a possibility that extracts of herbal medicines such as Evodia Fruit, Sappan Wood, Incised Notopterygium Rhizome, Schisandra Fruit, Great Burdock Achene, Angelica Dahurica Root and Rhubarb contain metabolism-dependent inhibitors of CYP3A4. Grapefruit juice shows a potent inhibition for CYP3A4. Consumption of grapefruit juice has been shown to inhibit the metabolism of certain drugs, which are normally subject to modification by CYP3A4.[9] The furanocoumarins, bergamottin, dihydroxybergamottin, GF-I-1 and GF-I-4, have been isolated from grapefruit juice and identified as mechanism-based inhibitors of CYP3A4.[10] These compounds have also been found in Angelica Dahurica Root

and Incised Notopterygium Rhizome.[11] Therefore, the preincubation time-dependent CYP3A4 inhibition observed with the extracts of Angelica Dahurica Root and Incised Notopterygium Rhizome may be attributable, at least in part, to the inhibitory action of these furanocoumarins. Furthermore the inhibitory effect of Licorice Root could be due to the presence of glabridin, which has been identified as a mechanism-based CYP3A4 inhibitor.[12]

A preincubation time-dependent inhibition of CYP2D6 was observed with 5 of the herbal medicines tested in this study (Angelica Dahurica Root, Clove, Coptis Rhizome, Evodia Fruit and Incised Notopterygium Rhizome). Hence, in this study, there were far fewer herbal medicines showing a preincubation time-dependent decrease in CYP2D6 activity in comparison to CYP3A4. Furthermore, the level of CYP2D6 inhibition was much less than that observed for CYP3A4.

In order to construct the reaction conditions, we used an NADPH-generating system as the NADPH source in this study. There is a possibility that direct inhibition of NADPH-cytochrome P450 reductase or G-6-P dehydrogenase in the NADPH-generating system was detected as an apparent inhibition of the CYP3A4 and CYP2D6 activities. If the inhibitory effect was direct against the NADPH-generating system, it was thought that there was a similar inhibitory effect between CYP3A4 and CYP2D6. However, both inhibitory effects were different for each herbal extract. In addition, seven herbal medicines, which showed a comparable or even greater CYP3A4 inhibitory effect than that of troleandomycin, were also examined using NADPH in place of the NADPH-generating system. The results obtained using the NADPH-generating system were almost the same as the result using NADPH (data not shown). From these results, it was thought that the inhibitory effect in the present study was connected directly to CYP.

Some metabolism-dependent inhibitors are metabolized to a compound(s) whose inhibitory action are more potent than that of the parent species. For example, the metabolites of amiodarone[13] and diltiazem[14] have been reported to be more potent inhibitors of CYP than their parent compounds. The experimental method employed in the present study enables us to determine whether a drug is metabolized to a metabolite(s) having more potent inhibitory effects than the parent drug, but not to determine whether the inhibition by a drug is reversible or not. Accordingly, further studies are needed to examine the reversibility of the inhibitory action of the herbal medicines examined in this report. These investigations probably require isolation and identification of the component(s) responsible for the inhibitory action of the herbal medicines.

The present study suggests that extracts of several herbal medicines, such as Evodia Fruit, Sappan Wood, Incised Notopterygium Rhizome, Schisandra Fruit, Great Burdock Achene, Angelica Dahurica Root and Rhubarb contain metabolism-dependent inhibitors of CYP, especially CYP3A4. To evaluate the potential risk of these herbal medicines inducing a serious adverse reaction to drugs in humans, it is important to identify components of the extract having metabolism-dependent inhibitory effects. Identification of inhibitory components in Evodia Fruit extract and extracts of other herbal medicines is currently in progress. We then aim to examine the reversibility of CYP inhibition using the isolated components.

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Japanese abstract

26種の生薬のシトクロム P450 3A4 (CYP3A4) 及び 2D6 (CYP2D6) に対する阻害作用を調べた。生薬エキス粉末から調製されたメタノール可溶性画分を NADPH 生成系存在下，ヒト肝ミクロソームとプレインキュベーションした後の CYP3A4 の残存活性 (erythromycin *N*-demethylation 活性) と CYP2D6 の残存活性 (dextromethorphan *O*-demethylation 活性) を測定した。その結果，16種の生薬がCYP3A4活性をプレインキュベーション時間依存的に低下させた。中でも，呉茱萸による CYP3A4 活性の低下作用が最も顕著であった (30分間プレインキュベーションした後の活性の残存率は 22.3%)。次いで，蘇木，羌活，五味子，牛蒡子，白芷，大黄が顕著な低下を示した (30分間プレインキュベーションした後の活性の残存率は，それぞれ 40.6, 41.2, 53.4, 47.1, 53.4, 59.2%)。これら7種生薬による CYP3A4 活性低下作用は，CYP3A4 に対する不可逆的阻害剤である troleandomycin による残存率 (49.4%) に匹敵した。CYP2D6 活性に対してプレインキュベーション時間依存的な活性の低下を示した生薬は，5種であった。最も CYP2D6 活性の低下作用が大きかった生薬は，羌活であり，30分間プレインキュベーションした後の活性の残存率は 61.9% であった。以上の結果から，呉茱萸，蘇木，羌活，五味子，牛蒡子，白芷，大黄等の複数の生薬エキス中に，CYP3A4 に対する metabolism-dependent inhibitor が含まれていることが示唆された。

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