Isoniazid is a mechanism-based inhibitor of cytochrome \( P_{450} 1A2, 2A6, 2C19 \) and 3A4 isoforms in human liver microsomes

Abstract Objective: In order to evaluate the inhibitory effects of isoniazid on cytochrome \( P_{450} \) (CYP) mediated drug metabolism, the in vitro inhibitory potency and specificity as well as the reduced nicotinamide adenine dinucleotide phosphate (NADPH)-, time- and concentration dependency of isoniazid as an inhibitor of the activity of the major human CYP isoforms were studied.

Methods: Using pooled human liver microsomes, the in vitro inhibitory effects of isoniazid on CYP1A2 (phenacetin \( O\)-deethylation), CYP2A6 (coumarin 7-hydroxylation), CYP2C9 (tolbutamide hydroxylation), CYP2C19 (S-mephenytoin 4'-hydroxylation), CYP2D6 (dextromethorphan \( O\)-demethylation), CYP2E1 (chlorzoxazone 6-hydroxylation) and CYP3A4 (midazolam 1'-hydroxylation) activities were examined.

Results: After a 15-min preincubation without NADPH, isoniazid reversibly inhibited microsomal CYP2C19- and CYP3A4-mediated reactions with apparent \( K_i \) values of 36 \( \mu M \) and 73 \( \mu M \), respectively. However, isoniazid had only weak inhibitory effects on the five other CYP-mediated reactions \( (K_i > 110 \mu M) \). After a 15-min preincubation with NADPH, isoniazid showed an increased inhibitory potency toward CYP1A2, CYP2A6, CYP2C19 and CYP3A4 activities \( (K_i = 56, 60, 10 \text{ and } 36 \mu M, \text{ respectively}) \). In addition, the inactivation of CYP1A2, CYP2A6, CYP2C19 and CYP3A4 by isoniazid was NADPH-, time- and concentration dependent, and was characterised by \( K_{inact} \) values of 0.11, 0.13, 0.09 and 0.08 min\(^{-1}\), and \( K_i \) values of 285, 173, 112 and 228 \( \mu M \), respectively.

Conclusions: As the peak plasma concentrations of isoniazid are around 30–50 \( \mu M \), isoniazid at clinically relevant concentrations reversibly inhibits CYP2C19 and CYP3A4 activities, and mechanistically inactivates CYP1A2, CYP2A6, CYP2C19 and CYP3A4 in human liver microsomes. Co-administration of isoniazid and drugs that are primarily metabolised by these CYP isoforms, particularly by CYP2C19 and CYP3A4, may result in significant drug interactions.

Keywords: Cytochrome \( P_{450} \); Inhibition; Interaction; Isoniazid

Introduction

Isoniazid is widely used in the treatment and prophylaxis of tuberculosis. The major metabolic pathway of isoniazid in man is acetylation [1], but cytochrome \( P_{450} \) (CYP) enzymes may also contribute to the metabolism of isoniazid [2]. Isoniazid is thought to be an inhibitor of CYP enzymes [3] because it has been reported to decrease the clearance of a number of therapeutic agents such as phenytoin [4, 5], theophylline [6, 7], diazepam [8], triazolam [9], paracetamol [10, 11], chlorzoxazone [12], carbamazepine [13], vincristine [14] and disulfiram [15]. These drugs are substrates of CYP2C9, CYP2C19, CYP1A2, CYP2E1 or CYP3A4 [16].

Studies of rat liver microsomes suggest that isoniazid may be a mechanism-based inhibitor of CYP enzymes [17]. However, there have been few in vitro studies in which the inhibitory effects of isoniazid on human CYP activities were investigated and it is not known whether the inhibition is NADPH- (reduced nicotinamide adenine dinucleotide phosphate), time- and concentration dependent, which are key features of mechanism-based inactivation. Therefore, we examined the in vitro inhibitory potency and specificity as well as the NADPH-, time- and concentration dependency of isoniazid for the major human CYP isoforms in pooled human liver microsomes using selective catalytic marker reactions for CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4.
Materials and methods

Materials

Isoniazid (isonicotinic acid hydrazide), phenacetin, paracetamol, coumarin, 7-hydroxycoumarin, tolbutamide, chloroxazone and NADPH were purchased from Sigma Chemical Co. (St. Louis, Mo.). Hydroxytolbutamide, 6-hydroxychloroxazone, 5-mephenytoin and 4'-hydroxymephenytoin were purchased from Ultratine Chemicals (Manchester, UK). Dextromethorphan and dextrophan were obtained from Orion Pharma (Espoo, Finland). Midazolam and 1'-hydroxymidazolam were kindly provided by Hoffmann-La Roche (Basel, Switzerland). Pooled human liver microsomes (prepared from five male and five female human liver microsomal samples), known to contain high levels of CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4, were obtained from Gentest Corp. (Woburn, Mass.). The liver samples have been obtained from several organ procurement organisations, which collect tissues in accordance with all pertinent regulations and obtain permission from the donors' families prior to organ collection. The procedures of these organisations have all been reviewed and approved by their respective institutional Human Subjects Committee. Other chemicals and reagents were obtained from Merck (Darmstadt, Germany).

Effects of isoniazid on CYP activities

The microsomal incubation conditions used to study the activities of CYP1A2 (phenacetin O-deethylation), CYP2A6 (coumarin 7-hydroxylation), CYP2C9 (tolbutamide hydroxylation), CYP2C19 (5-mephenytoin 4'-hydroxylation), CYP2D6 (dextromethorphan O-deethylation), CYP2E1 (chloroxazone 6-hydroxylation) and CYP3A4 (midazolam 1'-hydroxylation) have been described previously [18]. The time of incubation and concentration of microsomal protein (100 μg ml⁻¹) for each assay were determined to be in the linear range for the rate of metabolite formation.

All incubations were performed in duplicate and the mean values were used. A difference of less than 10% between the duplicate assays was used as the acceptance criterion. Briefly, each incubation was performed with 20 μl pooled human microsomal protein in an incubation medium containing 0.1 M sodium phosphate buffer (pH 7.4) and 5 mM MgCl₂. The final incubation volume was 0.2 ml. Isoniazid [0-250 μM], prepared in water with the minimal use of methanol (0.2%, v/v) was preincubated with the incubation medium at 37°C for 15 min, either in the presence or absence of 1.0 mM NADPH (in studies with phenacetin O-deethylation, coumarin 7-hydroxylation, 5-mephenytoin 4'-hydroxylation and midazolam 1'-hydroxylation, isoniazid was preincubated with the incubation medium for 0, 2, 5, 10 and 15 min in the presence of 1.0 mM NADPH before adding corresponding marker substrates). An equal volume of methanol (0.2%, v/v) was added to the non-inhibitor controls. After the preincubation, typical substrates were added either with or without 1.0 mM NADPH for measurements of the corresponding marker activities. The substrate stock solutions were prepared in different solvents as described in a previous study [18], and the final organic solvent content in each sample did not exceed 1% (v/v). After incubation at 37°C for a specific period of time, the reaction was quenched by adding an appropriate chemical to precipitate the proteins as described previously [18]. The incubation mixtures were then centrifuged for 5 min at 10,000 g. An aliquot of the supernatant fraction was subjected to analysis using high-performance liquid chromatography (HPLC).

HPLC analysis

Assays for the respective products of CYP marker reactions were carried out using HPLC [18]. The HPLC system consisted of a Pharmacia LKB 2150 pump, a Hewlett-Packard 1050 autosampler, a Hewlett-Packard 3396 integrator, a SPD-10A V Shimadzu UV detector (for analysis of CYP1A2, CYP2C9, CYP2C19, CYP2E1 and CYP3A4 activities) or a RF-551 Shimadzu fluorescence detector (for analysis of CYP2A6 and CYP2D6 activities). The intra- and inter-day coefficients of variation for all assays were less than 7% at relevant concentrations (n = 6).

Data analysis

The apparent inhibitory constant (KI) values were calculated using non-linear regression analysis with Systat for Windows 6.0.1 (SPSS Inc., Chicago, Ill.). Different models of enzyme inhibition, i.e. competitive, noncompetitive, uncompetitive and mixed-type inhibition, were fitted to the kinetic data [19]. An assessment of goodness of fit of the models was made using the size of the residual sum of squares and the random distribution of the residuals, the standard error and the 95% confidence interval of the parameter estimates. When necessary, an F test was performed to determine whether there was a significant difference in the size of the residual sum of squares between models [20]. In the case of preincubation (time)-dependent CYP inactivation, the apparent half-life for inactivation (t₁/₂) was estimated from linear regression analysis of the natural logarithm of residual enzyme activity against preincubation time. The concentration required for half-maximal inactivation (Kᵢ) and the maximal rate of inactivation at saturation (Kᵢmax) were estimated by fitting data to the following equation [21]:

\[ t_{1/2} = \frac{0.693(1+K_{i}/I)}{K_{i max}} \]

where I is the concentration of inactivator.

Results

After a 15-min preincubation without NADPH, isoniazid preferentially inhibited CYP2C19- and CYP3A4-mediated reactions (Fig. 1a, Table 1). The double-reciprocal plots and Dixon plots consistently indicated that isoniazid competitively inhibited CYP2C19 activity with a Kᵢ of 36 μM and uncompetitively inhibited CYP3A4 with a Kᵢ value of 73 μM (Fig. 2, Fig. 3). With concentrations ranging from 10 μM to 100 μM, isoniazid showed no remarkable inhibitory effect on CYP1A2, CYP2A6, CYP2C9, CYP2D6 and CYP2E1 activities (Fig. 1a). However, with concentrations higher than 100 μM, isoniazid exhibited weak reversible inhibitory effects on these five CYP isoformal activities (Table 1). The estimated Kᵢ values and the types of inhibition are given in Table 1.

After a 15-min preincubation with NADPH, isoniazid showed an increased inhibitory potency toward CYP1A2, CYP2A6, CYP2C19 and CYP3A4 activities (Fig. 1b, Table 1). The double-reciprocal plots and Dixon plots of isoniazid for CYP2C19 and CYP3A4 activities after a 15-min preincubation with or without NADPH are presented in Fig. 2 and Fig. 3. The estimated Kᵢ values of isoniazid for CYP1A2, CYP2A6, CYP2C19 and CYP3A4 activities were markedly lower in the presence of NADPH than in the absence of NADPH (Table 1). In addition, the inactivation of these CYP isoformal by isoniazid was found to be preincubation time- and concentration dependent (Fig. 4). The Kᵢmax and Kᵢ values are given in Table 2.