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Technical Note

Disposition of Antipyrine and Acetaminophen Given Alone and in Combination to Human Subjects

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INTRODUCTION

Antipyrine is a widely used model compound to assess hepatic metabolic functional capacity or changes in hepatic capacity secondary to pharmacologic interventions (1–5). This compound is rapidly and completely absorbed from the gastrointestinal tract and metabolized almost exclusively in the liver (1–5). It is not bound appreciably to plasma proteins and is distributed rapidly throughout body water (1). Urinary excretion of antipyrine's major metabolites may reflect the activity of at least three oxidative microsomal pathways: two hydroxylation [3-hydroxymethylantipyrine (HMA), 4-hydroxyantipyrine (OHA)] and one demethylation [norantipyrine (NORA)]. These metabolites then undergo sequential phase II glucuronidation or sulfation reactions (6,7).

Acetaminophen, a commonly used analgesic, is eliminated mainly by metabolism through the formation of glucuronide and sulfate conjugates with minor elimination via oxidative metabolism after normal doses (8–10). Acetaminophen has also been utilized as a model compound alone or in combination with other probe drugs to quantitate conjugation capacity in humans (11–14).

Simultaneous assessment of conjugative and oxidative metabolic capacity using acetaminophen and antipyrine may be of utility if their respective pharmacokinetic profiles remain unaltered after coadministration. However, Blyden and colleagues recently reported that acetaminophen plasma and urinary pharmacokinetics were altered during coadministered with antipyrine in normal volunteers (15). No significant alteration of antipyrine plasma or urinary metabolite disposition were noted (15). In their study urine was collected for only 24 hr after drug coadministration. A longer collection time, suggested by Danhof, Briemer, and others, may be required to detect ultimate changes in antipyrine metabolite profiles (6,7,16). The present study recharacterizes the pharmacokinetics of plasma acetaminophen, plasma antipyrine, and urinary antipyrine metabolites after single-dose, acetaminophen-antipyrine coadministration. Urine collection was extended to 36 hr after dose administration.

METHOD

Subjects

Eight normal healthy volunteers (seven males, one female), with a mean age (±SD) of 39.5 ± 13.7 years and a mean weight of 75.9 ± 16.4 kg, participated in the study. A medical history, a physical exam, and hematologic and biochemical laboratory profiles were performed on each subject prior to and at the end of the study. The study was approved by the Institutional Review Board of Hennepin County Medical Center. Each subject granted a written informed consent before participating in the study.

Drug Administration

Each subject received antipyrine alone (treatment A), acetaminophen alone (treatment B), and acetaminophen and antipyrine together (treatment C). At least 1 week elapsed between each drug administration. The sequence of administration was randomized. Full pharmacokinetic assessments were completed at the time of each study. The subjects fasted for at least 12 hr prior to and 4 hr after drug dosing.

On the day of treatment A, each subject received 1000 mg of antipyrine (USP) in 6 oz of water as an oral solution. Venous blood samples were obtained prior to and at 0.5, 1, 2, 4, 8, 12, 24, and 36 hr after administration. All urine was collected for 36 hr after drug administration. During treatment B, each subject received 650 mg of acetaminophen as two 325-mg tablets (McNeil Laboratories) with 6 oz of water. Venous blood samples were obtained at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, and 12 hr after drug administration. During treatment C, each subject received 650 mg of acetaminophen and 1000 mg of antipyrine orally as tablets and solution, respectively. Venous blood samples were obtained at 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, and 36 hr after dosing. Blood samples were collected in heparinized tubes and the plasma was separated within 5 min of sample collection at 4°C. All urine was collected immediately prior to dose administration

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and for 36 hr after dosing. All samples were frozen at −70°C until analyzed.

Assay

Antipyrine and acetaminophen concentrations in plasma as well as unchanged antipyrine and its major metabolites in urine were determined using high-performance liquid chromatographic methods. The plasma assays utilized a single-step acetonitrile extraction and a C-18 μ-Bondapak column (300, 3.9 mm, 10 μm) from Waters Chromatography (Waters Associates, Milford, MA). Detection wavelength was 254 nm. For antipyrine, the mobile phase was 47:53 acetonitrile:0.05 m*M* KH_2PO_4 (pH 6) buffer by volume flowing at 1 ml/min. The internal standard utilized for antipyrine analysis was 4-nitroacetanilide. For acetaminophen, the mobile phase was 10:90 acetonitrile:0.05 m*M* KH_2PO_4 (pH 3) by volume. The flow rate was set at 1.5 ml/min. 3-Acetamidophenol was used as the internal standard. The method has within-day and between-day coefficients of variation of less than 10% for both compounds and detection limits of 50 ng/ml for each of antipyrine and acetaminophen.

Urinary excretion of antipyrine, HMA, OHA, and NORA were measured using a separate HPLC method (17). The highest within-day coefficients of variation for antipyrine, HMA, OHA, and NORA between 6 and 60 μg/ml were 6.3, 8.6, 4.0, and 5.6, respectively. Between-day coefficients were 7.1, 8.4, 9.0, and 11.0%, respectively. Free concentrations of metabolites were measured by assay of urine samples without glucuronidase/arylsulfatase hydrolysis.

Pharmacokinetic Analysis

The plasma pharmacokinetic parameters of both acetaminophen and antipyrine were estimated using a noncompartmental approach. The area under the curve (AUC) up to the last collection time point was estimated using the trapezoidal method. The area of the tail was estimated by dividing the concentration of each drug in plasma at the last observed time point by the terminal disposition rate constant. The terminal disposition rate constant was estimated using nonlinear regression analysis of the plasma drug concentration–time data. Apparent total body clearance (TBC/*F*) was calculated from the relationship DOSE/$AUC_{0-\infty}$. Apparent volume of distribution at steady state (Vd_{ss}/F) was derived from the relationship between TBC/*F* and mean residence time (18). The maximum concentration (C_{max}) in plasma and time to maximum plasma concentration (t_{max}) were determined by visual inspection. The amount of unchanged antipyrine or its metabolites in urine was estimated as a percentage of the total amount recovered normalized to the molar weight of antipyrine.

Statistical Analysis

The pharmacokinetic parameters of each drug were compared between the different phases of the study using the paired Student's *t* test and the computer program SPSSPC$^+$. Significant differences were assumed when $P < 0.05$

RESULTS

Both plasma antipyrine and acetaminophen concentrations declined monoexponentially over time after drug administration (Figs. 1 and 2). The C_{max} and t_{max} did not change with coadministration compared to the separate administration of each drug (Table I).

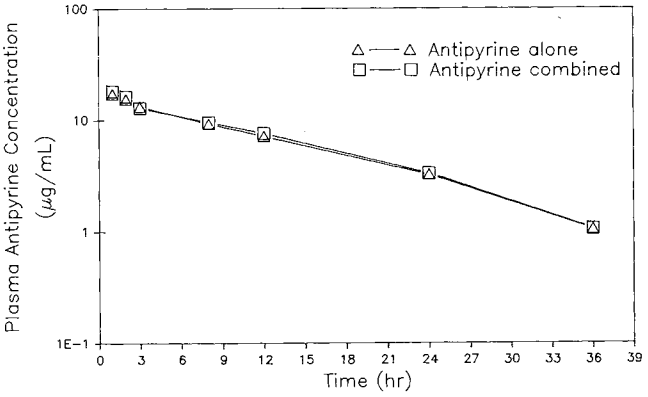

Fig. 1. Plasma antipyrine concentration–time plots after oral administration of 1 g antipyrine solution alone (△) or 1 g antipyrine solution combined with a 650-mg acetaminophen tablet (□) in a normal volunteer.

The TBC/*F*, Vd_{ss}/F, and $t_{1/2}\beta$ of plasma antipyrine were not significantly altered when administered in combination with acetaminophen (Table I). However, small but significant changes in plasma acetaminophen pharmacokinetic parameters were observed with antipyrine coadministration. Plasma acetaminophen $t_{1/2}\beta$ increased ($P = 0.019$), TBC/*F* decreased ($P = 0.004$), and Vd_{ss}/F decreased ($P = 0.013$) (Table I).

Total 36-hr urinary recovery of unchanged antipyrine and metabolites as a percentage of the dose administered were no different between antipyrine alone (53.1 ± 6.8%) and acetaminophen coadministration (56.2 ± 14.9%). However, small but significant changes were observed in the fractional recoveries of antipyrine and metabolites during the coadministration phase. Mean percentage fractional excretion of OHA decreased from 50.4 to 45.8% with increases in unchanged antipyrine from 7.1 to 9.3% and HMA from 19.3 to 21.8%. NORA percentages were unchanged (Table II). The

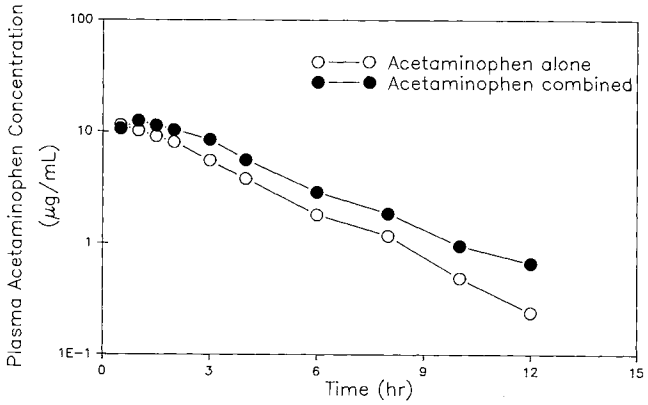

Fig. 2. Plasma acetaminophen concentration–time plots after oral administration of 650-mg acetaminophen tablets alone (○) or 650-mg acetaminophen tablets combined with 1 g of antipyrine solution (●) in a normal volunteer.

TABLE I. Pharmacokinetic Parameters of Antipyrine and Acetaminophen Administered Alone and in Combination (n = 8)

	C_{max} (mg/L)	T_{max} (hr)	$t_{1/2}$ (hr)	TBC/F (ml/min/kg)	Vd_{ss}/F (L/kg)
Antipyrine alone	29.2 ± 18.4	1.1 ± 0.4	12.2 ± 2.8	0.58 ± 0.27	0.58 ± 0.26
Antipyrine combined	31.2 ± 17.1	1.5 ± 1.1	13.8 ± 5.3	0.52 ± 0.25	0.52 ± 0.20
P value	0.251	0.402	0.279	0.107	0.307
Acetaminophen alone	7.3 ± 2.1	1.1 ± 0.6	3.1 ± 1.3	5.3 ± 1.8	1.4 ± 0.3
Acetaminophen combined	7.9 ± 2.4	1.0 ± 0.7	3.5 ± 1.0	3.8 ± 1.2	1.1 ± 0.2
P value	0.289	0.785	0.019	0.004	0.013

ratio of glucuronidated HMA remained constant between treatment A (61.7 ± 8.8%) and treatment C (61.3 ± 7.6%). No unconjugated NORA or OHA was detected in urine. The total amounts of unchanged antipyrine and metabolites recovered in the 0- to 24-hr urine collection period were significantly lower than that during the 0- to 36-hr urine collection period. During treatment A the total amounts recovered were 456.4 ± 74.5 and 530.5 ± 68.2 mg for the 24-hr versus the 36-hr period, respectively ($P < 0.001$).

DISCUSSION

The search for model markers which may be administered alone or in combination to characterize hepatic function in patients with various disease states has intensified in the last few years. This study was conducted to assess the effect, if any, of concomitant administration of acetaminophen and antipyrine on the pharmacokinetics of each agent.

Coadministration of acetaminophen and antipyrine resulted in small but significant decreases in acetaminophen TBC/F and Vd_{ss}/F and an increase in $t_{1/2}\beta$. These results are in agreement with those of Blyden *et al.* (15). Additionally, Blyden and colleagues reported that the fractional urinary recovery of acetaminophen glucuronide was preferentially reduced with antipyrine coadministration.

The TBC/F, $t_{1/2}\beta$, and Vd_{ss}/F of antipyrine in plasma were not affected by the presence of acetaminophen in the same subjects, again similar to the report of Blyden and colleagues (15). In contrast, our studies using 36-hr urine collections demonstrate that small but significant differences in unchanged antipyrine, HMA and OHA excretion ratios are present with concurrent administration of antipyrine and acetaminophen. The urine collection time of 24 hr used in the study by Blyden *et al.* (15) may not have been long enough to establish actual metabolite excretion ratios. In our studies, the total amounts recovered at 24 hr were significantly different from the 36-hr recoveries. Using our data from the 0- to 24-hr urine collection periods, unchanged antipyrine approaches statistical significance but fails to achieve P = 0.05. The P values for HMA and OHA were slightly larger using the 24-hr collection data (Table II).

Coadministration of acetaminophen with antipyrine resulted in a decrease in the percentage of antipyrine which is eliminated as OHA and compensatory increases in elimination of unchanged antipyrine and HMA. The mechanism or mechanisms of this interaction remains speculative. Possibilities include (1) antipyrine competition with minor acetaminophen metabolism at the P-450-level and (2) antipyrine metabolite competition with major acetaminophen metabolism via glucuronide conjugation. Both acetaminophen and OHA form ether glucuronides via a single hydroxyl group attached to a cyclic structure. This suggests that acetaminophen and OHA may share a common route of glucuronidation. Data reported by Blyden and colleagues documenting preferential inhibition of acetaminophen glucuronide fractional clearance after antipyrine–acetaminophen coadministration further supports this concept (15). Competition for this conjugative route could exert a feedback effect causing selected alterations in the parallel oxidative metabolism of antipyrine. In a compensatory manner, both unchanged antipyrine and HMA urinary excretion may increase as our data suggest. The percentage of conjugated HMA remained unchanged. This lack of change is not surprising given the nonether linkage that HMA forms with glucuronic acid and the probable multiplicity and specificity of the human glucuronyltransferase system (19).

The results of this study reinforce the concept that the interpretation of antipyrine metabolite excretion rates is somewhat dependent upon duration of urine collection time. Longer collection times (i.e., 36 hr or greater) may be required in order to characterize accurately ultimate antipyrine urine metabolite profiles in interaction studies and states of

Table II. Percentage of Total Recovery for Antipyrine and Major Metabolites, 24- Versus 36-Hour Urine Collections (Mean % ± SD)

	24-hr collection			36-hr collection		
% of total recovered	Antipyrine alone	Antipyrine/ acetaminophen	P	Antipyrine alone	Antipyrine/ acetaminophen	P
Unchanged AP	7.4 ± 3.3	10.0 ± 5.8	0.062	7.1 ± 3.0	9.3 ± 4.7	0.043
HMA	18.0 ± 3.1	20.2 ± 3.2	0.006	19.3 ± 2.9	21.8 ± 3.1	0.003
NORA	24.2 ± 5.7	23.3 ± 5.0	0.551	23.2 ± 6.1	23.2 ± 5.0	0.994
OHA	50.4 ± 3.1	46.4 ± 4.0	0.041	50.4 ± 3.8	45.8 ± 3.9	0.031

altered physiology. Furthermore, concurrent assessment of urinary antipyrine metabolite profiles and plasma antipyrine pharmacokinetics increases the sensitivity of the antipyrine test for detecting changes in oxidative activity.

Acetaminophen and antipyrine have been utilized as model markers for examining conjugative and oxidative capacities of the liver. These data indicate that the disposition of both antipyrine and acetaminophen are altered when the two compounds are administered together. Other compounds which undergo glucuronidation may have the potential to alter antipyrine oxidative metabolic profiles in urine without a detectable effect on its plasma disposition. Further studies should be conducted to define possible relationships between oxidative and conjugative reactions.

In conclusion, antipyrine and acetaminophen coadministration caused changes in disposition of both agents. This limits the utility of these agents in the simultaneous assessment of phase I and phase II microsomal activity.

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REFERENCES

1. M. V. Relling, W. R. Crom, J. A. Pieper, G. C. Cupit, G. K. Rivera, and W. E. Evans. *Clin. Pharmacol. Ther.* 41:651–660 (1987).
2. A. W. Harman, D. B. Frewin, B. G. Priestly, and C. B. J. Alexander. *Br. J. Clin. Pharmacol.* 7:45–48 (1979).
3. A. P. S. Narange, D. V. Datta, and V. S. Mathur. *Int. J. Clin. Pharmacol.* Ther. Toxicol. 23:28–32 (1985).
4. P. Spoelstra, M. W. E. Teunissen, A. P. Janssens, B. Weeda, W. Van Duijn, C. W. Koch, and D. D. Breimer. *Eur. J. Clin. Invst.* 16:321–327 (1986).
5. R. A. Branch, C. M. Herbert, and A. E. Read. *Gut.* 14:569–573 (1973).
6. M. Danhof, A. van Zuilen, J. K. Boeijinga, and D. D. Breimer. *Eur. J. Clin. Pharmacol.* 21:433–441 (1982).
7. M. Danhof and D. D. Breimer. *Br. J. Clin. Pharmacol.* 8:529–537 (1979).
8. J. R. Mitchell, D. J. Jollow, W. Z. Potter, D. C. Davis, J. R. Gillette, and B. B. Brodie. *J. Pharmacol. Exp. Ther.* 187:185–194 (1973).
9. J. O. Miners, J. Attwood, and D. J. Birkett. *Clin. Pharmacol. Ther.* 35:480–486. (1984).
10. J. T. Slattery and G. Levy. *Clin. Pharmacol. Ther.* 25:184–195 (1979).
11. K. E. Anderson, J. Schneider, E. J. Pantuck, C. B. Pantuck, G. H. Mudge, R. M. Welch, A. H. Conney, and A. Kappas. *Clin. Pharmacol. Ther.* 34:369–374 (1983).
12. M. J. Brodie, A. R. Boobis, E. L. Toverud, W. Ellis, S. Murray, C. T. Dollery, S. Webster, and R. Harrison. *Br. J. Clin. Pharmacol.* 9:523–525 (1980).
13. C. A. Shively and E. S. Vesell. *Clin. Pharmacol. Ther.* 18:413–424 (1975).
14. E. J. Triggs, R. L. Nation, A. Long, and J. J. Ashley. *Eur. J. Clin. Pharmacol.* 8:55–62 (1975).
15. G. T. Blyden, D. J. Greenblatt, B. W. LeDuc, and J. M. Scavone. *Eur. J. Clin. Pharmacol.* 35:413–417 (1988).
16. J. A. H. Forrest, J. A. Clements, and L. F. Prescott. *Clin. Pharmacokinet.* 7:93–107 (1982).
17. J. V. St. Peter and W. A. Awni. *J. Chromatogr. Biomed. Appl.* 494:424–427 (1989).
18. M. Rowland and T. N. Tozer. *Clinical Pharmacokinetics,* Lea & Febiger, Philadelphia, 1989, pp. 415–416.
19. S. Dragacci, J. Thomassin, J. Magdalou, H. Souhaili El Amri, P. Boissel, and G. Siest. *Eur. J. Clin. Pharmacol.* 32:485–491 (1987).