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Pharmacokinetic Interactions Between Isoniazid and Theophylline in Rats

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Abstract—Pharmacokinetic interactions between isoniazid and theophylline were studied in male Wistar rats, 206 ± 17 g. Concomitant oral administration of 2 × 5 mg kg^{-1} isoniazid accelerated slightly the disposition of theophylline (10 mg kg^{-1}, i.v.) whereas 2 × 25 mg kg^{-1} isoniazid slowed it marginally. The differences in distribution volume, systemic clearance and area under the concentration–time curve (AUC) between the high and the low dose, however, were statistically significant. One week pretreatment with 10 mg kg^{-1} isoniazid tended towards inhibition (significant decrease of systemic clearance, increase of AUC) and 50 mg kg^{-1} to acceleration (decrease of half-life, mean residence time and AUC, increase of systemic clearance) of theophylline disposition. After oral pretreatment with 20 mg kg^{-1} theophylline, neither the kinetics of free isoniazid (50 mg kg^{-1}, i.v.) and the amount acetylated nor the acetylation indices differed from the controls. There was no evidence that concomitant or subacute administration of different doses of isoniazid affects major metabolic pathways of theophylline or that prolonged theophylline treatment interacts with the *N*-acetylation capacity.

The bronchospasmolytic drug theophylline is characterized by a narrow therapeutic range, high interindividual variation in plasma concentration, and often unpredictable clearance (Hendeles & Weinberger 1983; Stavric 1988). Plasma concentrations below 55 μmol L^{-1} may be associated with inadequate therapy while those above 110 μmol L^{-1} potentially produce serious side-effects, mainly of the gastrointestinal, cardiovascular and central nervous sytems. Theophylline is thought to be primarily oxidized by two isozymes (cytochrome P450IA and P450IIB) of the hepatic microsomal mono-oxygenase system via *N*-demethylation and 8-hydroxylation pathways (Williams et al 1979; McManus et al 1988; Matthew & Houston 1990a, b). The differences in plasma levels of theophylline in man are mainly caused by the metabolic variability of these enzymes which depends on the subject's exposure to agents with inducing or inhibiting properties (Hendeles & Weinberger 1983; Upton 1991a, b).

Isoniazid is a widely used antituberculous drug. Its biotransformation in man involves polymorphic *N*-acetylation and an oxidative toxifying pathway (Weber & Hein 1979; Timbrell et al 1980; Iwainsky 1988). Isoniazid has been found to influence the activity of hepatic microsomal monooxygenases depending on the duration of treatment. It is an inhibitor during acute adminstration (Brodie et al 1981; Muakkassah et al 1981; Grech-Belanger et al 1983) and a novel type of inductor of cytochrome P450IIE after prolonged treatment (Rice & Talcott 1979; Gadeholt 1984; Ryan et al 1985). In view of this, the need to look for new drug interactions with isoniazid has been stressed by Baciewicz & Self (1985). Whether the impact of isoniazid on the monooxygenase system is relevant for the metabolism of theophylline is not yet fully understood. Studies so far conducted in man on isoniazid–theophylline kinetic interactions has shown conflicting results (Thompson et al 1982; Höglund et al 1987; Samigun et al 1990). In the reviews of Baciewicz & Self (1985) and Jonkmann & Upton (1984), further controlled studies have been recommended.

The purpose of this investigation was to evaluate how concomitant administration and subacute pretreatment of isoniazid effect the metabolic rate of theophylline which has been used as a probe to study functions of oxidative drug metabolism. Further, the possible effect of theophylline pretreatment on isoniazid disposition was also considered. Isoniazid was given in doses of 10 and 50 mg kg^{-1}. The lower dose is microbiologically active in man whereas the higher one is optimal for microsomal enzyme induction in rats (Bartmann 1988; Hoffmann & Pankow 1991).

Materials and Methods

Animals

Male Wistar rats, 206 ± 17 g (Schönwalde, Berlin), were kept under standard conditions (6 rats to a plastic cage) with free access to standard food and tap water. Animals were allowed to adapt to the conditions of the animal house for at least 14 days and randomized to 8 treatment groups (24 rats per group).

Study design

Theophylline kinetics were determined in the presence of 2 × 5 and 2 × 25 mg kg^{-1} isoniazid (Serva Feinbiochemica, Heidelberg, Germany) given orally immediately before and 4 h after intravenous administration of 10 mg kg^{-1} theophylline (Arzneimittelwerk, Dresden, Germany). To assess the effects of subacute isoniazid and theophylline pretreatment on each other's disposition, animals received either 0·9% NaCl solution (controls), 10 or 50 mg kg^{-1} isoniazid, or 20 mg kg^{-1} theophylline orally for 7 days (administration volume, 5 mL kg^{-1}). The kinetics were then determined 24 h after the last dosing by injecting 10 mg kg^{-1} theophylline or 50 mg kg^{-1} isoniazid (injection volume, 1 mL kg^{-1}) into the

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tail vein of the rats of the corresponding control and treatment groups. The following techniques were employed for drug administration and blood sampling. The respective test drugs were administered consecutively to the 24 rats belonging to one kinetic group at intervals of 1 min. Blood samples (about 1·5 mL) were drawn from the retro-orbital plexus using heparinized capillaries 30, 60, 90, 120, 240, 360, 480 and 600 min for theophylline and 15, 30, 45, 60, 80, 100, 120 and 180 min for isoniazid after drug administration. For blood sampling, generally 8 rats were punctured at any specific kinetic time point. None of the animals was punctured more than 3 times and the interval between two consecutive samplings was at least 50 min. One concentration time curve of the respective drug studied was constructed with blood samples of three rats which were randomly assigned before the beginning of the pharmacokinetic trial. Blood collection and intravenous administration were all performed under light ether anaesthesia. Serum samples were stored at −20°C until drug analysis.

Drug analysis

Theophylline serum concentrations were assayed by HPLC (Hewlett Packard, model 1084 B, USA, equipped with a variable UV-VIS detector set at 274 nm, column: 4·6 mm i.d. × 20 cm filled with 10 mm Lichrosorb RP-8 and temperature and flow rate set to 35°C and 1·6 mL min^{-1}, respectively). Serum standards or samples (200 μL) were mixed with 50 μL internal standard (100 μg mL^{-1} 8-methylcaffeine) and extracted twice with 3 mL acetylacetate. The pooled organic layers were evaporated to dryness and redissolved in 100 μL mobile phase (0·02 M KH_2PO_4, pH 4·0, and 25% methanol), 15 μL of which were injected into the HPLC. Quality controls for the method were: retention times (min): 3·23 for theophylline, 4·55 for caffeine and 6·35 for 8-methylcaffeine; linearity: between 2·52 and 257·5 μmol L^{-1} for theophylline and caffeine; recovery: 93% for theophylline, 86·5% for caffeine and 87·4% for the internal standard; and coefficient of variance: 1·6% for chromatographic, 5·7% for between measurements and 2·2% for day-to-day differences. Quantitative evaluation was made by using peak areas of the test drugs and internal standard for calculation.

Serum concentrations of free and total isoniazid were measured photometrically after formation of hydrazones with vanillin and *p*-dimethylaminobenzaldehyde, respectively (Maher et al 1957).

Statistical and pharmacokinetic analysis

Serum concentration-time profiles were constructed from blood samples of three rats together which were randomly assigned before the beginning of the study. Pharmacokinetic evaluation was made by fitting serum concentration–time data of the terminal slope by least square analysis to give elimination constants and half-lives. Areas under the curves (AUC) and areas under the moment curves (AUMC) were calculated by the trapezoidal rule and extrapolated to infinity. Mean residence times (MRT), relative clearances (CL) and volumes of distribution (Vd) were derived accordingly. A computer program was used for the kinetic analysis (Schiff 1985). Acetyl-isoniazid was the difference between total and free isoniazid. Means and standard deviations are given. For statistical evaluation, Wilcoxon's rank test was used with $P < 0.05$ as the level of significance.

Results

The influence of concomitant administration of isoniazid on theophylline disposition is demonstrated in Fig. 1 and Table 1. Both premedication schedules affected the kinetics of theophylline only slightly. Theophylline systemic clearance tended to be increased when 2 × 5 mg isoniazid was given concomitantly and was slightly reduced during 2 × 25 mg coadministration. However, differences of Vd, CL and AUC only reached the level of significance if the lower and the higher dose were compared.

In Fig. 2 and Table 2, the effect of one-week pretreatment with 10 and 50 mg kg^{-1} isoniazid, respectively, on theophylline kinetic profiles and parameters are shown. The lower dose of isoniazid increased the AUC values and decreased Vd and CL significantly. Half-lives remained unchanged. The higher dose of isoniazid, on the other hand, tended towards acceleration of theophylline elimination in which half-lives and MRT were decreased by about 1 h and CL values were increased by about 30%, without any change in Vd.

One-week premedication with 20 mg kg^{-1} theophylline neither altered the kinetics of free isoniazid nor of acetyl-

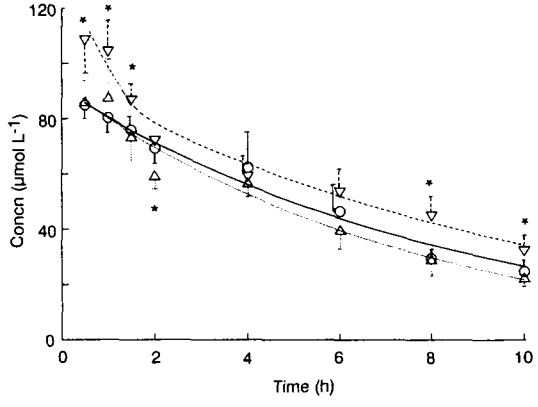

FIG. 1. Serum concentration-time profiles of theophylline (10 mg kg^{-1}, i.v.) in rats after concomitant oral administration of 2 × 5 (−△−) and 2 × 25 (···▽···) mg kg^{-1} isoniazid. *$P < 0.05$. Control −○−.

Table 1. Pharmacokinetic parameters of theophylline (10 mg kg^{-1}, i.v.) in rats during concomitant oral administration of 2 × 5 and 2 × 25 mg kg^{-1} isoniazid.

			Isoniazid premedication	
Parameters		Control	2 × 5 mg kg^{-1}	2 × 25 mg kg^{-1}
$t_{\frac{1}{2}}$	(h)	5·56 ± 0·78	4·90 ± 0·32	5·44 ± 0·78
Vd	(L kg^{-1})	0·55 ± 0·02	0·56 ± 0·04	0·47 ± 0·04*†
CL	(mL min^{-1} kg)	1·16 ± 0·17	1·32 ± 0·10	1·00 ± 0·09†
AUC	(mg h L^{-1})	142 ± 24	127 ± 10	168 ± 14†
MRT	(h)	7·97 ± 1·07	7·05 ± 0·45	8·00 ± 1·08

*$P < 0.01$ compared with controls. †$P < 0.01$ compared with 2 × 5 mg kg^{-1}.

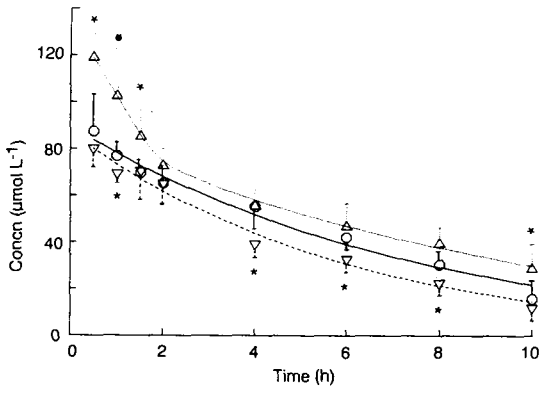

FIG. 2. Serum concentration-time profiles of theophylline (10 mg kg^{-1}, i.v.) in rats after oral pretreatment with 10 (—△—) and 50 (···▽···) mg kg^{-1} isoniazid for 7 days. *$P<0.05$. Control —○—.

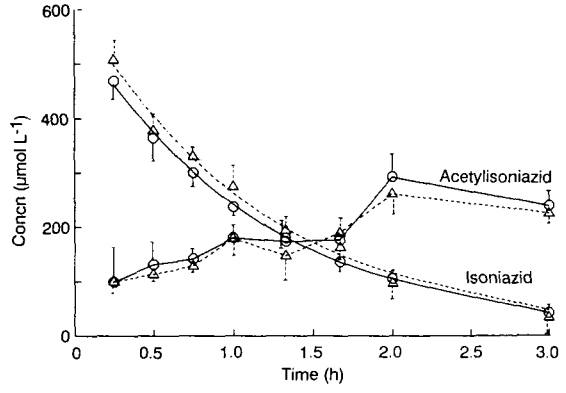

FIG. 3. Serum concentration-time profiles of isoniazid (50 mg kg^{-1}, i.v.) and acetylisoniazid in rats after oral pretreatment with 20 (···△···) mg kg^{-1} theophylline for 7 days. Control —○—.

Table 2. Pharmacokinetic parameters of theophylline (10 mg kg^{-1}, i.v.) in rats after oral premedication with 10 or 50 mg kg^{-1} isoniazid for 7 days.

Parameters		Controls	Isoniazid premedication 2 × 5 mg kg^{-1}	Isoniazid premedication 2 × 5 mg kg^{-1}
$t_{\frac{1}{2}}$	(h)	4·75 ± 0·45	4·75 ± 0·97	3·85 ± 0·43‡
Vd	(L kg^{-1})	0·55 ± 0·04	0·46 ± 0·05*	0·58 ± 0·06†
CL	(mL min^{-1} kg^{-1})	1·35 ± 0·09	1·14 ± 0·16*	1·75 ± 0·17*†
AUC	(mg h L^{-1})	124 ± 8	149 ± 20*	96 ± 9*†
MRT	(h)	6·83 ± 0·59	6·95 ± 1·28	5·54 ± 0·66*‡

*$P<0.01$ compared with controls. †$P<0.01$, ‡$P<0.05$ compared with 10 mg kg^{-1}.

Table 3. Pharmacokinetic parameters of isoniazid (50 mg kg^{-1}, i.v.) in rats after oral pretreatment with 20 mg kg^{-1} theophylline for 7 days.

Parameters		Control	Theophylline
$t_{\frac{1}{2}}$	(h)	0·74 ± 0·07	0·74 ± 0·07
Vd	(L kg^{-1})	0·63 ± 0·05	0·59 ± 0·04
CL	(mL min^{-1} kg)	9·83 ± 0·26	9·57 ± 1·76
AUC	(mg h L^{-1})	84·9 ± 2·2	91·7 ± 8·4
MRT	(h)	1·16 ± 0·08	1·14 ± 0·08

isoniazid (Fig. 3, Table 3). The acetylation indices (ratio of acetyl-isoniazid to total isoniazid) were not influenced by theophylline.

Discussion

Isoniazid is said to inhibit oxidative metabolism of a coadministered drug. Isoniazid was found to reduce the availability of functional cytochrome P450 in-vitro by binding transiently to the iron ligand and thereby forming an abortive complex (Muakkassah et al 1981, 1982). In man, isoniazid depressed the elimination of antipyrine (Brodie et al 1981; Grech-Belanger et al 1983) and several other drugs (Baciewicz & Self 1985) as well as endogenous substances (Brodie et al 1981; Parthe & Hagmann 1990).

In the present work, the effects of concomitant administration of isoniazid on theophylline systemic clearance were generally negligible and did not reach levels of statistical significance. These small changes, however, indicate that isoniazid only mariginally influences the well documented cytochrome P450-dependent oxidative pathways of theophylline (McManus et al 1988; Matthew & Houston 1990a, b).

Subacute pretreatment with isoniazid, on the other hand, influenced theophylline elimination in a different manner, even though these changes must be viewed as less pronounced; 10 mg kg^{-1} (corresponding to the microbiologically active dose in man (Bartmann 1988)) moderately inhibited theophylline elimination while 50 mg kg^{-1} (optimal for microsomal enzyme induction in rats (Pankow & Hoffmann 1989)), slightly enhanced it. Isoniazid hepatic microsomal enzyme induction after repeated treatment and its role in the bioactivation of several compounds could be confirmed. In contrast to the general argument that isoniazid acts as a potent inhibitor of drug oxidation mainly when given concomitantly, selective inhibition was also reported after prolonged treatment in rodents (Rice & Talcott 1979; Powell-Jackson et al 1982). It seems likely that neither mechanism of isoniazid would affect the major metabolic pathways of theophylline. When these changes are compared with theophylline clearance after phenobarbitone or polycyclic aromatic hydrocarbon pretreatment (Williams et al 1979; Matthew & Houston 1990a, b), the kinetic changes observed in the present study are too low to relate the results to any meaningful induction or inhibition of theophylline metabolism by isoniazid. Moreover, isoniazid-inducible cytochrome P450 (P450IIE1) has been shown to differ in many respects from cytochrome P450IIB and cytochrome P450IA (Rice & Talcott 1979; Gadeholt 1984; Ryan et al 1985; Hoffmann & Pankow 1991), the main isozymes thought to catalyse theophylline metabolism.

No comparative animal study is available for isoniazid and theophylline. In man, information is not unequivocal; some workers reported a significant decrease of theophylline clearance after one week pretreatment with therapeutic doses of isoniazid (Höglund et al 1987; Samigun et al 1990; Dal Negro et al 1988; Torreni et al 1989), while others obtained

contrary results (Thompson et al 1982). Further controlled studies were recommended to re-evaluate isoniazid and theophylline kinetic interactions (Jonkmann & Upton 1984; Baciewicz & Self 1985). However, direct comparison of these reports on man with animal data is difficult for a number of reasons. On the one hand, different designs of clinical studies make it difficult to differentiate whether the effect is due to repeated or concomitant intake. On the other hand, unlike the controlled animal studies, deviation of hepatic drug oxidation capacity from basal activities is likely in man and this is important since the effect of isoniazid on this system as well as the metabolism of theophylline may be influenced accordingly (Perry & Jenkins 1986; Dal Negro et al 1988). In conclusion, meaningful kinetic consequences of theophylline may not be expected as a result of isoniazid cotherapy under basal conditions compared with other important theophylline drug interactions listed in the literature (Upton 1991a, b).

The *N*-acetyltransferase of the liver catalyses the genetically controlled *N*-acetylation process of several drugs including isoniazid (Evans 1989). Apart from the genetic control, several factors modify the metabolic capacity of this pathway such as glucose loading, alcohol consumption or hydrocortisone pretreatment (Evans 1989). In this connection, the clinical pharmacokinetics of isoniazid is altered by a number of confounding factors (Weber & Hein 1979; Holdiness 1984). In the present study, it was assumed that theophylline with its multiple effects at the cellular level (Rall 1990) and interference at other sites such as further oxidative pathways of isoniazid (Höglund et al 1987) may alter one of these variables and hence the rate of acetylation. The present results, in which theophylline pretreatment for a week hardly influenced the disposition of free isoniazid and its acetylated metabolite, do not provide any evidence to support this hypothesis. A single study which addressed the problem under question failed to show a significant increase of isoniazid serum concentrations during theophylline treatment in volunteers (Höglund et al 1987).

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