Assessment of Doxylamine Influence on Mixed Function Oxidase Activity upon Multiple Dose Oral Administration to Normal Volunteers

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Abstract □ The primary purpose of this study was to assess the influence of doxylamine and phenobarbital on antipyrine/metabolites pharmacokinetics and 6β-hydroxycortisol urinary excretion. This study was conducted in 48 healthy male human volunteers (16 per treatment group) using a parallel study design. Treatment groups consisted of 12.5 mg of doxylamine succinate, placebo, or 30 mg of phenobarbital administered orally every 6 h for 17 days. Results indicate that no statistically significant differences were observed between the doxylamine and placebo groups that are indicative of enzyme induction. For the phenobarbital group, a significant increase for antipyrine total (36 versus 45 mL/h/kg) and nonrenal (35 versus 44 mL/h/kg) clearances and 6β-hydroxycortisol excretion (338 versus 529 μg) and a significant decrease in the terminal exponential half-life (11 versus 9 h) of antipyrine were observed.

Introduction

Doxylamine (2-[α-(2-(dimethylamino)ethoxy)-α-methylbenzyl]pyridine) succinate is an ethanolamine antihistamine indicated for nonprescription use as a hypnotic and as an antihistamine.[1,2] In a 2 year rodent bioassay study conducted by the National Center for Toxicological Research,[3−5] dose-related increases in benign liver and thyroid tumors were observed in B6C3F1 mice orally administered approximately 70 and 140 mg/kg/day of doxylamine succinate. In addition, histologic changes that were consistent with microsomal enzyme induction were observed (i.e., centrilobular hepatocellular hypertrophy), although no biochemical assessments of enzyme inducation were performed. In a subsequent study, doxylamine was shown to be a phenobarbital-type inducer of hepatic microsomal enzymes in B6C3F1 mice based on biochemical assays of enzyme activity and was shown to produce a 3−5-fold increase in thyroid stimulating hormone.[6] Additionally, in a study conducted in Fisher 344 rats, an increase in antipyrine total clearance was observed following oral administration of approximately 45 and 95 mg/kg/day of doxylamine for 8 days (personal communication, Robert C. Bookstaff, Procter and Gamble, Cincinnati, OH, January 25, 1995). Consistent with these findings are previous reports that have suggested a possible relationship between enzyme induction and alterations in thyroid and liver function.[7−9]

Antipyrine is an analgesic which is widely used as a marker of mixed function oxidase activity.[10−14] Antipyrine is predominately eliminated by oxidative metabolism to three major metabolites (3-(hydroxymethyl)antipyrine, norantipyrine, and 4-hydroxyantipyrine) which undergo further glucuronide conjugation.[11−14] As a result of the low hepatic extraction ratio and plasma protein binding (<10%) of antipyrine, total clearance for antipyrine is primarily a function of intrinsic metabolic clearance(s), making it an appropriate marker for oxidative metabolic induction/inhibition studies. The use of metabolic formation clearances for antipyrine metabolites also allows more specific identification of the cytochrome P-450 isozyme(s) involved (i.e., norantipyrine, CYP2C9/18; 4-hydroxyantipyrine, CYP3A4; and 3-(hydroxymethyl)antipyrine, CYP1A2 and CYP2C9/18 families).[15] In addition, since antipyrine and metabolites undergo subsequent conjugation, changes in the ratio of urinary recovery of unconjugated to conjugated product may serve as an indicator of metabolic conjugation, as suggested for 3-(hydroxymethyl)antipyrine.[16]

6β-Hydroxycortisol, a metabolite of cortisol, is another commonly used marker of enzyme induction.[17−20] This endogenous substance has been shown to be a specific marker for CYP3A induction,[21,22] which is one of the most abundant and commonly induced forms of cytochrome P450 in man.[20,21,23−26]

The purpose of this investigation was to determine whether doxylamine, administered at doses recommended in labeling, alters mixed function oxidase activity, as assessed using antipyrine/metabolites and 6β-hydroxycortisol, and to characterize the pharmacokinetics of doxylamine upon multiple dose, oral administration of 12.5 mg doxylamine succinate every 6 h.

Methods

This was a randomized open-label, positive and placebo-controlled, parallel-designed study conducted in 48 healthy male human volunteers (segregated into three treatment groups of 16 subjects each). Inclusion/exclusion criteria were established to avoid/minimize factors known to alter mixed function oxidase activity.[11,13,27−34] These criteria included inclusion of healthy male volunteers, on a normal diet and within a narrow age range (i.e., 18−40 years), and exclusion of smokers, subjects with positive drug screens, and subjects exposed to enzyme inducers or inhibitors within 30 days prior to the study. In addition, normal renal function (creatinine clearance) was also required in order to meet the underlying assumptions necessary to estimate metabolite formation clearances.[35] During the study, balanced diets were maintained and meals were prepared, excluding (e.g. cruciferous vegetables, charcoal broiled beef, etc.) or minimizing (e.g., protein) food stuffs known to alter mixed function oxidase activity.[36,37] This study was approved by the Institutional Review Board, prior to the start of the study.

Either 12.5 mg doxylamine (Pfizer, lot number 92001), 30 mg phenobarbital (Lilly, lot number 5D239B), or placebo (Forest Pharmaceuticals, lot number 8911) was orally administered with 240 mL of water every 6 h for 17 days (69 doses). In order to minimize the potential influence of food on absorption, drugs were administered at least 1 h before or 2 h after a meal. The dose of doxylamine was based on the range recommended within the OTC monograph for antihistamines.[38] The dose of phenobarbital was based on previous studies demonstrating induction.[19,39] The duration of dosing, which is longer than recommended in the OTC monograph for doxylamine, was also based on phenobarbital results which indicated that induction was consistently observed within 14 days.[19,39] Blood samples (lithium heparin) for doxylamine were obtained at 0, 0.5, 1, 2, 3, 4, 6, 8, 12, 16, and 24 h on days 1, 8, and 15; immediately prior to the morning dose on days 11 and 13; and at 0, 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 24, 36, and 48 h on day 18. Urine samples for doxylamine were pooled prior to dosing and over 24 h on days 1, 8, and 15 and over 48 h on day 18. Safety assessments included clinical laboratory tests [chemistry includingthyroid function (T4, T3 resin uptake, reverse T3, and TSH) and hematology].

Mixed function oxidase activity was assessed based on the pharmacokinetics of antipyrine and metabolites and the urinary recovery

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of 6β-hydroxycortisol and cortisol at baseline (7 days prior to doxylamine, phenobarbital, or placebo administration) and on days 1, 8, and 15. The pharmacokinetics of antipyrine were determined following 500 mg of antipyrine (Department of Pharmaceutics, University of Tennessee, lot 3252629) administered as a 5 min constant rate intravenous infusion. Blood samples (lithium heparin) for antipyrine were obtained at 0, 0.5, 1, 2, 4, 6, 12, 24, 48, and 72 h after the start of the infusion. Urine samples were pooled prior to dosing and over 72 h for antipyrine/metabolites (collected over sodium metabisulfite and stored at −70 °C) and over 24 h for 6β-hydroxycortisol and cortisol.

Bioanalytical Methods—The analysis of plasma samples for antipyrine was conducted as previously described.[40] The method included extraction with acetonitrile, addition of phenacetin as an internal standard, separation using isocratic reverse phase (C18) HPLC, and quantitation via UV detection at 254 nm. Approximate retention times for antipyrine and phenacetin were 9 and 17 min, respectively. The lower limit of quantitation was 0.1 mg/L with a coefficient of variation of 11% or less over the range of the standard curve.

Urine samples were analyzed for antipyrine and metabolites, prior to and following deconjugation using glusulase.[41] Following addition of sodium metabisulfite (preservative) and phenacetin (internal standard) to each sample, pH was adjusted using 1 M sodium acetate (pH 5) and subsequently extracted using ethyl acetate. Analytes were separated using isocratic reverse phase (C18) HPLC and quantitated using UV detection at 254 nm. Approximate retention times were as follows: antipyrine, 11 min; 3-(hydroxymethyl)antipyrine, 4.5 min; norantipyrine, 7.5 min; 4-hydroxyantipyrine, 17 min; and phenacetin, 26 min. The lower limits of quantitation were as follows: antipyrine, 3.5 mg/L; 3-(hydroxymethyl)antipyrine, 2 mg/L; norantipyrine, 7.5 mg/L; and 4-hydroxyantipyrine, 5 mg/L. The coefficients of variation for all analytes were <10%.

Plasma and urine samples were analyzed for doxylamine as follows. Following addition of *N*-propyldoxylamine (internal standard), samples were extracted using ethyl acetate/hexane. Analytes were separated using gas chromatography with nitrogen−phosphorus detection. Approximate retention times were as follows: doxylamine, 2 min; and *N*-propyldoxylamine, 3.5 min. The lower limit of quantitation was 2 ng/mL with a coefficient of variation of <10% and 15% for plasma and urine, respectively.

Urine samples were analyzed for 6β-hydroxycortisol using solid phase extraction (C18) and subsequent separation using a reverse phase (C18) HPLC method with UV detection at 254 nm. Approximate retention time for 6β-hydroxycortisol was 18 min. The lower limit of quantitation was 20 ng/mL with a coefficient of variation of <10%.

Urine samples were also analyzed for cortisol using 11-deoxycortisol as an internal standard, solid phase extraction (C18), and subsequent separation using a reverse phase (C18) HPLC method with UV detection at 254 nm. Approximate retention times were as follows: cortisol, 13 min; and 11-deoxycortisol, 22 min. The lower limit of quantitation was 10 ng/mL with a coefficient of variation of <8%.

Pharmacokinetic Analysis—Antipyrine plasma concentration−time data following intravenous administration were analyzed using PCNONLIN[42] and the following equation:

$$C = \sum_{i=1}^{n} \frac{C_i}{\lambda_i \, \mathrm{TI}} (1 - \mathrm{e}^{-\lambda_i b}) \mathrm{e}^{-\lambda_i \, \mathrm{ta}} \qquad (1)$$

where n is the number of exponentials necessary to characterize the plasma concentration−time profile; C_i is the ith coefficient; λ_i is the ith exponent; TI is the duration of the infusion; b is a second independent variable, equal to t during the infusion and TI after the infusion; t is time after the start of the infusion; and ta is the time after the end of the infusion. Initial parameter estimates were obtained from data after the end of the infusion, using ESTRIP.[43] Various weightings of the predicted plasma concentrations (1, $1/C$, and $1/C^2$) were used in the data analysis. Decisions on the appropriate weighting and the number of exponentials required to characterize the plasma concentration−time data were based on visual inspection of the randomness of scatter of the observed data about the fitted line and the sum of weighted squared residuals.[44,45] Steady-state volume of distribution, total clearance, and terminal exponential half-life were obtained from the coefficients and exponents using standard equations.[46−48] Antipyrine renal clearance was obtained from the amount of antipyrine recovered in urine, and the corresponding area under the plasma concentration−time profile.[46,47] Antipyrine nonrenal clearance was obtained as the difference between total and renal clearance. Metabolic formation clearances were obtained from the amount of metabolite (unconjugated and conjugated) recovered in urine, the dose administered, and antipyrine total clearance.[13] Urinary recoveries were adjusted for molecular weight (antipyrine, 188; 3-(hydroxymethyl)antipyrine, 204; norantipyrine, 174; and 4-hydroxyantipyrine, 204).[49] The ratio of conjugated to unconjugated for antipyrine and 3-(hydroxymethyl)antipyrine was determined from the cumulative amount recovered in urine, with and without hydrolysis.

Urinary recovery of 6β-hydroxycortisol and the ratio of 6β-hydroxycortisol to cortisol (adjusting for potential fluctuations in the daily amount of cortisol produced) were also analyzed.[19,20]

The maximum doxylamine plasma concentration (C_{max}) and the time at which the maximum occurred (T_{max}) were determined from visual inspection of the individual doxylamine plasma concentration−time profiles. Area under the plasma concentration−time curve over a dosing interval (AUC_τ) was determined using the linear trapezoidal rule.[46,47] The terminal exponential rate constant (λ_z) was estimated from linear least squares regression of the terminal phase of the log concentration−time profile from data following the last dose.[46] The terminal exponential half-life ($T_{1/2,z}$) was obtained as $0.693/\lambda_z$. All additional analyses were performed using conventional relationships.[46,47] Apparent oral clearance (CL_o) was obtained from the dose and AUC_τ at steady-state. Terminal volume of distribution (uncorrected for bioavailability; V_z/F) was obtained from CL_o and $T_{1/2,z}$. The accumulation ratio was obtained from the ratio of the steady-state AUC_τ to the AUC_τ following the first dose.[50]

Statistical Analysis—Demographics were analyzed using a Fisher's Exact Test for race and an ANOVA for age, height, body weight, and creatinine clearance. Change in mixed function oxidase activity and doxylamine pharmacokinetic parameters were compared using an ANOVA with Fisher's protected LSD procedure or a Kruskall−Wallis test with protected nonparametric multiple comparisons.[51] Within each treatment, changes from baseline were tested using a paired t-test or the Wilcoxon Signed Rank Test. When variables exhibited nonsystematic treatment differences, an analysis of linear trend was also performed. Assessment of doxylamine steady-state was performed using sequential contrasts.[52] All tests were performed at the $\alpha = 0.05$ level of significance.

Table 1—Mean (SD) Demographics for Subjects Administered Placebo, 12.5 mg of Doxylamine, or 30 mg of Phenobarbital Every 6 h for 17 days[a]

Treatment Group	Age (yr)	Height (cm)	BW (kg)	Race	CL_{cr} (mL/min)
Placebo	28.6 (6.11)	175 (7.01)	79.6 (8.77)	10 Caucasian 4 Black 1 Indian 1 Asian	138 (26.0)
12.5 mg of Doxylamine	27.4 (6.14)	178 (5.67)	77.6 (10.3)	14 Caucasian 1 Black 1 Hispanic	130 (29.4)
30 mg of Phenobarbital	26.3 (6.06)	178 (10.3)	76.2 (9.66)	14 Caucasian 1 Black 1 Indian	138 (35.1)

[a] CL_{cr} is creatinine clearance attained at baseline, and BW is body weight.

Results

Study Population—Demographics and creatinine clearance at baseline, for the doxylamine, phenobarbital, and placebo groups, are shown in Table 1. Statistical analysis of these data indicate no significant differences between groups for age, race, body weight, height, and creatinine clearance.

Safety Assessment—There were no clinically significant adverse findings for blood chemistry or hematologic parameters and no clinically significant differences in thyroid function parameters among the three treatment groups during drug administration.

Antipyrine/6β-Hydroxycortisol—Individual plasma concentration−time profiles for antipyrine were adequately char-

Table 2—Mean (SD) Antipyrine Pharmacokinetic Parameters and 6β-Hydroxycortisol Urinary Recovery in Subjects Administered Placebo Every 6 h for 17 days[a]

Parameters	Baseline	Day 1	Day 8	Day 15
V_{ss} (L/kg)	0.5153 (0.0645)	0.5115 (0.0648)	0.5255 (0.0655)	0.5475 (0.0610)
CL (mL/h/kg)	32.37 (7.78)	33.12 (9.00)	33.36 (8.85)	32.44 (8.25)
CL_r (mL/h/kg)	1.144 (0.7750)	1.140 (0.5609)	1.206 (0.8793)	1.077 (0.6232)
CL_{nr} (mL/h/kg)	31.22 (7.70)	31.98 (8.99)	32.15 (8.50)	31.36 (8.10)
$T_{1/2,z}$ (h)	12.16 (2.46)	11.82 (2.42)	12.28 (2.33)	13.10 (3.82)
AP_f/AP_t	0.870 (0.226)	0.870 (0.246)	0.949 (0.104)	0.935 (0.065)
$CL_{f,OHA}$ (mL/h/kg)	7.556 (3.421)	6.825 (2.922)	7.032 (3.404)	7.620 (4.026)
$CL_{f,NORA}$ (mL/h/kg)	3.622 (1.849)	3.432 (1.763)	3.569 (2.310)	3.941 (1.980)
$CL_{f,HMA}$ (mL/h/kg)	4.072 (1.927)	3.591 (1.835)	3.321 (1.905)	3.671 (1.938)
HMA_f/HMA_t	0.3016 (0.1356)	0.2998 (0.1331)	0.2878 (0.1252)	0.2938 (0.1280)
6β-OH (μg)	419.6 (467.2)	508.5 (372.4)	496.5 (500.1)	507.7 (597.3)
6β-OH/ Cortisol	6.235 (2.409)	5.576 (2.813)	6.974 (3.639)	6.108 (2.397)

[a] V_{ss} is steady-state volume of distribution; CL is total clearance; CL_r is renal clearance; CL_{nr} is nonrenal clearance; $T_{1/2,z}$ is the terminal exponential half-life; AP_f/AP_t is ratio of the amount of antipyrine (AP_f) to total (conjugates + parent) antipyrine (AP_t) recovered in urine; $CL_{f,OHA}$ is 4-hydroxyantipyrine formation clearance; $CL_{f,NORA}$ is norantipyrine formation clearance; $CL_{f,HMA}$ is 3-(hydroxymethyl)antipyrine formation clearance; HMA_f/HMA_t is ratio of the amount of 3-(hydroxymethyl)antipyrine (HMA_f) to total (conjugates + parent) 3-(hydroxymethyl)-antipyrine (HMA_t) recovered in urine; 6β-OH is 6β-hydroxycortisol; and 6β-OH/cortisol is the ratio of 6β-hydroxycortisol to cortisol.

Table 3—Mean (SD) Antipyrine Pharmacokinetic Parameters and 6β-Hydroxycortisol Urinary Recovery in Subjects Administered 12.5 mg of Doxylamine Every 6 h for 17 days[a]

Parameters	Baseline	Day 1	Day 8	Day 15
V_{ss} (L/kg)	0.4911 (0.0719)	0.5177 (0.0658)	0.5160 (0.0719)	0.5210 (0.0580)
CL (mL/h/kg)	33.53 (8.03)	34.52 (8.60)	32.92 (8.16)	33.17 (7.65)
CL_r (mL/h/kg)	0.8566 (0.3928)	0.9989 (0.4199)	0.8075 (0.3854)	0.8500 (0.4940)
CL_{nr} (mL/h/kg)	32.68 (7.80)	33.52 (8.38)	32.11 (8.03)	32.32 (7.38)
$T_{1/2,z}$ (h)	10.96 (2.59)	11.26 (2.52)	12.78 (4.08)	11.73 (2.82)
AP_f/AP_t	0.888 (0.096)	0.948 (0.080)	0.862 (0.212)	0.936 (0.162)
$CL_{f,OHA}$ (mL/h/kg)	7.812 (2.778)	8.009 (2.645)	7.981 (2.548)	8.083 (2.984)
$CL_{f,NORA}$ (mL/h/kg)	4.326 (1.457)	3.777 (1.382)	3.464 (1.796)	3.492 (1.414)
$CL_{f,HMA}$ (mL/h/kg)	4.077 (1.750)	4.151 (1.869)	4.111 (1.785)	4.113 (1.679)
HMA_f/HMA_t	0.3480 (0.0670)	0.3504 (0.0656)	0.3012 (0.0588)	0.3305 (0.0619)
6β-OH (μg)	271.1 (131.7)	286.0 (113.4)	245.9 (105.6)	262.0 (134.6)
6β-OH/ Cortisol	5.640 (2.351)	6.201 (2.002)	6.062 (2.226)	7.603 (3.544)

[a] V_{ss} is steady-state volume of distribution; CL is total clearance; CL_r is renal clearance; CL_{nr} is nonrenal clearance; $T_{1/2,z}$ is the terminal exponential half-life; AP_f/AP_t is ratio of the amount of antipyrine (AP_f) to total (conjugates + parent) antipyrine (AP_t) recovered in urine; $CL_{f,OHA}$ is 4-hydroxyantipyrine formation clearance; $CL_{f,NORA}$ is norantipyrine formation clearance; $CL_{f,HMA}$ is 3-(hydroxymethyl)antipyrine formation clearance; HMA_f/HMA_t is ratio of the amount of 3-(hydroxymethyl)antipyrine (HMA_f) to total (conjugates + parent) 3-(hydroxymethyl)-antipyrine (HMA_t) recovered in urine; 6β-OH is 6β-hydroxycortisol; and 6β-OH/cortisol is the ratio of 6β-hydroxycortisol to cortisol.

acterized by either a mono or biexponential function, using a weighting of $1/C^2$. Mean antipyrine and metabolites pharmacokinetic parameters at baseline and on days 1, 8, and 15 for placebo, doxylamine, and phenobarbital groups are summarized in Tables 2, 3, and 4, respectively. Statistical analysis of these parameters at baseline indicated no difference among groups except for norantipyrine formation clearance, which was higher in the phenobarbital group. However, since subsequent analyses were conducted on the change from baseline, this difference does not alter the interpretation of data (vide infra).

Statistical analyses indicate that antipyrine total clearance was significantly increased by phenobarbital, whereas no difference was observed between the placebo and doxylamine groups (Table 5 and Figure 1). Since no change in antipyrine renal clearance was observed among the three groups, and since renal clearance accounts for only a small portion of total clearance for antipyrine, changes in antipyrine total clearance reflect changes in antipyrine nonrenal clearance (i.e., metabolic clearance). The statistical analysis of formation clearances indicate that only the formation clearance for norantipyrine demonstrated a significant difference among groups. On day 8, neither phenobarbital nor doxylamine was significantly different from placebo. However, norantipyrine formation clearance for the doxylamine group was significantly lower than the phenobarbital group. By day 15, only the doxylamine group was significantly different from placebo (reduction in formation clearance); no difference existed between the phenobarbital and placebo groups. Linear trend analysis of these data indicate that norantipyrine formation clearance significantly increased over time for the phenobarbital group; no significant change was observed for the placebo or doxylamine groups, although the change (decrease) for norantipyrine formation clearance for the doxylamine group was of borderline significance ($p = 0.059$). Consistent with the multiple comparisons, the comparison of linear trends between groups indicated that doxylamine was significantly decreased from placebo and phenobarbital; the comparison of phenobarbital to placebo indicated an increase of borderline significance ($p = 0.055$).

The only other parameter to significantly differ among groups was the terminal exponential half-life for antipyrine. Statistical analysis indicated that on day 8 all three groups were significantly different from each other (doxylamine > placebo > phenobarbital). On day 15, the terminal exponential half-life of antipyrine was significantly decreased in the phenobarbital group, compared to the doxylamine and placebo groups, with no differences between the doxylamine and placebo groups. Linear trend analysis of these data indicate a significant decrease for the phenobarbital group and a significant increase for the doxylamine group. Comparing the linear trends among groups indicates that the phenobarbital group differed from both placebo and doxylamine groups; no difference existed between placebo and doxylamine groups. These results are consistent with changes observed for antipyrine total and nonrenal clearances.

Mean urinary recovery of 6β-hydroxycortisol and the ratio of 6β-hydroxycortisol to cortisol are also summarized in Tables 2–4. Statistical analysis of 6β-hydroxycortisol data indicated a significant increase for phenobarbital relative to doxylamine on day 8, with neither group differing from placebo (Table 5). On day 15, the phenobarbital group was significantly in-

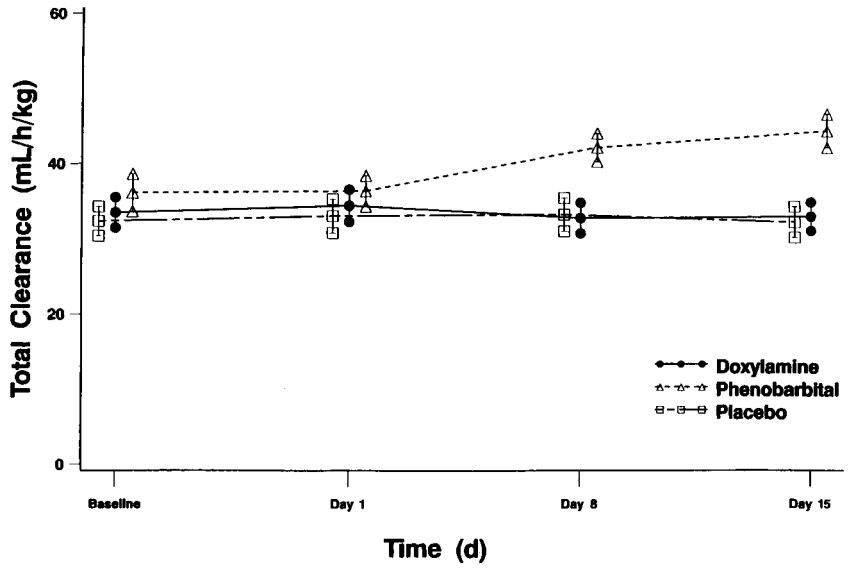

Figure 1—Mean (SE) antipyrine total clearance versus time for doxylamine, phenobarbital, and placebo groups.

Table 4—Mean (SD) Antipyrine Pharmacokinetic Parameters and 6β-Hydroxycortisol Urinary Recovery in Subjects Administered 30 mg of Phenobarbital Every 6 h for 17 days[a]

Parameters	Baseline	Day 1	Day 8	Day 15
V_{ss} (L/kg)	0.5266 (0.0777)	0.5299 (0.0715)	0.5484 (0.0550)	0.5441 (0.0566)
CL (mL/h/kg)	36.19 (9.98)	36.46 (8.23)	42.36 (7.55)	44.65 (9.04)
CL_r (mL/h/kg)	0.9911 (0.4750)	1.045 (0.3855)	1.063 (0.5867)	1.035 (0.6018)
CL_{nr} (mL/h/kg)	35.20 (9.97)	35.42 (8.02)	40.90 (7.44)	43.62 (8.72)
$T_{1/2,z}$ (h)	11.39 (2.56)	10.91 (2.35)	9.70 (1.89)	9.10 (2.02)
AP_f/AP_t	0.858 (0.204)	0.974 (0.071)	0.887 (0.203)	0.933 (0.085)
$CL_{f,OHA}$ (mL/h/kg)	9.133 (3.450)	9.280 (3.320)	10.33 (2.739)	10.91 (3.532)
$CL_{f,NORA}$ (mL/h/kg)	5.572 (1.748)	5.522 (1.748)	6.749 (3.109)	7.018 (2.808)
$CL_{f,HMA}$ (mL/h/kg)	4.579 (1.466)	4.530 (1.633)	4.597 (1.640)	4.723 (1.507)
HMA_f/HMA_t	0.3380 (0.0919)	0.3494 (0.0873)	0.3231 (0.1081)	0.3431 (0.0739)
6β-OH (μg)	338.0 (161.9)	348.8 (181.7)	460.4 (195.7)	528.9 (287.8)
6β-OH/ Cortisol	5.105 (1.487)	4.043 (1.572)	7.158 (3.629)	7.921 (3.787)

[a] V_{ss} is steady-state volume of distribution; CL is total clearance; CL_r is renal clearance; CL_{nr} is nonrenal clearance; $T_{1/2,z}$ is the terminal exponential half-life; AP_f/AP_t is ratio of the amount of antipyrine (AP_f) to total (conjugates + parent) antipyrine (AP_t) recovered in urine; $CL_{f,OHA}$ is 4-hydroxyantipyrine formation clearance; $CL_{f,NORA}$ is norantipyrine formation clearance; $CL_{f,HMA}$ is 3-(hydroxymethyl)antipyrine formation clearance; HMA_f/HMA_t is ratio of the amount of 3-(hydroxymethyl)antipyrine (HMA_f) to total (conjugates + parent) 3-(hydroxymethyl)-antipyrine (HMA_t) recovered in urine; 6β-OH is 6β-hydroxycortisol; and 6β-OH/cortisol is the ratio of 6β-hydroxycortisol to cortisol.

Table 5—Statistical Analyses of the Change from Baseline for the Pharmacokinetic Parameters for Antipyrine/Metabolites and Urinary Recovery of 6β-Hydroxycorticosteroid and Cortisol

	Change from Baseline[b]		
Parameter[a]	Day 1	Day 8	Day 15
V_{ss}	Plac Pb Dox	Plac Pb Dox	Pb Dox Plac
CL	Pb Plac Dox	Dox Plac Pb	Dox Plac Pb
CL_r	Plac Pb Dox	Dox Pb Plac	Plac Dox Pb
CL_{nr}	Pb Plac Dox	Dox Plac Pb	Dox Plac Pb
$T_{1/2,z}$	Dox Pb Plac	Pb Plac Dox	Pb Dox Plac
AP_f/AP_t	Plac Dox Pb	Dox Pb Plac	Dox Plac Pb
$CL_{f,OHA}$	Plac Pb Dox	Plac Dox Pb	Plac Dox Pb
$CL_{f,NORA}$	Dox Plac Pb	Dox Plac Pb	Dox Plac Pb
$CL_{f,HMA}$	Plac Pb Dox	Plac Pb Dox	Plac Dox Pb
HMA_f/HMA_t	Plac Dox Pb	Dox Plac Pb	Dox Plac Pb
6β-OH	Dox Pb Plac	Dox Plac Pb	Dox Plac Pb
6β-OH/Cortisol	Pb Plac Dox	Plac Dox Pb	Plac Dox Pb

[a] V_{ss} is steady-state volume of distribution; CL is total clearance; CL_r is renal clearance; CL_{nr} is nonrenal clearance; $T_{1/2,z}$ is the terminal exponential half-life; AP_f/AP_t is ratio of the amount of antipyrine (AP_f) to total (conjugates + parent) antipyrine (AP_t) recovered in urine; $CL_{f,OHA}$ is 4-hydroxyantipyrine formation clearance; $CL_{f,NORA}$ is norantipyrine formation clearance; $CL_{f,HMA}$ is 3-hydroxymethylantipyrine formation clearance; HMA_f/HMA_t is ratio of the amount of 3-(hydroxymethyl)antipyrine (HMA_f) to total (conjugates + parent) 3-(hydroxymethyl)-antipyrine (HMA_t) recovered in urine; 6β-OH is the amount of 6β-hydroxycorticosteroid recovered in urine; 6β-OH/cortisol is the ratio of the amount of 6β-hydroxycorticosteroid to the amount of cortisol recovered in urine. [b] DOX is doxylamine, PB is phenobarbital, and PLAC is placebo. Treatments listed in order of change from baseline. A common underline indicates no significant difference between those treatment groups.

creased relative to both the placebo and doxylamine groups. No difference existed between doxylamine and placebo groups. When these data were adjusted for cortisol production, only phenobarbital was significantly increased relative to placebo on day 15. No other comparisons were significant.

Doxylamine—The mean trough doxylamine plasma concentration–time profile is illustrated in Figure 2. Statistical analysis of the trough concentration–time profile indicated that steady-state was achieved by day 8. Individual doxylamine pharmacokinetic parameters on days 1, 8, 15, and 18 are summarized in Table 6. Statistical analyses indicated no time related change for doxylamine oral clearance or for C_{max} after day 1. For renal clearance, a significant difference between days 1 and 8 was observed. However, trend analysis of this parameter indicated no consistent change over time. Comparison of renal clearance estimates on day 18, using 6 and 48 h collection intervals, indicated a significantly higher renal clearance for the 48 h interval. This result suggests a time dependency for doxylamine renal clearance, consistent

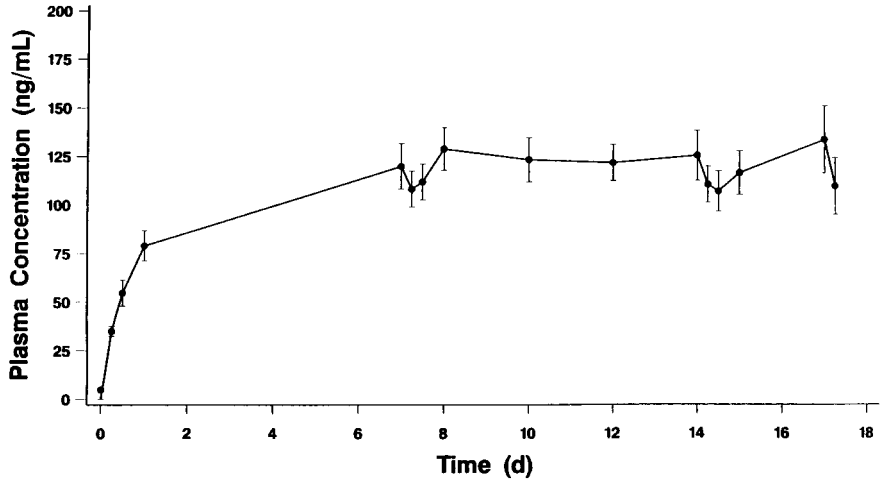

Figure 2—Mean (SE) trough doxylamine plasma concentration–time profile upon multiple dose oral administration of 12.5 mg of doxylamine succinate every 6 h to healthy male volunteers.

Table 6—Mean (SD) Doxylamine Pharmacokinetic Parameters upon Multiple Dose Oral Administration of 12.5 mg of Doxylamine Succinate Every 6 h to Healthy Male Volunteers

	Day			
Parameter[a]	1	8	15	18
$AUC_{(0-6h)}$ (ng h/mL)	240.1 (68.7)	854.9 (214.5)	787.8 (153.9)	826.4 (327.6)
C_{max} (ng/mL)	62.28 (19.52)	195.8 (52.84)	168.9 (24.55)	196.1 (67.70)
t_{max} (h)	2.19 (1.22)	1.47 (0.95)	1.53 (1.16)	1.90 (1.20)
$T_{1/2,z}$ (h)	NA NA	NA NA	NA NA	14.22 (3.56)
CL_o (mL/h/kg)	NA NA	137.1 (30.64)	145.6 (29.38)	151.2 (47.30)
$CL_{r(0-6h)}$ (mL/h/kg)	75.89 (31.11)	118.0 (62.55)	107.2 (84.57)	95.71 (47.83)
$CL_{r(0-48h)}$ (mL/h/kg)	NA NA	NA NA	NA NA	126.8 (64.49)
V_Z/F (L/kg)	NA NA	NA NA	NA NA	3.179 (1.446)
Accumulation ratio	NA NA	3.68 (1.13)	3.50 (1.21)	3.44 (0.91)

[a] $AUC_{(0-6h)}$ is the area under the plasma concentration–time profile from time zero to 6 h, C_{max} is the maximum plasma concentration; t_{max} is the time that the maximum plasma concentration occurs; $T_{1/2,z}$ is the terminal exponential half-life; CL_o is the oral clearance; $CL_{r(0-xh)}$ is the renal clearance from time zero to *x* h; V_Z/F is the terminal volume of distribution uncorrected for bioavailability; and accumulation ratio is the ratio of $AUC_{(0-6h)}$ to the $AUC_{(0-6h)}$ on Day 1; NA, Not Applicable.

with the diurnal variation observed in trough plasma concentrations (Figure 2).

Discussion

Antipyrine has been extensively used as a marker for mixed function oxidase activity[10–14] with more recent identification of the isozymes associated with each metabolic pathway.[15] The approximate pharmacokinetic parameters of antipyrine in humans include a total clearance of 40 mL/h/kg, a steady-state volume of distribution of 0.5 L/kg, a renal clearance of 1 mL/h/kg, and a terminal exponential half-life of 12 h. The approximate formation clearances for the major metabolites of antipyrine include the following: 3-(hydroxymethyl)antipyrine, 4 mL/h/kg; norantipyrine, 6 mL/h/kg; and 4-hydroxyantipyrine, 10 mL/h/kg. The antipyrine pharmacokinetics obtained at baseline are in good agreement with those previously reported (vide supra).

As previously reported,[39,53] phenobarbital administration resulted in a significant increase in antipyrine total and nonrenal clearances, consistent with enzyme induction. For the doxylamine group, no increase in antipyrine total clearance or metabolic formation clearances compared to placebo was observed, indicating no change in CYP1A2, CYP2C9/18, and CYP3A4 isozymes, which are some of the more commonly drug inducible forms of cytochrome P450.[54,55] Changes in formation clearance for antipyrine metabolites upon phenobarbital administration were generally consistent with those previously reported.[11] No change has been reported for the formation clearances of 3-(hydroxymethyl)antipyrine and 4-hydroxyantipyrine. However, norantipyrine formation clearance has been reported to increase upon phenobarbital administration,[11] consistent with induction of cytochrome P450 2C in man.[15] In the present study, a linear trend analysis suggested an increase in norantipyrine formation clearance for the phenobarbital group. No significant differences among the three groups for the urinary recovery ratios of conjugated to unconjugated antipyrine and 3-(hydroxymethyl)antipyrine indicate no change in glucuronidation.

The only other change in antipyrine pharmacokinetics was a decrease in the terminal exponential half-life for the phenobarbital group. Since the terminal-exponential half-life is a dependent parameter[56,57] inversely related to total clearance and directly related to volume of distribution, the decrease observed in the phenobarbital group was related to the increased antipyrine total clearance.

6β-Hydroxycortisol is extensively used as an indicator of enzyme induction for cytochrome P450 3A.[17–20] Normal daily urinary excretion of 6β-hydroxycortisol is 90−400 μg/m^2/day and the normal daily urinary excretion of cortisol is 50 μg/day (Nichols Laboratory). These values are similar to that observed in the present study. Previous studies have indicated that phenobarbital administration results in a significant increase in 6β-hydroxycortisol excretion (adjusted and unadjusted for cortisol production), as was observed in this study.[19] No difference was observed between the doxylamine and placebo groups for the urinary recovery of 6β-hydroxycortisol (adjusted or unadjusted for cortisol production). The apparent discrepancy between the results for phenobarbital with the two markers of CYP 3A (6β-hydroxycortisol and 4-hydroxyantipyrine) may represent differences in sensitivity in measuring CYP 3A induction.

Although subjects were not stratified based on metabolic function at baseline, the randomization of subjects appeared adequate since no differences were observed in baseline parameters except for a significantly higher formation clearance for norantipyrine for the phenobarbital group. Since this

isozyme (2C9/18) is associated with only 5% poor metabolizers[58−61] and no difference in formation clearance for norantipyrine was observed at baseline between the placebo and doxylamine groups, it seems unlikely that the lack of an effect on norantipyrine formation clearance in the doxylamine group was due to poor metabolizers. In addition, the higher norantipyrine formation clearance in the phenobarbital group does not appear to represent an induced population, since this is the only group in which an increase in formation clearance was observed (vide infra).

Doxylamine pharmacokinetics in humans have previously been assessed upon single dose oral administration.[62−65] The pharmacokinetic parameters of doxylamine include an oral clearance of approximately 180 mL/h/kg, a renal clearance of approximately 100 mL/h/kg, and a terminal exponential half-life of 10 h. In the present study, steady-state was obtained within 1 week (earliest time observed after the first day of dosing). The oral and renal clearances (∼150 and ∼110 mL/h/kg, respectively) observed in this study are in good agreement with those previously observed. However, the terminal exponential half-life was slightly longer (14 h vs 10 h), probably due to the longer duration of sample collection (48 h vs 24−30 h).

In conclusion, results from this study indicate that no differences suggestive of enzyme induction were observed in the pharmacokinetics of antipyrine/metabolites or the urinary recovery of 6β-hydroxycortisol, between the doxylamine and placebo groups, when doxylamine is administered at doses for the duration recommended within labeling. Consistent with previous observations, results also indicate that phenobarbital is an inducer of mixed function oxidase activity (cytochrome P450 2C and 3A). For doxylamine, pharmacokinetic results obtained upon multiple dose oral administration are in good agreement with those previously reported upon single dose oral administration.

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