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OXYMORPHONE METABOLISM AND URINARY EXCRETION IN HUMAN, RAT, GUINEA PIG, RABBIT, AND DOG

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ABSTRACT:

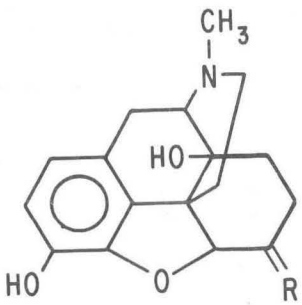
Oxymorphone was extensively metabolized by human, rat, dog, and guinea pig and to a lesser extent by rabbit. The most abundant metabolite in urine for all species was conjugated oxymorphone (12.7–81.7% administered dose) followed by 6β- and 6α-carbinols produced by 6-keto reduction of oxymorphone. 6β-Oxymorphol (0.2–3.1%) was found in the urine of all species, whereas 6α-oxymorphol (0.1–2.8%) was found only in human, rabbit, and guinea pig. Small amounts of free oxymorphone (≤10%) were excreted by all species except rabbit, which excreted 31.7%. Overall recoveries of oxymorphone and metabolites from urine ranged from 15–96%, of which >80% was excreted in the first 24 hr by all species except dog. Only 35% was excreted by dog during the first day. Stereoselectivity of 6-keto- reduction was observed for all species with the 6β-carbinol metabolite being most abundant in the urine of all but guinea pig. Considerable individual variability occurred in the excretion of free and conjugated oxymorphone by six human subjects following oral dosing. Species trends in the metabolism of 6-keto-opioids are discussed.

OM[1] is a semisynthetic narcotic analgesic derived from thebaine. The hydrochloride salt (Numorphan, Endo Pharmaceuticals, Inc.) is available for parenteral administration and is approximately 10 times more potent than morphine (1–4). The oral to parenteral potency ratio of OM in humans is about 1:10 which makes oral OM approximately equianalgesic to im administered morphine (1).

Although OM has been used clinically for the relief of pain for many years, no reports of its metabolism and urinary excretion have appeared. This study of OM in humans and laboratory animal species was undertaken as part of an ongoing study of the metabolism of 6-keto-opioids, *i.e.* narcotic agonists and antagonists with a morphinan-6-one skeletal structure. Thus far, the metabolism of naltrexone (5, 6), naloxone (6), hydromorphone (7), and hydrocodone (8) in humans, dogs, rats, guinea pigs, and rabbits has been reported. This article describes the metabolic profile and urinary excretion of OM in humans and laboratory animals following a single dose of OM.

Materials and Methods

Chemicals. Drugs and chemicals were obtained from the following sources: chloroform (preserved with 1% ethanol) and isopropanol, Burdick and Jackson Laboratories, Inc., Muskegon, MI; Tri-Sil Z, Pierce Chemical Co., Rockford, IL; OM, Endo Laboratories, Inc., Garden City, NY; 6β-hydrocodol (IS), Drug Addiction Laboratory, University of Virginia. 6αOM and 6βOM were synthesized by the selective reduction of OM as reported (9) (structure 1). The structure and purity of all drugs were checked by thin layer chromatography and GC/MS.



OM: R = O
6βOM: R = OH, H
6αOM: R = H, OH

STRUCTURE 1

Prior to use, chloroform was treated with calcium hydroxide (USP-FCC food grade, J. T. Baker Chemical Co., Phillipsburg, NJ) to eliminate phosgene-related impurities (10).

Animals and Human Subjects. The animals used in this study consisted of one male (9.6 kg) and one female (7.5 kg) mongrel beagle dogs (approximately 3 years old), six male Wistar albino rats (198 ± 5 g, 6–9 months old), six male Hartley albino guinea pigs (322 ± 30 g, 6–9 months old), and four male New Zealand albino rabbits (2.5 ± 0.1 kg, 10–12 months old). The six humans in this study were healthy, adult male federal prison volunteers from whom informed consent was obtained under NIH Guidelines; all were former narcotic addicts who were drug-free at the time of the study and had been so for at least 1 week. The age (years) and weight (kg), respectively, of each subject were as follows: subject 1, 34, 77.1; subject 2, 33, 118.8; subject 3, 29, 74.8; subject 4, 43, 76.2; subject 5, 38, 86.2; subject 6, 41, 74.8.

Abbreviations used are: OM, oxymorphone (4,5α-epoxy-3,14-dihydroxy-17-methylmorphinan-6-one); 6αOM, 6α-oxymorphol; 6βOM, 6β-oxymorphol; GC/MS, gas chromatography/mass spectrometry; CI, chemical ionization; MF, mass fragmentography; TMS, trimethylsilyl; IS, internal standard.

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Drug Administration and Urine Collection. Animals were housed in metal cages with stainless steel urine collection pans. Control urine was collected for 24 hr prior to drug administration. A single dose of OM·HCl (2.5 mg base/kg) was administered sc and urine was collected in 24-hr aliquots for 2 days. After each collection, the pans were rinsed with water, which was added to the urine. Feces were discarded.

Control urine from the six human subjects was collected for 24 hr prior to drug administration. OM·HCl (10 mg) was administered orally in solution and urine collections were made at 2, 4, 8, 12, 24, 48, 72, 96, and 120 hr following drug administration. During the intervals, subjects' urine specimens were combined for each interval.

Urine specimens were pooled for each laboratory animal species but not for the six human subjects. Following collection, the samples were frozen until time of analysis.

Gas Chromatography-Mass Spectrometry. Mass spectra were obtained on a Finnigan model 3300 quadrupole GC/MS operating in the methane CI mode. The system was interfaced to a Finnigan model 6000 interactive data system. Electron energy was 80 eV. The GC consisted of a 2 mm × 1.52 m glass column packed with 3% OV-225 on Gas-Chrom Q (100/120 mesh) coupled by a glass capillary tube to the mass spectrometer. Methane served as reagent and carrier gas at a flow rate of 20 ml/min. Temperatures of the injector, column, and ion source were as follows: 250, 200, and 100°C, respectively.

Quantitative measurements were made by MF; ions specific for each compound at its respective retention time were as follows: OM, m/z 502, 5.9 min; 6βOM, m/z 504, 4.7 min; 6αOM, m/z 504, 5.2 min; IS, m/z 374, 7.2 min. Plots of peak height ratios of compound/IS *vs.* concentration (0–2.0 μg/ml for OM; 0–1.0 μg/ml for metabolites) were linear throughout the concentration range. The lower limit of detection for each compound was approximately 20 ng/ml. Daily standard curves were constructed from five standard urine samples which were processed in the same manner as drug specimens. Least squares regression of these data provided slopes and intercepts for calculation of drug content of samples.

Urine Hydrolysis and Extraction. Urine aliquots (10 ml) were treated with IS (20 μg, 6β-hydrocodol) and extracted with and without acid hydrolysis. For hydrolysis, samples were mixed with concentrated HCl in a 10:1 ratio and heated in an autoclave at 115°C and 1.27 kg/cm^2 pressure for 20 m in a manner similar to that reported for the acid hydrolysis of morphine (11). Following hydrolysis and adjustment to pH 10 with sodium hydroxide, the hydrolyzed samples were processed in the same manner as those without hydrolysis.

The extraction procedure was identical to that used for the extraction of naltrexone (5) with the exception that the pH of the aqueous phases was adjusted to 10.0 ± 0.1 rather than 9.5 and the solvent, chloroform/isopropanol (9:1, v/v), was substituted for chloroform. Extracts were evaporated to dryness under a stream of nitrogen and transferred with methanol to acylation tubes (Regis Chemical Co., Morton Grove, IL). The methanol was evaporated and the residue was treated with Tri-Sil Z (100 μl). The sample tube was sealed and heated for 3 hr at 90°C. Samples were maintained at 90°C until just prior to analysis by GC/MS.

Extraction Efficiency. Preliminary studies were performed to determine the optimum conditions for extraction of OM, 6βOM, and 6αOM from urine with chloroform/isopropanol. Extraction efficiencies were highest in the pH range 9.5–10.5 with chloroform/isopropanol in a 9:1 (v/v) ratio. Recoveries ± SE at pH 10.0 for the entire extraction procedure were as follows: OM, 73.4 ± 1.2%; 6βOM, 67.9 ± 0.4%; 6αOM, 72.6 ± 0.4%.

Results

Identification of OM and Metabolites in Urine. OM, conjugated OM, and 6-keto reduction metabolites (6βOM and conjugated 6βOM) appeared immediately in human urine (0–2-hr collection) following the administration of a single 10-mg oral dose of OM·HCl. These substances also appeared in the 0–24-hr urines of rat, dog, guinea pig, and rabbit following a single sc dose of OM·HCl (2.8 mg/kg). Small amounts of free and conjugated 6αOM were found in human, guinea pig, and rabbit urine. The identities of OM and the metabolites were established by comparison of GC retention times and methane GC/MS-CI spectra of the silyl derivatives obtained from drug urine extracts with equivalent extracts of authentic standards (fig. 1). Conjugated metabolites were cleaved by an acid hydrolysis procedure (11) prior to analyses. Initial studies on the stability of OM, 6βOM, and 6αOM during acid hydrolysis ruled out the possibility of artifact formation or loss from hydrolysis. No other metabolites were detected by these methods.

Assay Development. A reliable quantitative assay was developed for OM, 6βOM, and 6αOM in urine. The method consisted of extraction of untreated or acid-hydrolyzed urine by an established procedure (5) followed by measurement with methane CI-MF. Various GC liquid phases (OV-225, OV-17, Silar-5CP, SE-30) were tested for separation of drug components. GC columns packed with OV-225 provided the best resolution of all phases tested. The $(M - 15)^+$ ions at m/z 502 and 504 for OM and 6-hydroxy metabolites were selected for monitoring by MF on the basis of their relative abundance (fig. 1). Confirmatory molecular ions at m/z 517 and 519 were monitored on an occasional basis to ensure identification. Typical MF scans are shown in fig. 2 for extracts of standards, and drug urine of human and animals.

Quantitative Studies. The amount of unchanged OM excreted in urine varied from about 2% (administered dose) for human and rat to 31.7% for rabbit (table 1). Dog and guinea pig excreted 5.3 and 10%, respectively. Conjugated OM was present in substantial quantities for all species. Rabbit (11.7%) and rat (12.7%) excreted almost equivalent amounts, whereas human (44.1%), dog (56.4%), and guinea pig (81.7%) excreted considerably larger amounts. Metabolic reduction of the 6-keto group of OM occurred to a small extent in all species. Guinea pig and rabbit produced the largest amount of reduced metabolites (about 4% total free and conjugated 6αOM and 6βOM) followed by human (3.0%), dog (1.6%), and rat (0.2%). Stereoselectivity of reduction was found for all species with 6βOM being the favored isomer by all animals except guinea pig.

Total drug and metabolite present in the 0–120-hr samples for humans and the 0–48-hr samples for laboratory animals ranged from a low of 14.9% for rat to a high of 95.8% for guinea pig. Intermediate recoveries were found for rabbit (47.3%), human (49.0%), and dog (63.3%). Of these totals, greater than 80% was excreted in the first 24-hr period following drug administration for all species except dog which showed a greater excretion of OM and metabolites during the second day.

Considerable intersubject variability was present for the six human subjects in their excretion pattern of OM and metabolites (table 2). Free OM excretion over a 5-day period ranged from 0.3 to 2.5%. Excretion of conjugated OM ranged from 27.2 to 61.3%. Free 6βOM ranged from 0.1 to 0.7%. Excretion of conjugated 6βOM was less variable with a range of 2.1 to 3.0%. Small amounts of conjugated 6αOM were detected in three subjects.

OM, conjugated OM, and conjugated 6βOM were detectable through 5 days and reached cumulative mean excretions of 1.9, 44.1, and 2.6%, respectively (fig. 3). Free 6βOM was detectable only through 48 hr at a mean cumulative excretion of 0.3%. The half-life ($t_{1/2}$) of free OM was estimated from urinary excretion data of three subjects. With the Sigma-Minus method for calculation of $t_{1/2}$, (12) the $t_{1/2}$ ± SE was 8.9 ± 0.4 hr. The mean $t_{1/2}$ ± SE from urinary excretion rate plots for the same three subjects was 7.6 ± 2.0 hr.

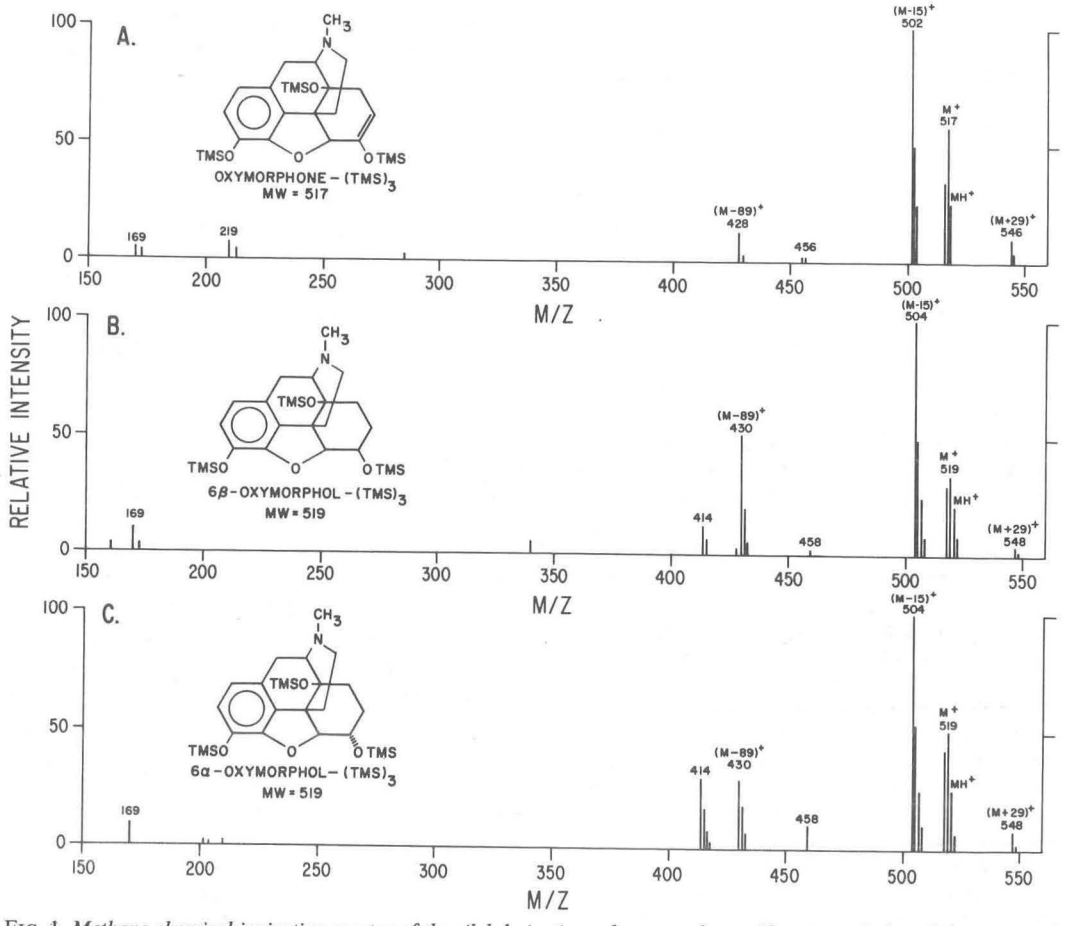

FIG. 1. *Methane chemical ionization spectra of the silyl derivatives of oxymorphone, 6β-oxymorphol, and 6α-oxymorphol.*

TABLE 1

Recovery of drug and metabolites from urine following a single dose of oxymorphone hydrochloride

These data represent the means of triplicate determinations and are expressed as percentage of administered dose. Conjugated (Conj) drug and metabolites were determined by subtraction of free from total concentration after acid hydrolysis.

Species (*N*)	Dose/Route	Time	OM		6βOM		6αOM		Total	Total First 24 hr
			Free	Conj	Free	Conj	Free	Conj		
		hr			%				%	%
Human (6)	10 mg (oral)	0–120	1.9	44.1	0.3	2.6	0	0.1	49.0	82
Rat (6)	2.5 mg/kg (sc)	0–48	2.0	12.7	0.1	0.1	0	0	14.9	97
Dog (2)	2.5 mg/kg (sc)	0–48	5.3	56.4	0.4	1.2	0	0	63.3	35
Guinea pig (6)	2.5 mg/kg (sc)	0–48	10.0	81.7	0.3	1.0	0.5	2.3	95.8	94
Rabbit (6)	2.5 mg/kg (sc)	0–48	31.7	11.7	2.0	1.1	0.6	0.2	47.3	99

Discussion

The metabolite profile of OM in humans and laboratory animals is similar in a number of respects to that observed for other 6-keto-opioids. The high degree of conjugation of OM for all species except rabbit is found with only minor exception for naltrexone (6), naloxone (6), and hydromorphone (7). The free phenolic group common to each of these substances is highly accessible for conjugation (presumably as the glucuronide) by liver microsomal enzymes similar to that found for morphine (13). The low ratio of conjugated material to intact OM in the rabbit appears to be an exceptional case since more conjugate than free parent was found in urine following naltrexone (6), naloxone (6), hydromorphone (7), and hydrocodone (8) administration. Saturation of enzyme systems does not appear to be the reason for the low conjugate/parent excretion ratios of OM in rabbit since the dosage (2.5 mg/kg) was intermediate in the dosage range of the other 6-keto-opioids, all with high conjugate/parent ratios. The dosage in the other studies varied from approximately 1–5 mg/kg (6–8). It seems more likely that differences in liver glucuronyltransferase activity toward OM *vs.* the other 6-keto-opioids account for the observed differences in glucuronidation. Separate enzymes have been isolated from rabbit liver microsomes for the glucuronidation of morphine and *p*-nitrophenol (13) indicating narrow substrate specificities within this family of conjugating enzymes.

The presence of the free phenolic group of OM also appears to preclude significant *N*-dealkylation, similar to that found for naltrexone (6), naloxone (6), and hydromorphone (7). Methylation of the phenolic group substantially increases *N*-demethylation as evidenced by the *N*-demethylation of hydrocodone (8) and oxy-

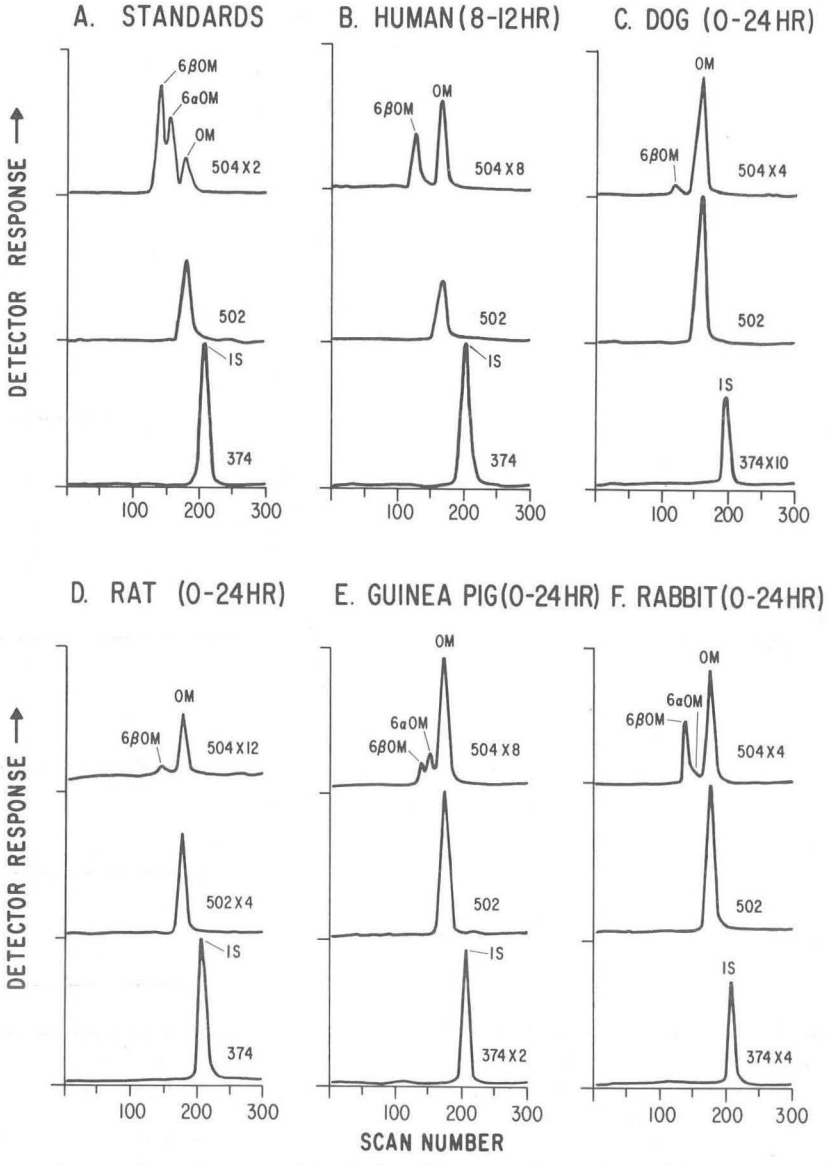

FIG. 2. *Mass fragmentography recordings of extracts of standards and human and animal urine following oxymorphone administration.*

TABLE 2

Urinary excretion of oxymorphone and metabolites in six human subjects

These data are expressed as percentage of dose and represent the cumulative means of triplicate determinations of samples collected over a 120-hr period following a single 10-mg oral dose of oxymorphone hydrochloride.

Subject	OM		6βOM		6αOM		Total
	Free	Conj	Free	Conj	Free	Conj	
			%				%
1	2.5	28.5	0.6	2.8	0	0.1	34.5
2	5.0	27.2	0.2	2.5	0	0.2	35.1
3	0.6	63.1	0.1	2.1	0	0	65.9
4	0.7	48.8	0.2	2.4	0	0.3	52.4
5	0.3	42.0	0.7	2.8	0	0	45.8
6	2.4	54.9	0.2	3.0	0	0	60.5
Mean ± SE	1.9 ± 0.7	44.1 ± 5.9	0.3 ± 0.1	2.6 ± 0.1	0	0.1 ± 0.1	49.0 ± 5.3

codone (14), the *O*-methyl ether of OM. Whether these effects of the phenolic group on *N*-dealkylation are a result of differences in enzyme specificities or differences in substrate accessibility due to the higher lipid solubilities of the ethers *vs.* free phenols remains unclear.

Reduction of the ketone group of OM to 6α- and 6β-carbinols also occurred for naltrexone (6), naloxone (6), and hydromorphone (7) for all species. Reduction of hydrocodone also was evident for all species except dog (8). The degree of reduction of OM, naltrexone, and naloxone varied from species to species, but

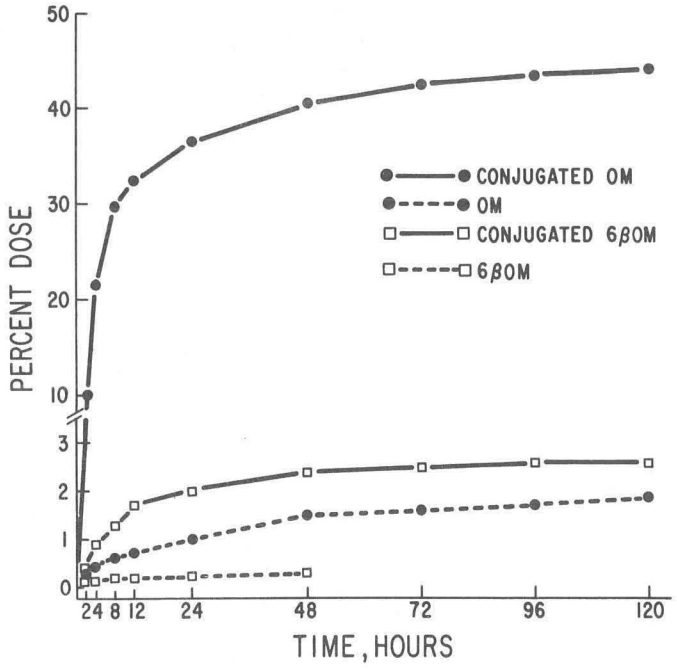

FIG. 3. *Mean cululative urinary excretion of free and conjugated oxymorphone and 6β-oxymorphol for six human subjects following a 10-mg oral dose of oxymorphone hydrochloride.*

was consistently low (<10% of administered dose) for rat, dog, and rabbit. A highly variable rate of reduction has been found for humans and guinea pigs with up to 40% of the dose of naltrexone being excreted by humans as reduced metabolites (5). The selectivity of reduction (6α- *vs.* 6β-epimer formation) also was variable with the 6β-carbinol being highly favored by most species except guinea pig.

Overall urinary recoveries of OM and metabolites were quite comparable with those found for other 6-keto-opioids. Recoveries for the 6-keto-opioids for rat range from 3–17% (6, 8), whereas recoveries for the remaining species generally range from 30–60% (6–8) of administered dose. It is likely that the remainder of the dose was present as polar unidentified metabolites as well as being partially eliminated in feces. The moderate recovery of OM (~50%) and other 6-keto-opioids from human urine following oral dosing indicates that these compounds are well absorbed from the gastrointestinal tract. Excretion of free OM in urine was low for all species (2–10%) in this study except rabbit. This also appears to be an exception for this species since the rabbit generally has excreted significantly more conjugated metabolite than free parent drug in the 6-keto-opioid series (6–8).

Individual variation in human excretion of OM and metabolites was quite similar to that found for hydromorphone (7), the chemical derivative of OM without a 14-hydroxy group. Like hydromorphone, excretion of free OM varied over a 10-fold range within six subjects, whereas conjugated OM varied only over an approximate 2-fold range. Also, mean keto reduction for these two compounds was less than 3%.

Emerging species trends from these and previous studies on the metabolism of 6-keto-opioids indicate that of the five mammalian species: a) conjugation (glucuronidation) activity is greatest in the dog, but is high in human, guinea pig, and rabbit; b) 6-ketoreductase activity is high in human and guinea pig followed by rabbit and is almost nonexistent in rat and dog; c) 6-keto reduction of opioids with a free phenolic group is stereoselective; reduction generally favors formation of the 6-β-hydroxymetabolite for all species except guinea pig; d) *N*-dealkylation of 6-keto-opioids with a free phenolic group is not a significant metabolic pathway but becomes important with *O*-methyl ethers such as hydrocodone for human and dog. Perhaps further metabolic studies of the opioids will add to these generalizations of species trends in metabolism.

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