Negligible Excretion of Unchanged Ketoprofen, Naproxen, and Probenecid in Urine

R. A. UPTON ˣ, J. N. BUSKIN, R. L. WILLIAMS, N. H. G. HOLFORD, and S. RIEGELMAN

Received March 20, 1980, from the *Drug Studies Unit, University of California, San Francisco, CA 94143.* Accepted for publication May 5, 1980.

Abstract □ On the average, 0.6% of a dose of ketoprofen or naproxen or 1.2% of a dose of probenecid was found in the urine of normal male volunteers assayed immediately after its collection. Between ~60 and 85% of the dose of these drugs can be excreted in the urine as conjugates, which rapidly hydrolyze at body temperature, at room temperature, and even during frozen storage, thereby regenerating the parent drug. Since urine collections involved sample retention in the bladder at 37° for collection intervals as long as 2–3 hr, the given percentages excreted unchanged probably are overestimates. It is possible that no unchanged ketoprofen, naproxen, or probenecid is excreted in urine. This study contrasts with previous reports of up to 50% of a dose of ketoprofen and 15–17% of doses of naproxen and probenecid being excreted in urine as the parent compound. Those reports probably reflect primarily the duration of frozen sample storage between collection and assay along with the urine collection schedules employed, the speed of the clinical procedures, and the analytical procedures used. Attention should be given to potential conjugate hydrolysis whenever the pharmacokinetics of carboxylic acids are studied.

Keyphrases □ Ketoprofen—urinary excretion of unchanged drug □ Naproxen—urinary excretion of unchanged drug □ Probenecid—urinary excretion of unchanged drug

Previous reports suggested that up to 50% of a dose of ketoprofen (1–4), 15% of a dose of naproxen (5–7), and 17% of a dose of probenecid (8–10) are excreted in urine as the unchanged drug. It also has been claimed that the percentage of each of these drugs excreted unchanged is variable among individuals or within individuals (2, 5, 10) and that the percentage excreted unchanged might be depressed with concurrent administration of other drugs (6). In contrast, this report presents evidence that, even in normal individuals, the renal excretion of ketoprofen, naproxen, and probenecid is negligible. Data reported previously probably reflected the collection, storage, and handling of urine samples instead of the urinary excretion of these compounds.

EXPERIMENTAL

The data were derived from several studies in which ketoprofen, naproxen, or probenecid was given orally to adult male volunteers judged to be normal after a physical examination, an extensive blood screen, and urinalysis. Participants received other drugs only where specifically stated.

Urine was analyzed within 30 min of serial collections from 0 to 1, 1 to 2, 2 to 3, 3 to 4, and 4 to 6 hr after the 13th dose of a regimen of 50 mg of ketoprofen[1] given every 6 hr or after the 13th dose of a regimen of 500 mg of probenecid[2] given every 6 hr. Urine was analyzed within 30 min of collection from 0 to 1, 1 to 2, 2 to 3, 3 to 6, 6 to 8, 8 to 10, and 10 to 12 hr after the seventh dose of a regimen of 500 mg of naproxen[3] given every 12 hr.

Ketoprofen, naproxen, probenecid, and their conjugates were analyzed by a sensitive high-pressure liquid chromatographic (HPLC) assay described previously (11). Conjugates were analyzed as the parent drug after simple alkaline hydrolysis (11).

[1] Orudis capsules, Ives Laboratories, New York, N.Y.
[2] Benemid tablets, Merck Sharp and Dohme, West Point, Pa.
[3] Naprosyn tablets, Syntex Laboratories, Palo Alto, Calif.

Table I—Urinary Excretion of Ketoprofen, Naproxen, and Probenecid in an Interdose Interval at Steady-State Plasma Concentrations with Urine Assayed Immediately after Collection

Dose and Interval	Volunteer	Percent of Dose Unchanged	Percent of Dose as Conjugates
Ketoprofen, 50 mg, 6 hr	1	0.5	67.9
	2	0.5	70.7
	3	0.7	82.9
	4	0.8	77.7
	5	0.5	69.1
Mean ± *SD*		0.6 ± 0.1	73.7 ± 6.4
Naproxen, 500 mg, 12 hr	6	0.4	64.4
	7	0.9	61.7
	8	0.4	42.5
	9	0.7	58.6
Mean ± *SD*		0.6 ± 0.2	56.8 ± 9.8
Probenecid, 500 mg, 6 hr (with ketoprofen regimen)	10	1.3	57.0
	11	1.4	33.4
	12	1.0	40.2
Mean ± *SD*		1.2 ± 0.2	43.5 ± 12.1

RESULTS

Table I shows the percentage of a dose of ketoprofen, naproxen, or probenecid appearing unchanged or as alkali-hydrolyzable conjugates in urine at steady-state plasma concentrations. On the average, only 0.6% of the ketoprofen dose, 0.6% of the naproxen dose, and 1.2% of the probenecid dose appeared in urine as the unchanged drug, but 74, 57, and 44% of these drugs were excreted as conjugates, respectively. None of the five people taking ketoprofen alone and none of the four subjects taking naproxen alone excreted >0.9% of unchanged drug in the urine.

The three individuals taking probenecid were simultaneously following the described ketoprofen regimen. None excreted >1.4% of the dose of probenecid (mean, 1.2%) or >0.2% of the ketoprofen dose as the unchanged drugs.

In an additional three individuals taking the ketoprofen regimen with aspirin[4] (975 mg every 6 hr), the maximum urinary excretion of unchanged ketoprofen observed was 0.9% of the dose (0.8 ± 0.1%), but 59.7 ± 3.6% was excreted as conjugates. When three of the four individuals taking naproxen undertook a further naproxen regimen together with 300 mg of cimetidine[5] given every 6 hr, the maximum urinary excretion of naproxen was 0.7% (0.5 ± 0.2%), and 65.6 ± 8.3% was excreted as conjugates. The data on the urinary excretion of ketoprofen, naproxen, and probenecid were derived from 14 volunteers.

Some samples were stored at −15° for assay at various dates after collection (Table II). Over a period of <2 months, the ketoprofen and naproxen content of these samples increased up to threefold at the apparent expense of the alkali-hydrolyzable conjugates, presumably as a result of conjugate hydrolysis, even in the frozen state. Furthermore, since a different sample of the same urine collection was stored for each repetition of the assay, the progressive accumulation in content of the unchanged drug (Table II) was specifically an effect of storage rather than of repeated freezing and thawing, which also appear to have an effect. When samples were frozen and then thawed after 12 hr, a period during which (by reference to Table II) one might expect a negligible increase in unchanged drug specifically due to storage, the content of unchanged ketoprofen increased by 14.2 ± 5.9% (n = 4), and the content of naproxen increased by 17.1 ± 14.6% (n = 3).

[4] Unbuffered 325-mg tablets, Eli Lilly & Co., Indianapolis, Ind.
[5] Tagamet tablets, Smith Kline and French Laboratories, Philadelphia, Pa.

0022-3549/80/1100-1254$01.00/0

Table II—Urine Sample Content after Storage at −15°

Sample Designation	Day of Assay	Concentration of Unchanged Drug, μg/ml	Concentration of Unchanged Drug plus Conjugates, μg/ml[a]	Increase in Unchanged Drug	
				μg/ml/day	μg/day/μg of conjugates
Ketoprofen 1	0	0.196	19.0		
	26	0.357	17.6	0.00619	0.000330
Ketoprofen 2	0	0.0728	3.39		
	28	0.0922			
	54	0.211			
	106	0.228	3.40	0.00160	0.000482
	$r = 0.904$, $\alpha(1) < 0.05$				
Ketoprofen 3	0	1.66	165.0		
	28	3.23		0.0561	0.000343
Ketoprofen 4	0	0.0281	1.26		
	28	0.0436			
	54	0.0864			
	106	0.362	1.26	0.00325	0.00263
	$r = 0.935$, $\alpha(1) < 0.05$				
Mean ± *SD*				0.0168 ± 0.0263	0.00094 ± 0.00112
Naproxen 1	0	22.0	679		
	8	26.3			
	22	30.9	676		
	60	46.7	728	0.405	0.000616
	$r = 0.999$, $\alpha(1) < 0.001$				
Naproxen 2	0	3.28	607		
	8	4.71	565		
	50	10.4	587	0.140	0.000232
	$r = 0.999$, $\alpha(1) < 0.025$				
Naproxen 3	0	2.32	321		
	1	3.09	289		
	43	7.67	315	0.117	0.000367
	$r = 0.994$, $\alpha(1) < 0.05$				
Mean ± *SD*				0.221 ± 0.160	0.000406 ± 0.000195

[a] Micrograms per milliliter of unchanged drug after alkaline hydrolysis (11).

Because all samples other than those assayed on Day 0 were stored frozen, their assay was subject to the effects not only of storage but also of freezing and thawing. As a result, elucidation of the kinetics of conjugate hydrolysis might be complex, requiring more detailed data than are presented here. However, regardless of whether the hydrolysis rates observed are interpreted as zero or first order, they are sufficient to account for the levels of unchanged drug that have been reported in the literature. A zero-order rate of production of 17 ng/ml/day (the average zero-order rate from Table II) leads to a 5% apparent excretion of unchanged ketoprofen in an individual who had taken a 50-mg dose and produced 1.5 liters of urine to be assayed 3 months later. A first-order daily rate of production of 0.9 ng of ketoprofen/μg of conjugates (Table II) would lead to an apparent 6% excretion of unchanged ketoprofen when the urine of an individual excreting 74% (Table I) of the dose as conjugates is assayed after frozen storage for 3 months. Similar calculations show that an apparent excretion of naproxen of ~4% could arise by delaying the assay of urine samples by 3 months. These calculations indicate that after 8 months of frozen storage, 13% of the dose as unchanged ketoprofen might be expected.

Figure 1 shows the apparent urinary excretion of unchanged ketoprofen by 12 volunteers (each taking three single 50-mg doses) whose 24-hr urine was stored at −15° for 20–100 days between collection and assay. A linear correlation between the apparent ketoprofen excretion by each volunteer[6] and the time of storage of the urine is significant ($r = 0.6$, $p < 0.0005$, $n = 32$). As predicted, after 3 months of storage, ~4–5% of the dose appeared to be in the urine as unchanged ketoprofen. In another study, 12 additional volunteers (each taking three single 50-mg doses), whose urine was frozen shortly after collection and thawed for the first time after 8 months, appeared to excrete 9.6 ± 2.0% ($n = 36$) of the dose unchanged.

Greater rates of hydrolysis were observed at room temperature (Table III). Samples doubled in their content of unchanged ketoprofen or naproxen within 4–5 hr. (The pH of eight urine samples taken from three volunteers at various times within 24 hr after dosage ranged between 5.4 and 6.7 immediately after collection.) Therefore, analytical units not alerted to the potential facile hydrolysis of ketoprofen or naproxen conjugates during extended processing of urine in the clinic or during sample workup might report even higher percentages of the drug excreted unchanged than the figures discussed here.

Furthermore, hydrolysis rates even higher than those at room temperature were observed at 37° (Table IV). Therefore, the extent of excretion of unchanged ketoprofen, naproxen, and probenecid shown in Table I, while less than that reported previously, probably is still an overestimate since urine collections at 2–3-hr intervals involve sample retention in the bladder at 37° for sufficient time for significant conjugate hydrolysis. It is possible that no unchanged drug is excreted into urine.

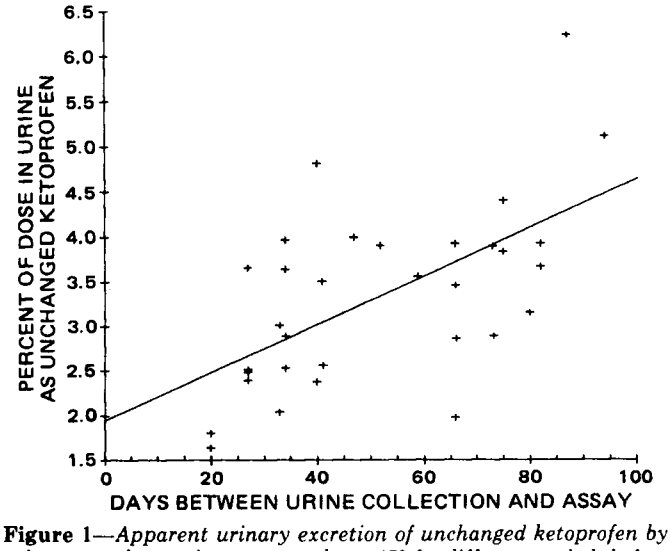

Figure 1—*Apparent urinary excretion of unchanged ketoprofen by volunteers whose urine was stored at −15° for different periods before assay.*

[6] Data on the third dose of four of the individuals are incomplete.

Table III—Urine Sample Content after Storage at Room Temperature

Sample Designation	Time of Assay, hr	Concentration of Unchanged Drug, μg/ml	Concentration of Conjugated Drug, μg/ml[a]	Increase in Unchanged Drug	
				μg/ml/hr	μg/hr/μg of conjugates
Ketoprofen 1	0	0.179	27.9		
	3.5	0.351			
	11	0.726			
	23	1.27			
	28	1.41		0.0448	0.00161
	$r = 0.998$, $\alpha(1) < 0.0005$				
Ketoprofen 2	0	0.148	32.3		
	3.5	0.337			
	11	0.625			
	23	1.23			
	28	1.37		0.0444	0.00137
	$r = 0.998$, $\alpha(1) < 0.0005$				
Mean ± *SD*				0.0446 ± 0.0003	0.00149 ± 0.00017
Naproxen 1	0	22.0	657		
	1	25.2			
	2	28.9		3.49	0.00531
	$r = 0.999$, $\alpha(1) < 0.025$				
Naproxen 2	0	3.28	604		
	5	6.93			
	11	10.1		0.616	0.00102
	$r = 0.996$, $\alpha(1) < 0.05$				
Naproxen 3	0	1.21	208		
	1	1.43			
	10	3.99			
	23	6.98		0.253	0.00121
	$r = 0.999$, $\alpha(1) < 0.025$				
Mean ± *SD*				1.45 ± 1.77	0.00251 ± 0.00242
Probenecid 1	0	13.9	203		
	3.5	19.2			
	11	20.6			
	23	33.3			
	28	36.0		0.787	0.00376
	$r = 0.985$, $\alpha(1) < 0.0025$				
Probenecid 2	0	42.8	332		
	3.5	45.6			
	11	52.1			
	23	69.3			
	28	77.1		1.23	0.00359
	$r = 0.994$, $\alpha(1) < 0.0005$				
Probenecid 3	0	4.14	138		
	3.5	4.60			
	11	5.70			
	23	7.61			
	28	7.73		0.128	0.000903
	$r = 0.993$, $\alpha(1) < 0.0005$				
Mean ± *SD*				0.715 ± 0.555	0.00275 ± 0.00160

[a] As the equivalent concentration of unchanged drug.

For ketoprofen and naproxen, at least three conjugates apparently are excreted in urine. If urine is assayed by the previously reported HPLC method (11) with the hydrolysis step omitted and acidic extraction conditions used as for plasma analysis, peaks are seen at 1.8 (largest), 2.0, and 2.9 (smallest) times the retention time for ketoprofen or at 2.5 (largest), 3.1 (smallest), and 4.1 times the retention time for naproxen. After extracts of urine from subjects taking either ketoprofen or naproxen were chromatographed, fractions of the eluent, each containing one of the three peaks, were collected. An aliquot of each eluent fraction was rechromatographed to ensure that fractions were not cross-contaminated with other conjugates or with the parent drug. The remaining part of each fraction underwent alkaline hydrolysis and then reextraction.

Further chromatography revealed a ketoprofen or a naproxen peak alone. For the first and second conjugate peaks of ketoprofen and the first and third conjugate peaks of naproxen, the conjugate gave rise to the parent compound on an equal peak area basis (to within 20%). For the other peaks (the smallest) from either compound, this finding was less certain due to the less straightforward concentration and extraction procedures that were necessary. Equivalence of peak areas confirms that conjugation does not involve the major chromophoric moiety but rather the carboxylic function and, hence, the ease of subsequent conjugate hydrolysis.

DISCUSSION

A negligible fraction of the dose of ketoprofen, naproxen, or probenecid is excreted in the urine as the unchanged drug, but a large fraction (up to 85%) appears as conjugates. Glucuronides of ketoprofen (12) and naproxen (8) were reported previously. Evidence in this study suggests that there are at least three conjugates of ketoprofen and three of naproxen. These conjugates are rapidly hydrolyzed to the parent compound by mild alkaline conditions (11). Hydrolysis of one or more conjugates of each drug appears to occur even in urine stored frozen, the most commonly employed mode of storage. Furthermore, freezing and thawing appear to promote hydrolysis, and even more rapid hydrolysis occurs at room temperature or at 37°.

Earlier reports of significant urinary excretion of unchanged ketoprofen, naproxen, and probenecid probably reflect not only the time of frozen storage between collection and assay but also the urine collection schedules employed, the speed of the clinical procedures, and the analytical procedures used, particularly the conditions and speed of the sample workup. The previously reported variability in ketoprofen, naproxen, and probenecid urinary excretion (2, 5, 10) probably is related to variability in these conditions, especially the time between collection and assay. Differences between different experimental groups might have

Table IV—Urine Sample Content after Storage at 37°

Sample Designation	Time of Assay, hr	Concentration of Unchanged Drug, μg/ml	Concentration of Conjugated Drug, μg/ml[a]	Increase in Unchanged Drug μg/ml/hr	Increase in Unchanged Drug μg/hr/μg of conjugates
Ketoprofen	0	0.196	18.8		
	0.25	0.235			
	0.5	0.276			
	0.75	0.335			
	1.0	0.375			
	2.0	0.502		0.155	0.00825
	$r = 0.994$, $\alpha(1) < 0.0005$				
Naproxen	0	22.0	657		
	0.25	23.6			
	0.5	26.2			
	0.75	27.6			
	1.0	30.2			
	2.0	40.3		0.24	0.0141
	$r = 0.997$, $\alpha(1) < 0.0005$				
Probenecid 1	0	20.6	203		
	12	51.7		2.59	0.0128
Probenecid 2	0	52.1	332		
	12	167		9.58	0.0288
Probenecid 3	0	5.7	138		
	12	12.9		0.600	0.00435
Mean ± *SD*				4.26 ± 4.7	0.0153 ± 0.0124

[a] As the equivalent concentration of unchanged drug.

a similar source, particularly where clinical and analytical schedules were not both randomized.

Since virtually no naproxen appears to be excreted unchanged in urine, reports of a decrease in naproxen excretion with concomitant doses of other drugs such as probenecid (6) must be questioned. The apparent decrease in unchanged naproxen excretion probably is a direct consequence of reduced naproxen conjugate excretion, which results in a diminished rate of postexcretion conjugate hydrolysis. The previously reported increase in renal clearance of probenecid with metabolic alkalosis (6, 9, 13) induced by intravenous sodium bicarbonate may largely result from improved conditions for conjugate hydrolysis in the alkaline urine in the bladder.

The ease of hydrolysis of the conjugates described probably is attributable to their being carboxylic acid esters. The greater apparent excretion of unchanged drug found for probenecid possibly is related to its being a benzoic acid, a stronger acid than the alkanoic acids, and it therefore forms a conjugate more susceptible to hydrolysis in urine between the times of excretion and assay. Data describing urinary excretion of other carboxylic acids, including many nonsteroidal anti-inflammatory drugs, should be interpreted with caution unless adequate attention was given to potential conjugate hydrolysis both *in vivo* and *in vitro*. The best safeguard at present is immediate assay after short urine collection intervals. If this procedure is not possible, immediate frozen storage for short periods might yield insignificant hydrolysis. It is conceivable, but by no means can it be assumed, that temperatures of $<-15°$ might permit negligible hydrolysis.

The virtual absence of unchanged compound in the urine may arise because alternative forms of elimination are extremely rapid. However, the total plasma clearances of ketoprofen and naproxen are only 5 and 0.7 liters/hr, respectively (5, 13). Even if these clearances are considered to be totally metabolic, extraction ratios of only 0.06 and 0.01 are apparent, respectively (assuming 1:1 partitioning of these drugs between plasma and the red blood cells). Ketoprofen, naproxen, and probenecid all are highly lipophilic compounds, a characteristic discusssed in a previous report (11), where it was noted that they can be extracted into ether and can be retained on lipophilic chromatographic columns even when they are almost completely ionized in water. Such compounds are potentially highly susceptible to renal tubular reabsorption (10). Perhaps reabsorption from the glomerular filtrate is so efficient that virtually no ketoprofen, naproxen, or probenecid is excreted into urine as such.

Since plasma rather than urine concentrations are more central to most pharmacokinetic interpretations, a similar ease of hydrolysis in plasma samples might lead to even more misleading descriptions of the general pharmacokinetic behavior of these drugs. However, in analyzing ketoprofen or naproxen in plasma immediately upon collection of blood samples, the ratio of the total concentration (parent drug and its conjugates) to that of unchanged ketoprofen or naproxen, respectively, was only 1.42 ± 0.14 (11 samples from one subject at various times up to 24 hr postdosing) or 1.10 ± 0.05 (39 samples from three subjects). If immediate assay did in fact reflect the relative proportions of compounds in the bloodstream, then even complete hydrolysis upon storage would affect the apparent ketoprofen or naproxen plasma levels only slightly, at least by comparison with the potential change in urine concentrations. After ~2 months of storage at −15° there appeared to be little change in the ketoprofen samples (total drug to parent drug ratio of 1.37 ± 0.08), but repeating the analysis for ketoprofen in five samples between 3 and 6 months after collection (involving further freezing and thawing) revealed that the content of unchanged ketoprofen had increased by 10–25%. Repeated analysis for naproxen only 2 months after collection showed that the conjugates had almost disappeared, with a commensurate increase (~10%) in the content of unchanged drug.

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ACKNOWLEDGMENTS

Ms. A. F. Fast managed the clinical sections of this study.