Pharmaceutical Research, Vol. 8, No. 12, 1991

Research Article

The Pharmacokinetics of Antipyrine and Three of Its Metabolites in the Rabbit: Intravenous Administration of Pure Metabolites

John V. St. Peter,[1,2] Yusuf Abul-Hajj,[2] and Walid M. Awni[1-3]

Received January 24, 1991; accepted June 24, 1991

Antipyrine (AP) is a commonly used probe of oxidative metabolism. Indirect evidence demonstrates formation rate limited disposition of its metabolites. Kinetic studies using antipyrine and its major metabolites 3-hydroxymethylantipyrine (HMA), norantipyrine (NORA), and 4-hydroxyantipyrine (OHA) were completed to investigate the metabolic fate of preformed antipyrine metabolite and to demonstrate directly formation rate-limited metabolite disposition *in vivo*. Bolus injections of antipyrine and preformed metabolites (40–50 mg/kg) were administered to male, New Zealand white rabbits. Plasma and urine were analyzed using HPLC. These studies demonstrate that HMA, NORA, and OHA are formation rate limited in the rabbit. NORA appears to undergo further extensive oxidative and conjugative metabolism. Unknown additional peaks were detected in urine after NORA dosing but not after HMA or OHA administration. Mass spectroscopy of the unknown HPLC eluents identified potential structures of these NORA metabolites.

KEY WORDS: antipyrine; metabolites; rabbit; oxidation; conjugation; pharmacokinetics.

INTRODUCTION

The plasma disposition of antipyrine, a compound with a low hepatic extraction ratio and minimal protein binding, is used as a marker of oxidative metabolism in man and animals (1,2). Antipyrine oxidative metabolism appears to be under the control of multiple isoenzymes of the cytochrome P-450 system (2–4). The relative activity of the enzymatic pathways involved in antipyrine oxidation in humans has been estimated by quantifying antipyrine metabolites in urine (1,5–9). Antipyrine metabolite excretion and elimination are usually assumed to be more rapid than its oxidative formation (i.e., formation rate limited). However, direct validation of this assumption by assessing the disposition of both parent compound and preformed metabolites after intravenous administration of antipyrine and its metabolites is very important. Total recovery of administered antipyrine dose is incomplete and generally ranges from 30 to 70%. Thus, studies examining the potential for further metabolism of metabolites may be helpful in accounting for total recovery of antipyrine dose in urine.

This report describes the plasma and urine disposition of antipyrine and its three main metabolites [3-hydroxymethylantipyrine (HMA), norantipyrine (NORA), and 4-hydroxyantipyrine (OHA)] after intravenous (iv) bolus administration of antipyrine and the preformed metabolites in a chronic venous-cannulated, bladder-catheterized rabbit model. Further, potential structures of unknown compounds detected in urine after administration of the preformed metabolites of antipyrine were elucidated using a combination of high-pressure liquid chromatography (HPLC) and mass spectrometry (MS).

MATERIALS AND METHODS

Materials

Antipyrine (Sigma, St. Louis, MO), NORA (Aldrich, Milwaukee, WI), and OHA (Aldrich, Milwaukee, WI) were obtained from commercial sources in the highest purity available. HMA was synthesized in our laboratory utilizing the method described by Buijs *et al.* (10) HMA purity was determined by differential scanning calorimeter, mass spectrometry, infrared spectroscopy, and HPLC. Mass spectra were determined on AEIMS-30 and Finnigan 4000 mass spectrometers. Infrared spectra were determined on a 5DXC FT-IR spectrometer using KBr discs.

Method

Male New Zealand White rabbits received single intravenous bolus doses of either HMA (40 mg/kg), NORA (50

[1] The Drug Evaluation Unit, Department of Medicine, Division of Nephrology, Hennepin County Medical Center, Minneapolis, Minnesota 55415.

[2] College of Pharmacy, University of Minnesota, Minneapolis, Minnesota 55455.

[3] To whom correspondence should be addressed at The Drug Evaluation Unit, Hennepin County Medical Center, 701 Park Avenue South, Minneapolis, Minnesota 55415.

0724-8741/91/1200-1470$06.50/0

mg/kg), or OHA (50 mg/kg) on 1 day and antipyrine (50 mg/kg) on another day. The antipyrine dose was selected based upon previous reports in rabbits which described plasma disposition of antipyrine after single doses (11–15). Approximately equimolar doses of preformed metabolites were chosen based upon availability of compound and limited prior information about plasma disposition of one of the metabolites (OHA) (16). Preformed metabolite solutions were prepared immediately prior to injection by diluting exact amounts of each metabolite in a minimal amount of ethyl alcohol, USP 95%, and further diluting to a volume of no more than 7 ml with 0.9% sodium chloride for injection. The marginal ear vein was used for drug administration and blood sampling. Ear veins were cannulated using an iv catheter (I-Cath Delmed, New Brunswick, NJ). Catheter patency was maintained by instillation of small volumes of diluted heparinized saline solution (100 units/ml, preservative-free heparin sodium injection, LyphoMed). Bolus doses of drug were administered over 45 to 90 sec.

Blood samples (1.5 ml) were drawn at 1, 3, 5, 7, 9, 12, 15, 20, 30, 45, 60, 75, 90, and 120 min after metabolite injections. Blood samples were collected at 1, 3, 5, 7, 10, 20, 30, 60, 120, 180, 240, and 360 min after antipyrine administration. Samples were immediately centrifuged at 3000 rpm for 5 min. The plasma was then separated and frozen at −70°C until analyzed.

A size 8 FR, pediatric Foley catheter (Bard, Murray Hill, NJ) was placed in each rabbit bladder via the urethra to facilitate urine collection. Bladders were irrigated with 15–20 ml of warm (37°C) saline immediately prior to dosing with metabolite or antipyrine. Total urine was collected for approximately 24 hr after dosing. Multiple bladder irrigations were carried out at various points during all urine collections. All urine samples were collected over an antioxidant (sodium metabisulfite). Total volumes of urine were determined for all collection intervals. Aliquots (20–50 ml) of well-mixed sample were frozen at −70°C until analyzed.

Sample Analysis

Plasma and urine were analyzed for antipyrine and/or HMA, NORA, and OHA using a previously described method (17). Free and conjugated urine metabolites were determined by assay of samples with and without glucuronidase-arylsulfatase (Glusulase).

Fractional isolates of the unknown peaks in urine, after NORA administration, were collected from HPLC eluent (Fig. 1). Two peaks (5 and 2) were extracted from the mobile phase utilizing ethyl acetate. Four other peaks (1, 6, 3, and 4) were extracted utilizing methylene chloride. Several fractions of each peak were combined and dried under nitrogen.

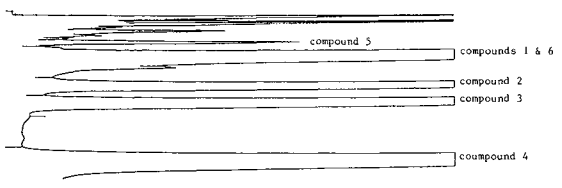

Fig. 1. Chromatogram of rabbit urine after administration of 50 mg/kg of norantipyrine intravenously.

Powders from each peak (0.5–1 mg) were subjected to mass spectrometry for potential compound identification.

Data Analysis

All plasma concentration–time data were analyzed using compartmental techniques, weighted (1/observed concentration) nonlinear regression and the computer program PCNONLIN. Antipyrine, HMA, and OHA plasma data were best fit to a two-compartment open model, while NORA data were fit to a one-compartment open model. Noncompartmental techniques were also used to analyze the data. Estimates of clearance, volume of distribution at steady states (V_{dss}), and terminal elimination half-life were equivalent to estimates from the compartmental methods. Thus, only compartmental analysis is presented.

The total-body clearance (TBC) for each compound was determined from dose/AUC, where dose is the amount of antipyrine or metabolite administered and AUC is the antipyrine or metabolite area under the plasma concentration-time profile from time 0 to infinity. For the two-compartment model, V_{dss} was estimated from known relationships between the central compartment volume of distribution (V_c) and the intercompartmental distribution rate constants.

Since none of the metabolites was detected in plasma after antipyrine administration, metabolite formation clearances (Cl_f) were estimated from $Met/AUC_{antipyrine}$, where Met is the amount of metabolite recovered in urine after antipyrine administration and $AUC_{antipyrine}$ is the antipyrine area under the plasma concentration–time profile from time 0 to infinity. The amount of each metabolite was converted to the equivalent weight of antipyrine utilizing the molecular weight of antipyrine and its metabolites.

Renal clearance (Cl_r) of antipyrine or unconjugated metabolite was estimated from Amt/AUC, where Amt is the amount of antipyrine or free metabolite recovered in urine and AUC is the respective antipyrine or metabolite area under the plasma concentration–time profile from time 0 to infinity.

All parameters are presented as mean ± standard deviation.

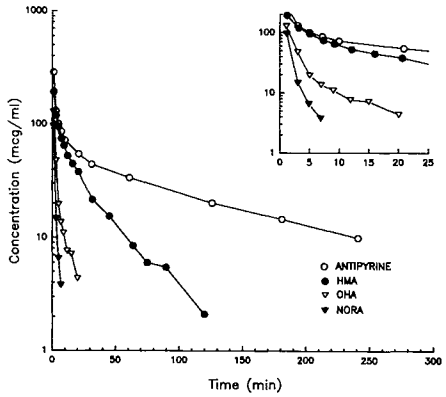

Fig. 2. Mean plasma concentration–time profiles for antipyrine, HMA, OHA, and NORA after iv bolus administration of each respective agent. Inset depicts the same curves from time 0 to 20 min. In each case, only the administered compound was detected in rabbit plasma.

Table I. Plasma Antipyrine Pharmacokinetic Parameters and Urinary Metabolite Recovery in Rabbits After Intravenous Administration of 50 mg/kg Antipyrine

	Rabbit No.								
	1	2	3	4	5	6	7	Mean	SD
Weight (kg)	3.25	2.64	3.04	3.24	3.42	2.76	2.71	3.01	0.31
AUC (mg · min/L)	5961.7	14519.7	4994.4	9401.0	5827.7	6562.0	3888.9	7307.9	3605.4
TBC (ml/min/kg)	8.70	3.83	10.62	5.85	9.11	6.71	11.20	8.00	2.66
$t_{1/2\alpha}$ (min)	1.43	1.94	1.84	2.53	0.90	3.06	1.22	1.85	0.75
$t_{1/2\beta}$ (min)	88.89	103.23	40.86	75.95	48.01	71.89	40.27	67.02	24.68
V_c (L/kg)	0.23	0.15	0.26	0.12	0.09	0.31	0.20	0.19	0.08
V_{dss} (L/kg)	1.05	0.54	0.59	0.55	0.56	0.66	0.60	0.65	0.18
Cl_r (ml/min)	0.37	0.03	0.10	0.07	NA[a]	0.08	0.21	0.14	0.13
	Urinary metabolite recovery (equivalent weight)								
HMA (%)[b]	1.6	5.5	4.3	6.7	NA	8.6	5.9	5.5	2.4
NORA (%)[b]	2.3	4.6	5.2	6.4	NA	9.7	7.2	5.9	2.5
OHA (%)[b]	5.8	22.8	45.1	29.9	NA	26.0	26.8	26.1	12.6
Antipyrine (%)[b]	1.3	0.3	0.3	0.4	NA	0.4	0.7	0.6	0.4
Total recovery (%)[b]	11.0	33.2	54.9	43.4	NA	44.7	40.6	38.0	15.0

[a] Not available.
[b] Percentage of dose recovered in urine.

RESULTS

Plasma Pharmacokinetics

Antipyrine metabolites were not detected in plasma after antipyrine administration. Mean plasma concentration–time curves for antipyrine, OHA, NORA, and HMA after direct administration of each of these compounds to rabbits are shown in Fig. 2. Estimated pharmacokinetic parameters for antipyrine, OHA, NORA and HMA are listed in Tables I, II, III, and IV, respectively. Antipyrine total-body plasma clearance, terminal-phase elimination half-life (t½), and volume of distribution at steady state (V_{dss}) in rabbits were 8 ± 2.7 ml/min/kg, 67 ± 25 min, and 0.65 ± 0.18 L/kg, respectively. The total percentage of the antipyrine dose recovered in urine as antipyrine and free and conjugated HMA, OHA, and NORA was 38.0 ± 15.0%.

Plasma clearances of the preformed metabolites were all greater than plasma antipyrine clearance and followed the order NORA > OHA > HMA > antipyrine. Metabolite formation clearances, estimated after administration of antipyrine, were all substantially lower than their respective plasma elimination clearances. Metabolite formation clearances as well as antipyrine and metabolite total-body clearances and renal clearances are listed in Table V.

The total percentages of the dose recovered in urine after administration of the preformed metabolites as free and conjugated OHA, NORA, and HMA were 51.8 ± 3.1, 30.3 ± 10.5, and 86.1%, respectively. The total percentage of the NORA dose recovered in urine as free and conjugated NORA and the newly identified metabolites was 48.3 ± 10.8%. OHA recovered in urine was all in the conjugated form, whereas NORA and HMA were recovered as both free and conjugated moieties. Individual amounts of glucuronidated or sulfated compounds were not determined as the enzyme used for this assay procedure contained both glucuronidase and arylsulfatase.

Table II. Plasma Pharmacokinetic Parameters for 4-Hydroxyantipyrine (OHA) in Rabbits After Intravenous Administration of 50 mg/kg OHA

	Rabbit No.					
	1	2	3	4	Mean	SD
Weight (kg)	2.94	3.23	3.04	2.71	2.98	0.21
AUC (mg · min/L)	806.9	431.1	443.7	796.1	619.46	210.31
TBC (ml/min/kg)	61.95	115.97	112.69	62.83	88.36	30.02
$t_{1/2\alpha}$ (min)	1.09	1.20	0.59	1.74	1.16	0.47
$t_{1/2\beta}$ (min)	11.93	11.83	5.48	12.53	10.44	3.32
V_c (L/kg)	0.13	0.32	0.15	0.31	0.23	0.10
V_{dss} (L/kg)	0.38	0.93	0.42	0.71	0.61	0.26
Total recovery (%)[a]	48.6	50.4	55.7	52.7	51.8	3.1
Free OHA (%)[b]	0	0	0	0	0	—
Conjugated OHA (%)[b]	100	100	100	100	100	—

[a] Percentage of dose recovered in urine.
[b] Percentage of total amount recovered.

Table III. Plasma Pharmacokinetic Parameters for Norantipyrine (NORA) in Rabbits After Intravenous Administration of 50 mg/kg NORA

	Rabbit No.				
	1	2	3	Mean	SD
Weight (kg)	3.37	3.34	2.86	3.19	0.29
AUC (mg · min/L)	294.9	326.0	446.3	355.75	79.93
TBC (ml/min/kg)	169.54	153.34	112.08	144.99	29.63
$t_{1/2}$ (min)	1.30	0.65	0.37	0.77	0.47
V_{dss} (L/kg)	0.32	0.14	0.06	0.17	0.13
Total recovery (%)[a]	42.2	22.7	25.9	30.3	10.5
Free NORA (%)[b]	19.1	47.2	34.5	33.6	14.1
Conjugated NORA (%)[b]	80.9	52.8	65.5	66.4	14.1
Cl_r (ml/min)	46.1	51.0	27.1	41.4	12.6

[a] Percentage of dose recovered as free and conjugated NORA in urine.
[b] Percentage of total amount recovered.

Unknown Peak Isolation and Identification

HPLC chromatograms of urine obtained after NORA administration revealed several unidentified peaks, whereas no unidentified peaks were detected after HMA or OHA administration. Fractional isolates of the unknown peaks, from urine after NORA administration, were collected (Fig. 1). Two of these peaks were most efficiently extracted from the mobile phase by utilizing ethyl acetate (peak 5, retention time = 5 min; peak 2, retention time = 12 min), while the other four peaks were extracted utilizing methylene chloride (peak 1, retention time = 7.4 min; peak 6, retention time = 8.9 min; peak 3, retention time = 14 min; and peak 4, retention time = 23 min). Several fractions of each peak were then combined and dried under nitrogen. The dried powders for each peak, 0.5–1 mg, were then subjected to mass spectrometry for compound identification.

Mass spectral determinations (molecular ions and fragmentation patterns) of fractions 1–6 (Fig. 1) were carried out and the structures of NORA metabolites were tentatively identified as shown in Fig. 3. Compound 6 gave a parent ion (M^+) at m/z 174 and a fragmentation pattern identical to that of NORA, which indicates that compound 6 is the enol form of NORA. The molecular ion fragment $(M)^+$ at m/z 216 and $(M-42)^+$ at m/z 174 indicates the presence of an acetate group in compound 3. All other fragmentation peaks support the structure of compound 3 as the acetate metabolite of NORA. Although it is possible that compound 3 may be the N-acetylated derivative of NORA, we think that compound 3 is, in fact, the O-acetylated enol metabolite of NORA since we showed unequivocally that compound 6 exists as the enol form of NORA. Compound 2 showed a molecular ion at m/z 234. Low-resolution FAB-MS of compound 5 gave a parent ion $(M)^+$ at m/z 288 and a fragment ion at m/z $(M-80)^+$ corresponding to the desulfated form of compound 5. A molecular ion peak using low-resolution FAB-MS of compound 4 was observed at m/z 402 and a fragment ion at m/z 241 $(M-160 + H)^+$, suggesting the presence of a glucosidic moiety in compound 4.

DISCUSSION

The plasma antipyrine $t_{1/2}$, V_{dss}, and total-body clearance from this study are in agreement with previously published values in rabbits (12,13). The total plasma clearance of antipyrine is the cumulative clearance via oxidative metabolism and renal excretion of unchanged antipyrine. Likewise, the plasma elimination clearances of preformed HMA, NORA, and OHA, after iv administration, are the cumulative clearances of each respective metabolite via further metabolism and renal excretion. In all three cases the mean metabolite plasma elimination clearance was greater than that of the parent compound, antipyrine. The order of values of plasma clearances of the compounds was NORA > OHA > HMA > antipyrine. Additionally, all mean estimates of metabolite formation clearances were substantially less than their respective total-body clearances and 4- to 20-fold less than antipyrine plasma elimination clearance (Table V). Therefore, this study demonstrates that HMA, NORA, and OHA elimination is formation rate limited in the rabbit.

Our results are in agreement with the only other published investigation we can find which documents antipyrine metabolite disposition after administration of preformed metabolite in men and dog (16). In this very early study on the disposition of antipyrine, Brodie and Axelrod administered

Table IV. Pharmacokinetic Parameters for 3-Hydroxymethylantipyrine (HMA) in Rabbits After Intravenous Administration of 40 mg/kg HMA

	Rabbit No.		
	1	2	Mean
Weight (kg)	3.04	2.95	2.99
AUC (mg · min/L)	3073.8	2093.8	2583.82
TBC (ml/min/kg)	13.01	19.10	16.06
$t_{1/2_\alpha}$ (min)	2.06	1.82	1.94
$t_{1/2_\beta}$ (min)	19.56	16.25	17.91
V_c (L/kg)	0.16	0.18	0.17
V_{dss} (L/kg)	0.32	0.37	0.35
Total recovery (%)[a]	84.1	88.1	86.12
Free HMA (%)[b]	13.7	13.3	13.50
Conjugated HMA (%)[b]	86.3	86.7	86.50
Cl_r (ml/min)	4.54	6.56	5.55

[a] Percentage of dose recovered as free and conjugated HMA in urine.
[b] Percentage of total amount recovered.

Table V. Metabolite Formation Clearances, Total-Body Clearances, and Renal Clearances (Mean ± SD)

	Metabolite formation clearance (ml/min)	Total-body clearance (ml/min)	Renal clearance, free compound (ml/min)
HMA	1.01 ± 0.67	47.91[a]	5.55[a]
NORA	1.14 ± 0.80	467.66 ± 131.17	41.4 ± 12.6
OHA	5.30 ± 4.91	267.16 ± 105.95	0.00
Antipyrine	NA	24.23 ± 8.43	0.14 ± 0.13

[a] Mean values only.
[b] Not applicable.

OHA orally to three human subjects and intravenously to one dog. These investigators detected OHA in plasma after intravenous administration in the dog. An estimated OHA $t_{1/2}$ of 8.7 min in the dog is similar to the terminal mean OHA $t_{1/2}$ (10.4 min) estimated in our rabbit study. However, OHA was not detected in plasma after oral administration in humans. Urine collected for 24 hr after dosing revealed OHA recovery of ≥92% in all three humans. The mean OHA recovery (51.8%) from rabbits in our study was approximately half that observed by Brodie and Axelrod in humans. Species differences in metabolic pathways between humans and rabbits may account for this discrepancy in recovery. However, analytical methodology differences may also be important. We have utilized a specific HPLC method for the measurement of OHA, while Brodie and Axelrod utilized a nonspecific spectrophotometric method. It is possible that these authors were able to measure OHA and some, yet unidentified, metabolites of OHA using their nonspecific method, while we measured only OHA. The higher percentage recovery in urine utilizing the spectrophometric method compared to the HPLC method suggests that OHA may be metabolized further to yet unidentified metabolites. Further work is needed to examine the possible existence of such metabolites of OHA.

Our study of NORA disposition revealed that this metabolite undergoes further oxidative and conjugative metabolism after preformed NORA administration. These processes involved hydroxylation of NORA and further methylation, sulfation, acetylation, and glucosidation. The potential for further metabolism of N-demethylated antipyrine structures has previously been suggested by researchers using radiolabeled antipyrine compounds in humans (18). Further, sulfation of NORA has been described previously, however, the sulfated structure was defined as a C5-enol monosulfate instead of the paraphenol monosulfate proposed in this study (19).

The presence of multiple pathways of metabolism for NORA is consistent with its extremely rapid plasma clearance and low urine recovery. The total urinary recovery of NORA as free NORA, conjugated NORA, and free and conjugated new identified metabolites accounted for only 48.3 ± 10.8% of the administered NORA dose. This low urine recovery could be attributed to alternate excretion routes such as bile or feces, which were not collected in this study. However, antipyrine metabolite excretion appears to be mainly renal (20,21). It may also be due to yet other unidentified metabolites of NORA. The hydroxylation of NORA and further acetylation, methylation, sulfation, and glucosidation demonstrates the rather complicated nature of antipyrine metabolism with regard to the NORA pathway in the rabbit.

Early studies of antipyrine metabolism in man, done by Danhof and colleagues, demonstrated a delay in the urinary excretion of HMA (1). Similarly, it is interesting to note that rabbit HMA total-body clearance is substantially slower than that of OHA and NORA and only twofold greater than that of antipyrine (see Table V). Conjugation of HMA is less extensive than that of OHA and NORA. HMA is usually renally excreted as both free and conjugated moieties. Free amounts of OHA and NORA, if detected, are generally in very small quantities. The mean total urinary recovery of HMA as free and conjugated HMA was 86.1% of the HMA dose administered to rabbits. Continued metabolism of HMA has been documented in man, however, this pathway is thought to contribute minimally to the total metabolic pic-

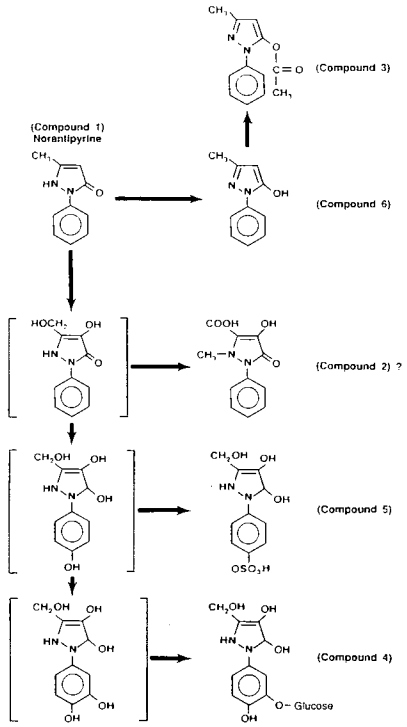

Fig. 3. Proposed structures of norantipyrine metabolites which were recovered in urine. Intermediate structures are enclosed in brackets.

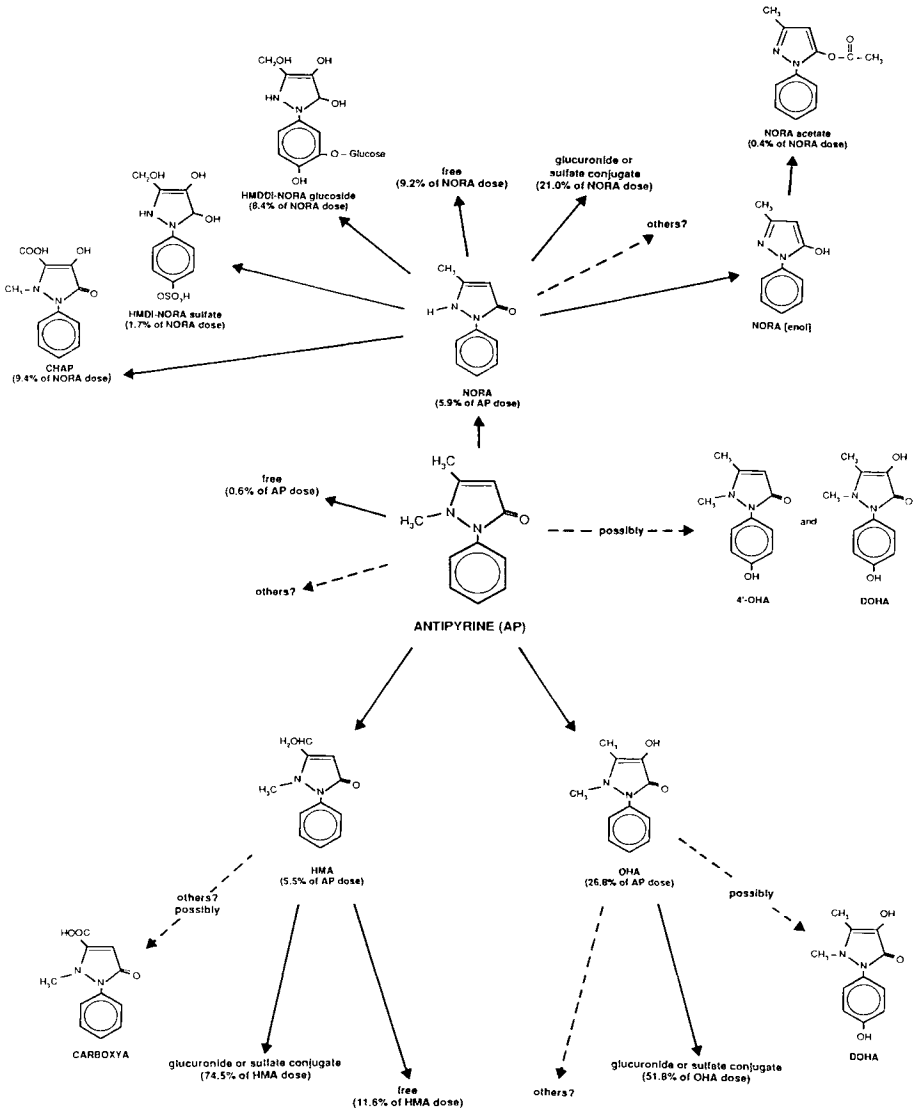

Fig. 4. Diagram representing the mass balance and the various pathways of metabolism after administration of antipyrine and each of its main identified preformed metabolites to rabbits. The ratio in parentheses reflects the mean percentage urinary recovery for each pathway from this study. Other possible pathways of metabolism are indicated by dashed arrows and some possible structures are from human data. AP, antipyrine; HMA, 3-hydroxymethyl antipyrine; OHA, 4-hydroxyantipyrine; 4′-OHA, 4′-hydroxyantipyrine; DOHA, dihydroxyantipyrine; NORA, norantipyrine; CARBOXYA, 3-carboxyantipyrine; CHAP, 3-carboxy-4-hydroxyantipyrine; HMDi NORA sulfate, 3-hydroxymethyl-4′-sulfate-dihydronorantipyrine; HMDDi NORA glucoside, 3-hydroxymethyl-3′,4′-dihydroxy-3′-glucoside-dihydronorantipyrine.

ture (1,3). Our study suggests that this is also the case in the rabbit.

Combining all the results of our study with previously known information, we can put together an overall picture on the possible metabolic fate of antipyrine and its metabolites in rabbits. A diagram of the mass balance and the various possible pathways of metabolism of antipyrine and its metabolites after administration of the parent compound and each of the main identified preformed metabolites (i.e., HMA, OHA and NORA) to rabbits is shown in Fig. 4. This diagram shows that the identified pathways of elimination of antipyrine, NORA, and OHA accounted for only approximately 40–50% of the administered doses of these compounds. Various other unidentified metabolic pathways may exist for these three compounds. In rabbits, almost the entire dose of HMA, (i.e., 86.1% of dose) can be accounted for by either free or conjugated HMA eliminated in urine. Possibly, 3-carboxy antipyrine (Fig. 4) may account for the remaining percentage of HMA dose as has been suggested for humans (3).

This study further demonstrates the complexity of antipyrine metabolism and elimination. Antipyrine plasma clearance is reflective of the net effect of more than one metabolic pathway. The potential for interaction at these metabolic steps should not be overlooked when utilizing antipyrine as a probe agent. It is possible that one or more than

one metabolic pathway may change without a noticeable effect on the overall plasma clearance of antipyrine (7). Significant compensatory changes in separate antipyrine metabolic pathways may occur. Thus, characterization of the main individual antipyrine metabolite disposition may provide more specific information about metabolic pathway activity than total plasma clearance alone. Concurrent assessment of urinary antipyrine main metabolite profiles and plasma antipyrine disposition increases the sensitivity of the antipyrine test for detecting possible changes in oxidative activity.

The question can be raised regarding the appropriateness of antipyrine as a probe agent of hepatic oxidative metabolism. On one hand, since antipyrine is metabolized through a wide range of pathways, the reduction of antipyrine metabolism must be substantial before one can see any change in the total plasma clearance of antipyrine. Therefore, some might conclude that antipyrine is not a sensitive marker of hepatic function. On the other hand, it may be considered an ideal marker of hepatic metabolism for the sole reason of being such substrate for a variety of enzymatic pathways. In either case, urinary metabolite recovery data should become an essential component of these studies.

CONCLUSION

Metabolite formation clearances for HMA, NORA, and OHA, estimated after administration of the parent compound, antipyrine, appear much slower than their respective total-body clearances, estimated after administration of preformed metabolite. This documents the formation rate-limited nature of these antipyrine metabolic pathways in a rabbit model. In the rabbit and potentially in humans, NORA appears to undergo further extensive oxidative and conjugative metabolism through multiple pathways which include methylation, sulfation, acetylation, and glucosidation. Other possible pathways of metabolism and elimination for antipyrine, NORA, and OHA need to be identified.

ACKNOWLEDGMENTS

This work was part of J.V.St.P.'s fellowship training in clinical pharmacokinetics with W.M.A. at The Drug Evaluation Unit.

The authors gratefully acknowledge Doug Tritschler for the assistance and technical expertise and Jan Lovick and Dee Lunzer for their help in typing the manuscript. We would also like to thank Dr. Cheryl Zimmerman and the anonymous reviewers for their very helpful comments.

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