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Inhibition of the metabolism of paracetamol by isoniazid

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1 The effect of isoniazid given daily for 7 days on paracetamol (acetaminophen) kinetics and metabolism was studied in 10 healthy volunteers. Paracetamol, 500 mg, was given before isoniazid, on day 7 of isoniazid administration, and 2 days after the last dose of isoniazid.
2 On day 7, isoniazid markedly inhibited the formation clearance of the glutathione and catechol metabolites by 69.7% and 62.2%, respectively. Total paracetamol clearance was lowered by 15.2%. There was no effect of isoniazid on the non-oxidative pathways of paracetamol elimination.
3 Two days after isoniazid was discontinued, paracetamol metabolism had returned to pre-isoniazid values.

Keywords isoniazid paracetamol interaction metabolism inhibitor

Introduction

Both paracetamol (Black, 1984; Hinson, 1980; Mitchell & Jollow, 1975; Prescott, 1983) and isoniazid (Mitchell *et al.*, 1976; Timbrell, 1979) can cause hepatotoxicity. Isoniazid causes liver necrosis in certain individuals at therapeutic doses and paracetamol is heptatotoxic in overdose. These two medications are likely to be taken concurrently. In the only previous study (Ochs *et al.*, 1984) isoniazid did not significantly affect the total clearance of paracetamol in 11 volunteers.

The known metabolic pathways of paracetamol are shown in Figure 1. The predominant pathways of paracetamol biotransformation are conjugation to form phenolic, glucuronide and sulphate metabolites, and microsomal oxidative pathways of metabolism (catalysed by cytochromes P-450) that lead to both catechol and glutathione metabolites (Andrews *et al.*, 1976; Black, 1984; Hinson, 1980; Mitchell *et al.*, 1974; Prescott, 1983; Slattery *et al.*, 1987). With large doses of paracetamol the conjugation pathways become saturated and a larger percentage of paracetamol is cleared through the oxidative pathways (Davis *et al.*, 1976; Mitchell *et al.*, 1974; Prescott, 1983; Slattery & Levy, 1979; Slattery *et al.*, 1987).

The putative toxic intermediate formed by cytochromes P-450 oxidation is *N*-acetyl-*p*-benzoquinone imine (NAPQI) which causes cell damage when glutathione is depleted (Albano *et al.*, 1985; Dahlin *et al.*, 1984). Hepatic microsomal cytochrome P450IIE1 (P-450j) has been shown to catalyse the formation of NAPQI and is induced by ethanol and isoniazid (Morgan *et al.*, 1983; Raucy *et al.*, 1989; Ryan *et al.*, 1986). Furthermore, pretreatment of Long Evans rats with isoniazid increased the paracetamol-induced hepatic necrosis score by 85% (Kalhorn, Slattery & Nelson, unpublished observations). It also is known that isoniazid can inhibit the microsomal oxidative metabolism of some drugs (Kutt *et al.*, 1970; Muakkassah *et al.*, 1981; Wright *et al.*, 1982). This study was designed to deter-

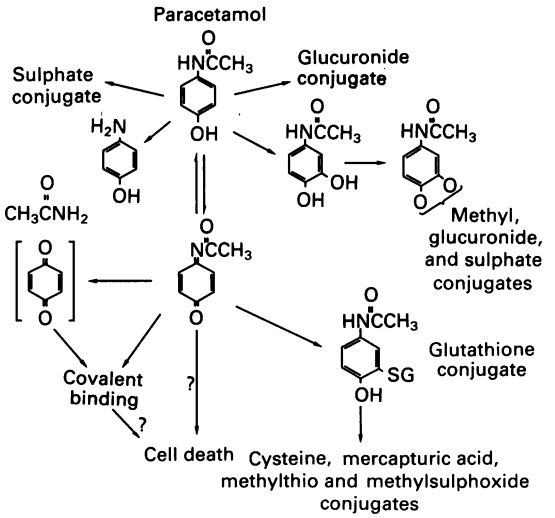

Figure 1 Pathways of metabolism of paracetamol. Sulphate and glucuronide conjugation are the two major routes of metabolism of paracetamol. The two oxidative pathways leading to the catechol and glutathione metabolites are quantitatively less important.

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mine the potential for isoniazid to induce the oxidative pathways of paracetamol in man, as well as to test the possibility that isoniazid inhibits paracetamol metabolism.

Methods

Volunteers

All subjects were healthy volunteers who gave written informed consent to the study which was approved by the Clinical Screening Committee for Human Experimentation, University of British Columbia. The study was limited to subjects within 15% of ideal body weight who were non-smokers and non-drinkers or who drank alcohol infrequently. Subjects were required to refrain from taking any medication or alcohol during the study and for 1 week prior to the study.

Protocol

Acetylator phenotype Sulphamethazine (10–12 mg kg^{-1}) was given orally to each of the ten fasted subjects followed by collection of a 6 h urine sample and a 6 h serum sample.

The urine was collected and stored at −20° C until the samples were analysed. Urine (1 ml) was diluted with water (9 ml) and the solution was filtered through a 0.45 μm filter. The plasma (100 μl) was mixed with acetonitrile (100 μl). After 2 min, 200 μl of water was added and the mixture was centrifuged in an Eppendorf centrifuge for 2 min. The supernatant (25 μl) was used as the sample in the subsequent h.p.l.c. analysis.

The samples were analysed by h.p.l.c. (Nouws *et al.*, 1985) by comparison with the standard solutions of equimolar sulphamethazine and acetylated sulphamethazine.

Calculations of the ratio of the concentration of the acetylated compound to those of the parent and acetylated drug were done for urine and plasma samples. Subjects were classified as slow acetylators if the ratio was less than 0.7 and 0.3 in the urine and plasma respectively.

Paracetamol-isoniazid interaction Isoniazid (300 mg) daily was administered for 7 days. Paracetamol (500 mg) was given on three occasions: the day prior to starting isoniazid (day 0), on the 7th day of isoniazid administration (day 7) and 48 h after the last dose of isoniazid (day 9).

The subjects fasted overnight and emptied their bladders just prior to taking paracetamol (500 mg with 200 ml of water) at approximately 08.00 h. A heparin lock was inserted into a forearm vein to take blood samples, 5 ml over sodium EDTA at 0, 1, 2, 3, 4, 5, 6, 8, 10 and 12 h after ingestion of paracetamol. Subjects continued to fast for a further 1.5 h. In addition a 24 h urine sample, collected over 3 g of ascorbic acid and maintained at 4° C, was collected on days 0, 6, 7 and 9. Sample volumes were measured and the samples were divided and stored at −70° C until analysis. Blood samples were taken for measurement of complete blood count, creatinine, aspartate amino transferase, alanine amino transferase, γ-glutamyl transferase, and alkaline phosphatase, on days 0 and 7. Twenty-four hour urinary creatinine excretion was measured.

Blood samples for the measurement of paracetamol were centrifuged and the plasma was frozen at −70° C until assay. Paracetamol and metabolites in urine and plasma were assayed by an h.p.l.c. method (Wilson *et al.*, 1982). The relative standard deviation of the method for the different compounds ranged from 2.2 to 4.9%.

The plasma clearance (CL) of paracetamol was calculated from the dose and area under the plasma concentration curve (AUC) assuming complete oral absorption. The formation clearances of the various metabolites and the renal clearance of paracetamol were calculated as the product of the fraction of the dose of the respective compound found in the urine and plasma drug clearance. Renal clearances of paracetamol sulphate and glucuronide were calculated from the ratio of the molar amount of conjugate found in urine to the AUC of the conjugate. Formation clearance through the toxic pathway was calculated as the sum of the formation clearances of the 3-cysteinyl, 3-mercapto and 3-methylthio conjugates. Formation clearance through the catechol pathway was calculated as the sum of the formation clearances of 3-hydroxy and 3-methoxy-paracetamol.

Statistics

Results are expressed as mean ± s.e. mean. The data were analysed by 2-way analysis of variance and paired Student's *t*-test, using the Bonferroni correction for multiple comparisons (Hays, 1981). $P < 0.05$ was accepted as an indication of significance.

Results

Ten healthy volunteers between the ages of 23 and 56 years were chosen for the study. There were equal numbers of men and women and equal numbers of rapid and slow acetylators. All ten subjects completed the protocol without adverse effects. The data from all subjects were analysed and comparison of paracetamol metabolism was made on days 0, 7 and 9.

Recoveries of paracetamol and its metabolites in the urine are shown in Table 1. All of the oxidative metabolites were markedly decreased on day 7 compared with day 0 and day 9 in all subjects. The urinary recoveries of the conjugated products were increased to a small but significant degree on day 7 compared with day 9. There was no statistical difference between day 0 and day 9 in the recovery of any of the metabolites. Total recovery on day 9 was slightly decreased as compared with day 7. This could not be explained by differences in 24 h creatinine recoveries, which were 1550 ± 130 mg, 1510 ± 110 mg and 1400 ± 80 mg on days 0, 7 and 9, respectively ($P \gg 0.05$).

Total paracetamol clearance was decreased significantly ($P < 0.01$) on day 7, 18.9 ± 1.7 l h^{-1} as compared with day 0, 22.2 ± 2.20 l h^{-1} and day 9, 22.2 ± 1.8 l h^{-1}. Formation clearances of the oxidative and non-oxidative

Table 1 Effect of isoniazid treatment on the recovery of paracetamol and its metabolites in urine (mean ± s.e. mean results).

	Urinary recovery (% of dose)		
Product	*Day 0*	*Day 7*	*Day 9*
Paracetamol glucuronide	46.59 ± 2.25	53.01 ± 3.05	44.48 ± 3.15**
Paracetamol sulphate	31.48 ± 3.22	34.64 ± 2.57	27.25 ± 2.47**
Paracetamol	3.71 ± 0.49	4.35 ± 0.44	3.34 ± 0.41**
3-hydroxy paracetamol	2.96 ± 0.31*	1.82 ± 0.17	2.98 ± 0.23**
3-*O*-methyl paracetamol	2.82 ± 0.37**	0.76 ± 0.11	2.76 ± 0.31**
3-cysteine paracetamol	2.91 ± 0.28**	0.908 ± 0.09	3.19 ± 0.27**
Paracetamol 3-mercapturate	2.74 ± 0.33**	1.09 ± 0.21	2.86 ± 0.34**
3-methylthio paracetamol	0.64 ± 0.10*	0.16 ± 0.02	0.80 ± 0.12**
Total percent recovered	93.86 ± 2.60	96.74 ± 1.47	87.65 ± 2.76*

*$P < 0.05$, **$P < 0.01$, as compared with day 7 by paired *t*-test with Bonferroni correction.

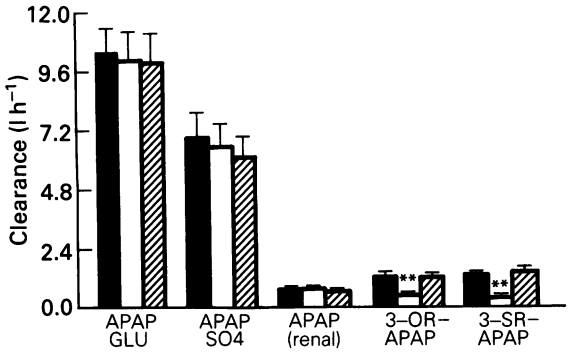

Figure 2 Effect of isoniazid administration on paracetamol (APAP) renal clearance and paracetamol metabolite formation clearance (mean ± s.e. mean, $n = 10$). Isoniazid, 300 mg, was administered daily for 7 days. Paracetamol metabolites: (APAP Glu) paracetamol glucuronide; (APAP SO4) paracetamol sulphate; (3-OR-APAP) sum of 3-hydroxy paracetamol plus 3-O-methyl paracetamol; (3-SR-APAP) sum of 3-cysteine paracetamol, paracetamol 3-mercapturate, plus 3-methylthio paracetamol. Comparisons were made by paired *t*-test with Bonferroni correction. **$P < 0.01$ from day 0 and day 9. ■ day 0, □ day 7 and □ day 9.

metabolites and paracetamol renal clearance are shown in Figure 2. There were no significant differences in paracetamol renal clearance or formation clearance of the conjugated compounds, but clearance through oxidative pathways was markedly diminished by isoniazid on day 7 ($P < 0.01$).

Subgroup analysis showed male *vs* female and rapid *vs* slow acetylator phenotype comparisons were not statistically significant. However, when both gender and phenotype were taken into account the extent to which the oxidative pathways were reduced on day 7 was significantly greater ($P < 0.02$, unpaired *t*-test) in slow acetylator females (20.3 ± 5.0%) than in rapid acetylator males (46.1 ± 4.6%). Rapid acetylator females (33.9 ± 4.5%) and slow acetylator males (34.0 ± 3.3%) were not significantly different from the other groups. This trend is consistent with a dose-related inhibitory effect of isoniazid.

None of the subjects demonstrated any appreciable change in clinical laboratory measurements. In particular γ-glutamyl transferase on day 0 was normal (28.0 ± 1.8 u l^{-1}) and was not significantly changed on day 7 (28.1 ± 2.0 u l^{-1}).

Discussion

The results of this study show that isoniazid is a potent inhibitor of the oxidative metabolism of paracetamol. These findings are in agreement with those of previous studies in which isoniazid was found to inhibit the oxidative metabolism of both phenytoin (Kutt *et al.*, 1970) and carbamazepine (Wright *et al.*, 1982). We have demonstrated that isoniazid is a more potent inhibitor of the oxidative metabolism of paracetamol than cimetidine (Mitchell *et al.*, 1984) or ethanol (Critchley *et al.*, 1983). Sub group analysis showing that the inhibition is greatest in slow acetylator females suggests that it is a dose-related effect.

The urinary recovery and formation clearance of the thiol metabolites is an index of the exposure to the oxidative metabolite, NAPQI, the major hepatotoxin. We expected from experiments in rats to see an induction of this oxidative pathway by isoniazid on day 9 as compared with day 0. There are a number of possible explanations for failure to demonstrate evidence of induction: (i) P450IIE1 does not contribute markedly to the oxidation of paracetamol in man, (ii) there are marked interindividual differences in the inducibility of the pathway in man, and (iii) residual inhibition or destruction of the P-450 enzymes responsible, prevented demonstration of the induction, i.e. without inhibition, formation clearance would have remained low on day 9.

The significant inhibition of the oxidative metabolism of paracetamol by isoniazid demonstrated in this study will not result in an adverse drug-drug interaction. Isoniazid decreases the total clearance of paracetamol by only 15%. This small effect of isoniazid on paracetamol clearance represents a clinically inconsequential drug interaction. If, however, isoniazid were ingested at the same time as an acute or chronic paracetamol overdose, a clinically useful drug interaction, the inhibition of formation of the toxic intermediate and

prevention of hepatotoxicity, might occur. The effect of paracetamol on the risk of isoniazid hepatotoxicity is presently unknown.

This study suggests that concomitant administration of isoniazid and paracetamol is not associated with an increased formation of the toxic metabolite of paracetamol; in fact the reverse was shown. We cannot be certain whether induction may occur under other conditions.

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References

Albano, E., Rundgren, M., Harrison, P. J., Nelson, S. D. & Moldéus, P. (1985). Mechanisms of *N*-acetyl-*p*-benzoquinone imine cytotoxicity. *Mol. Pharmac.*, **28**, 306–311.

Andrews, R. S., Bond, C. C., Burnett, J., Saunders, A. & Watson, K. (1976). Isolation and identification of paracetamol metabolites. *J. int. med. Res.* **4** (Suppl. 4), 34–39.

Black, M. (1984). Acetaminophen hepatotoxicity. *Ann. Rev. Med.*, **35**, 577–593.

Critchley, J. A., Dyson, E. H., Scott, A. W., Jarvie, D. R. & Prescott, L. F. (1983). Is there a place for cimetidine or ethanol in the treatment of paracetamol poisoning? *Lancet*, **i**, 1375–1376.

Dahlin, D. C., Miwa, G. T., Lu, A. Y. H. & Nelson, S. D. (1984). *N*-acetyl-*p*-benzoquinone imine: A cytochrome P-450 mediated oxidation product of acetaminophen. *Proc. Nat. Acad. Sci. (U.S.A.)*, **81**, 1327–1331.

Davis, M., Simmons, C. J., Harrison, N. G. & Williams, R. (1976). Paracetamol overdose in man: Relationship between pattern of urinary metabolites and severity of liver damage. *Quart. J. Med.*, **45**, 181–191.

Hays, W. L. (ed.) (1981). *Statistics, 3rd edition*, pp. 298–300. Holt, Rinehart and Winston Publishers.

Hinson, J. A. (1980). Biochemical toxicology of acetaminophen. *Rev. Biochem. Toxicol.*, **2**, 103–129.

Kutt, H., Brennan, R., Dehejia, H. & Verebely, K. (1970). Diphenylhydantoin intoxication. A complication of isoniazid therapy. *Am. Rev. resp. Dis.*, **101**, 377–384.

Mitchell, J. R. & Jollow, D. J. (1975). Metabolic activation of drugs to toxic substances. *Gastroenterology*, **68**, 392–410.

Mitchell, J. R., Thorgeirsson, S., Potter, W. Z., Jollow, D. J. & Keiser, H. (1974). Acetaminophen-induced hepatic injury: Protective role of glutathione in man and rationale for therapy. *Clin. Pharmac. Ther.*, **16**, 676–684.

Mitchell, J. R., Zimmerman, H. J., Ishak, K. G., Thorgeirsson, U. P., Timbrell, J. A., Snodgrass, W. R. & Nelson, S. D. (1976). Isoniazid liver injury: Clinical spectrum, pathology, and probable pathogenesis. *Ann. Intern. Med.*, **84**, 181–192.

Mitchell, M. C., Schenker, S. & Speeg, Jr., K. V. (1984). Selective inhibition of acetaminophen oxidation and toxicity by cimetidine and other histamine H_2-receptor antagonists *in vivo* and *in vitro* in the rat and in man. *J. clin. Invest.*, **73**, 383–391.

Morgan, E. T., Koop, D. R. & Coon, M. J. (1983). Comparison of six rabbit liver cytochrome P-450 isozymes in formation of a reactive metabolite of acetaminophen. *Biochem. Biophys. Res. Commun.*, **112**, 8–13.

Muakkassah, S. F., Bidlack, W. R. & Yang, W. C. T. (1981). Mechanism of the inhibitory action of isoniazid on microsomal drug metabolism. *Biochem. Pharmac.*, **30**, 1651–1658.

Nouws, J. F., Vree, T. B., Breukink, H. J., Baakman, M., Driessens, F. & Smulders, A. (1985). Dose dependent disposition of sulphadimidine and of its N4-acetyl and hydroxy metabolites in plasma and milk of dairy cows. *Vet. Quart.*, **7**, 177–186.

Ochs, H. R., Greenblatt, D. J., Verburg-Ochs, B., Abernethy, D. R. & Knüchel, M. (1984). Differential effects of isoniazid and oral contraceptive steroids on antipyrine oxidation and acetaminophen conjugation. *Pharmacology*, **28**, 188–195.

Prescott, L. F. (1983). Paracetamol overdosage. Pharmacological considerations and clinical management. *Drugs*, **25**, 290–314.

Raucy, J. L., Lasker, J. M., Lieber, C. S. & Black, M. (1989). Acetaminophen activation by human liver cytochromes P450IIE1 and P450IA2. *Arch. Biochem. Biophys.*, **271**, 270–283.

Ryan, D. E., Koop, D. R., Thomas, P. E., Coon, M. J. & Levin, W. (1986). Evidence that isoniazid and ethanol induce the same microsomal cytochrome P-450 in rat liver, an isozyme homologous to rabbit liver cytochrome P-450 isozyme 3a. *Arch. Biochem. Biophys.*, **246**, 633–644.

Slattery, J. T. & Levy, G. (1979). Acetaminophen kinetics in acutely poisoned patients. *Clin. Pharmac. Ther.*, **25**, 184–195.

Slattery, J. T., Wilson, J. M., Kalhorn, T. F. & Nelson, S. D. (1987). Dose-dependent pharmacokinetics of acetaminophen: Evidence of glutathione depletion in humans. *Clin. Pharmac. Ther.*, **41**, 413–418.

Timbrell, J. A. (1979). The role of metabolism in the hepatotoxicity of isoniazid and iproniazid. *Drug Metab. Rev.*, **10**, 125–147.

Wilson, J. M., Slattery, J. T., Forte, A. J. & Nelson, S. D. (1982). Analysis of acetaminophen metabolites in urine by high performance liquid chromatography with UV and amperometric detection. *J. Chromatogr.*, **227**, 453–462.

Wright, J. M., Stokes, E. F. & Sweeney, V. P. (1982). Isoniazid-induced carbamazepine toxicity and vice versa: A double drug interaction. *New Engl. J. Med.*, **307**, 1325–1327.

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