Aims In vitro data indicate that imipramine (IMI), a widely used tricyclic antidepressant drug, is N-demethylated by several isoforms of cytochrome P450, which include CYP3A. The aim of this study was to investigate the role of CYP3A in the in vivo N-demethylation of IMI.

Methods Healthy subjects were given troleandomycin (TAO), a selective inhibitor of CYP3A, 250 mg daily for 2 days before a single oral dose of 100 mg IMI was administered.

Results Pretreatment with TAO significantly increased the AUC of IMI by 59% (1971 ± 938 vs 3134 ± 2000 μg h−1, 95% confidence interval for difference between means: 218 to 2108 μg h−1, P < 0.05) and decreased its oral clearance by 30% (60.9 ± 27.4 vs 42.5 ± 22.7 l h−1, 95% confidence internal for difference between means: 7.2 to 31.7 l h−1, P < 0.05).

Conclusions We conclude that CYP3A may play an important role in the in vivo N-demethylation of IMI.

Keywords: imipramine, desipramine, CYP3A, Troleandomycin

Introduction

Imipramine (IMI) is a widely used tricyclic antidepressant drug [1], which is metabolized mainly by N-demethylation to its active metabolite desipramine (DMI) and by aromatic hydroxylation to 2-hydroxyimipramine and 10-hydroxy-imipramine [1, 2]. DMI is further oxidized to 2-hydroxy-desipramine and 10-hydroxydesipramine [2]. These metabolic pathways are catalyzed by several liver microsomal cytochromes P450. A major problem in the clinical use of this drug is that the large interpatient variations in plasma concentrations of IMI and DMI may cause differences in clinical response and toxicity, particularly cardiotoxicity [1, 3]. The major source of this variation is considered to be the interindividual differences in activities of the cytochromes P450 that catalyze IMI metabolism [4].

Several studies have indicated that the 2-hydroxylation of IMI and DMI cosegregate with polymorphic CYP2D6 activity which is responsible for the oxidation of debrisoquine and sparteine [5, 7, 8]. However, the N-demethylation and 10-hydroxylation of IMI are catalyzed partially by polymorphic N-mephenytoin 4'-hydroxylase (CYP2C19) both in vivo and in vitro [9–11]. Furthermore, the rate of IMI N-demethylation correlated highly with CYP1A2 activity (r = 0.88, P < 0.001) and CYP3A4 activity (r = 0.80, P < 0.02) in human liver microsomal preparations [12], and antibodies to CYP1A2 and CYP3A4 abolished 80% and 60% of N-demethylation activity, respectively [12]. The involvement of CYP1A2 and CYP3A4 has also been confirmed by another report [13]. In vitro studies have shown that fluvoxamine, an inhibitor of both CYP1A2 and CYP2C19, could inhibit IMI N-demethylation, even in poor metabolizers of mephenytoin [14–16].

Troleandomycin is a macrolide antibiotic, which has been shown to inhibit in vitro human CYPs 3A3, 3A4 and 3A5 in human liver microsomes. The activity of seven other CYP forms including CYP1A2 and CYP2C9 [18] was unaffected. Watkins and co-workers have shown that using the erythromycin breath test, CYP3A activity [19] was inhibited by 80% by treatment with 250 mg oral TAO in healthy volunteers [20]. Thus, the aim of this study was to investigate the effect of TAO on the pharmacokinetics of IMI in vivo to assess the role of CYP3A in the N-demethylation of IMI.

Methods

Subjects

Nine male Chinese aged 21–28 years were enrolled into the study. All subjects were healthy as assessed by medical history, physical examination, and routine laboratory tests. None of them smoked tobacco products or received regular medication and all subjects abstained from alcohol and grapefruit juice during the study. The study protocol was approved by the Ethics Committee of Hunan Medical University and informed written consent was obtained from each subject.

Protocol

The study was performed in two randomized phases separated by a washout period of 4 weeks. After an overnight fast, each subject was given 100 mg IMI (Huanghe Pharmaceutical Corp, Shanghai, PR China) orally on two
Table 1 The pharmacokinetic parameters (mean ± s.d.) of IMI and DMI in nine Chinese subjects with and without TAO treatment.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Without TAO</th>
<th>With TAO</th>
<th>95% CI of the mean difference</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>IMI</td>
<td></td>
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<tr>
<td>$t_{1/2}$ (h)</td>
<td>14.0 ± 5.1</td>
<td>18.0 ± 7.1</td>
<td>1.5–6.6</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>AUC ($\mu$g ml$^{-1}$ h)</td>
<td>1971 ± 938</td>
<td>3134 ± 2000</td>
<td>218–2108</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td>CL ($l h^{-1}$)</td>
<td>60.9 ± 27.4</td>
<td>42.8 ± 22.7</td>
<td>7.2–31.7</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td>DMI</td>
<td></td>
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</tr>
<tr>
<td>AUC ($\mu$g ml$^{-1}$ h)</td>
<td>1409 ± 703</td>
<td>962 ± 553</td>
<td>217.4–678.7</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Pharmacokinetic analysis
The elimination half-life ($t_{1/2}$) of IMI was calculated from least squares regression of the log plasma drug concentration versus time profile. The area under the concentration-time curve (AUC) was calculated using the log linear trapezoidal rule and extrapolated to time infinity (AUC) using the elimination rate constant ($k_{e}$). The apparent oral clearance (CL$_{o}$) was calculated by dividing the dose by AUC.

Statistical analysis
Data are expressed as mean ± s.d. with 95% confidence intervals (CI) for the mean differences. Statistical evaluation was performed using paired t-test. A P value less than 0.05 was required for rejection of the null hypothesis.

Results
Table 1 summarizes the pharmacokinetics of IMI and its N-demethylation metabolite DMI without and with TAO pretreatment in nine subjects. Figure 1a shows the corresponding plasma concentration-time profiles. Pretreatment with TAO significantly increased the AUC of IMI by 59% (1971 ± 938 vs 3134 ± 2000 $\mu$g $l^{-1}$ h, P < 0.05), prolonged...
is its elimination $t_{1/2}$ by 29\% ($14.0 \pm 5.1$ vs $18.0 \pm 7.1$ h, $P < 0.005$), and decreased its CL, by 30\% ($60.9 \pm 27.4$ vs $42.5 \pm 22.7$ l h$^{-1}$, $P < 0.005$). Figure 1b illustrates the plasma DMI concentration-time profile with or without TAO pretreatment. TAO decreased the AUC of DMI by 22\% ($14.0 \pm 7.0$ vs $962 \pm 553$ g l$^{-1}$ h, $P < 0.001$) and the amount of DMI excreted in 0–48 h urine ($0.91 \pm 0.74$ vs $0.41 \pm 0.26$ mg, $P < 0.05$).

The combination of TAO and IMI was well tolerated by the subjects, all of whom completed the study protocol. Slight gastrointestinal symptoms including nausea and gastric irritation were experienced by one subject 1 h after IMI administration alone and by two subjects 3 h after TAO and IMI co-administration. Drowsiness was experienced by all subjects.

Discussion
The present study examined the effects of TAO on the pharmacokinetics of single dose IMI and its active metabolite DMI. TAO significantly increased the AUC of IMI, decreased its oral clearance and decreased the AUC of DMI. Previous in vitro studies revealed that CYP1A2 and CYP3A4 are the major CYPs responsible for the N-demethylation of IMI, while the contribution of CYP2C to this metabolic route appears to be minor [12]. Since TAO is a selective inhibitor of CYP3A [18], the inhibitory effects of TAO on the N-demethylation of IMI to DMI observed in the present study suggested that CYP3A may play an important role in the in vivo N-demethylation of IMI.

Previous studies have shown that TAO inhibits the metabolism of a variety of drugs including carbamazepine, theophylline, methylphenidate, tizanidine and erythromycin [19, 22–26]. All these drugs are substrates of CYP3A. Such inhibitory effects are probably caused in part by the in vitro formation of an inactive cytochrome CYP-TAO metabolite complex [27, 28], which may also be the mechanism of inhibition by TAO of IMI metabolism.

In our study IMI showed a 6.2-fold variation in AUC which is similar to that reported by others [5]. This variation is likely to be due not only to the differing contributions of the polymorphic CYP 2D6 and 2C19, but also to those of CYP 1A2 and 3A4 whose content in the liver varies 10-fold between individuals [30].

Our data were similar to those reported previously in Chinese subjects taking the same dose of IMI [15]. However, the mean AUC and CL, value for IMI were somewhat higher and lower respectively than those in Caucasians [9–11]. It is well known that Asian patients are often intolerant to doses of IMI commonly used in Caucasian patients [31]. Intercultural differences in drug metabolism have been reported in a number of studies [32–34] and may result from variations in genetic background. For example, the prevalence of poor metabolizers of S-mephentoin 4’-hydroxylation is 2–5% in Caucasians but 13–23% in Chinese populations [35]. Recent studies reported a higher AUC and half-life for oral nifedipine, a substrate of CYP3A4 in South Asians than in Caucasians, suggesting that liver CYP3A4 might be expressed at a lower level in the former ethnic group [36, 37].

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References