Biotransformation and pharmacokinetics of ethylmorphine after a single oral dose

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- 1 The pharmacokinetics of ethylmorphine after administration of a single dose of the cough mixture Cosylan[®] were investigated in 10 healthy subjects.
- 2 The median urinary recovery of ethylmorphine and measured metabolites was 77% over 48 h. The median $t_{\max}$ of unchanged ethylmorphine was 45 min, and the terminal elimination $t_{1/2}$ was 2 h. Ethylmorphine-6-glucuronide was found to be the major metabolite.
- 3 Two subjects had significantly lower urinary recovery (0,48 h) of morphine and morphine-glucuronides than the remainder. Furthermore, these two had urinary metabolic ratios (MR_O) and partial metabolic clearances (CL_{mO}) for *O*-deethylation of ethylmorphine tentatively classifying them phenotypically as poor metabolisers of the debrisoquine/sparteine type.
- 4 Genotyping for cytochrome P450 (*CYP*) 2D6 alleles revealed five homozygote (wt/wt) and five heterozygote subjects. Two subjects phenotypically classified as poor metabolisers were genotypically *CYP2D6A*/wt and *CYP2D6D*/wt, respectively.
- 5 Serum and urine samples taken more than 8 and 24 h after administration of ethylmorphine respectively, contained morphine and morphine-glucuronides, but no ethylmorphine, ethylmorphine-6-glucuronide or (serum only) norethylmorphine. Norethylmorphine could be detected after hydrolysis of urine samples in all subjects. The urinary recovery of the active metabolites morphine and morphine-6-glucuronide after administration of ethylmorphine varied by a factor of 9 between individuals.
- 6 The wide variation in recovery of morphine and morphine-glucuronides after oral administration of ethylmorphine could not be explained simply by a difference in *CYP2D6* genotype. Constitutional variation in other enzymatic pathways involved in ethylmorphine metabolism is probably crucial. Ratios of morphine to parent drug cannot be used to distinguish the source of morphine after administration of ethylmorphine. Norethylmorphine should be included in urine assays for opiates in forensic toxicology, and no firm conclusions about the source of morphine are possible based on serum samples obtained more than 24 h after drug administration.

Keywords ethylmorphine morphine human oral dose metabolism pharmacokinetics *CYP2D6*

Introduction

Ethylmorphine is the 3-ethoxy congener of morphine, used mainly as an antitussive [1]. In addition it has an analgesic effect, and some potential for abuse [2]. In all these respects ethylmorphine resembles codeine which is the 3-methoxy homologue of morphine. It has recently been demonstrated that ethylmorphine is metabolised to morphine in humans [3], and that this *O*-deethylation co-segregates with the debrisoquine-type genetic metabolic polymorphism, suggesting that the CYP2D6 isozyme is involved [4]. The analgesic effect and abuse liability of ethylmorphine might therefore be linked to its metabolism to morphine. In turn, morphine may also mediate its effects through formation of active metabolites including the 6-glucuronide [5–7]. In addition morphine-3-glucuronide binds to opiate receptors with a low affinity [8, 9], and may be a functional antagonist of several of the effects of morphine [10, 11]. Thus, the overall effect of morphine and thereby also at least some effects of ethylmorphine, could depend upon the ex-

tent and pattern of glucuronidation. Ethylmorphine is metabolised by two other pathways, namely 6-glucuronidation to ethylmorphine-6-glucuronide and *N*-demethylation to norethylmorphine (Figure 1). The relative importance of the three different pathways of ethylmorphine metabolism has not previously been defined fully in humans.

Because ethylmorphine is abused in large doses for its euphoric potential the dose given in this study was high. The present study addressed this issue with special emphasis on the formation of morphine and morphine-glucuronides after administration of ethylmorphine.

Methods

Materials

Cosylan® (Parke-Davis) was purchased from a local pharmacy. Ethylmorphine hydrochloride was obtained

Figure 1 Metabolic pathways of ethylmorphine. Solid lines indicate formation of metabolites identified by reference substances, dotted lines indicate formation of putative metabolites identified by an increase in the amount of precursor after hydrolysis.

from Weiders Farmasøytiske A/S (Oslo, Norway). Codeine- d_3 hydrochloride, norcodeine- d_3 hydrochloride, morphine-3-glucuronide, morphine-6-glucuronide and normorphine were purchased from Sigma Chemical Co. (St Louis, MO, USA). Morphine hydrochloride was purchased from Norsk Medisinaldepot (Oslo, Norway). Pentafluoropropionic anhydride was purchased from Pierce (Rockford, IL, USA), butylacetate (glass distilled grade) from Rathburn (Walkerburn, Scotland), acetone (pro analysis) and dichloromethane (for chromatography) from Merck (Darmstadt, Germany). Acetonitrile (Fisons, England) was h.p.l.c.-grade. Norethylmorphine was synthesised from ethylmorphine according to the method of Xu, with a purity > 99% determined by h.p.l.c. [12]. Ethylmorphine-6-glucuronide was synthesised from ethylmorphine according to the method of Bugge, with a purity > 98% quantified by h.p.l.c. [13]. All other reagents were analytical grade, and used as received.

Subjects

Ten male volunteers (age 24 ± 1 years; weight, 71.1 ± 10.2 kg) were recruited to the study. Each was shown to be healthy on the basis of clinical history, physical examination, laboratory tests (haemoglobin, creatinine, ASAT, ALAT, γ -GT), and immunological screening for drugs in urine. All subjects were CYP2D6 genotyped. The study protocol was approved by the regional ethics board (Oslo, Norway) and all subjects gave written informed consent.

Medications

Cosylan® (Parke-Davis) 0.88 ml kg⁻¹ body weight was administered as a single oral dose. Cosylan® contains ethylmorphine hydrochloride 1.7 mg, extract from the cortex of *Cascara Sagrada* 50 mg, menthol 0.2 mg, concentrated ethyl alcohol 0.046 ml, sucrose 373 mg, sodium saccharine 1.09 mg, monohydric liquid glucose 399 mg, and benzoic acid 1.37 mg to 1 ml. The ethanol content of the dose (2.2 g for a person weighing 70 kg) gives a maximum blood alcohol concentration of 0.004% (1 mM), which is far below levels known to affect drug metabolism.

Protocol

Subjects abstained from food and fluids beginning at 24.00 h on the evening before the study. They arrived at the testing facility at 08.00 h on the study day, and received the oral dose of ethylmorphine at 09.00 h. Free access to sugar free soft drinks was allowed during the subsequent 8 h. A standard low-fat lunch was provided 4 h after the dose. Serum samples were drawn through an indwelling intravenous catheter immediately before dosing and thereafter at 15, 30 and 45 min and at 1, 2, 4 and 8 h, and at 24 and 48 h by puncture of the cubital vein. Urine samples were obtained immediately before dosing and thereafter collected from 0 to 4, 4 to 8, 8 to 24, and 24 to 48 h. An additional sample was collected after 7 days.

Adverse reaction data were collected using a non-directed adverse event questionnaire and by spontaneous report.

Analytical methods

Ethylmorphine and norethylmorphine were quantified in serum and urine by gas chromatography—mass spectrometry, using a Hewlett-Packard 5890 gas chromatograph, a 5970A mass selective detector and 59970 CS ChemStation software for serum analysis (Hewlett-Packard, USA) and a Unix-ChemSystem (59940 HP-UX) for urine analysis. The column and oven temperature program were as described previously [14]. Serum samples were centrifuged prior to solid-phase extraction on Analytichem Bond Elut Certify columns. Urine samples were extracted on Bound Elut Certify columns without prior centrifugation. The compounds were eluted with dichloromethane/isopropyl alcohol/NH₃ as recommended by the manufacturer. The eluates from the extraction columns were evaporated to dryness, and derivatised with pentafluoropropionic (PFP) anhydride as described previously [14]. Ethylmorphine-6-glucuronide and norethylmorphine-6-glucuronide in urine were measured indirectly by the same method after acid hydrolysis. Samples of 1.0 or 0.5 ml were mixed with an equal volume of 6N HCl and incubated at 80° C for 16 h. Selected ions were monitored in the EI mode at m/e 296 and 459 for ethylmorphine-PFP, m/e 355 and 591 for norethylmorphine-PFP, m/e 285 and 448 for codeine- d_3 -PFP and m/e 355 and 577 for norcodeine-PFP. Codeine- d_3 and norcodeine- d_3 were used as internal standards for ethylmorphine and norethylmorphine, respectively. The extraction recoveries in serum at 0.05 and 0.2 $\mu\text{mol l}^{-1}$ varied from 82 to 96% for ethylmorphine, norethylmorphine, codeine- d_3 and norcodeine- d_3 , with a precision of 3.6 to 18%, and accuracy from 7.7 to 11.5%. The limits of detection in serum were 0.013 and 0.014 $\mu\text{mol l}^{-1}$ for ethylmorphine and norethylmorphine, whereas the limits of determination were 0.039 and 0.038 $\mu\text{mol l}^{-1}$, respectively. The extraction recoveries in urine at 1.0 and 30.0 $\mu\text{mol l}^{-1}$ varied from 85 to 96% for ethylmorphine, norethylmorphine, codeine- d_3 and norcodeine- d_3 , with a precision of 7.7 to 13.7%, and accuracy from 6.4 to 12.6%. The limits of detection in urine were 0.055 and 0.061 $\mu\text{mol l}^{-1}$ for ethylmorphine and norethylmorphine, whereas the limits of determination were 0.18 $\mu\text{mol l}^{-1}$ for both compounds.

Serum and urine samples were analysed for ethylmorphine-6-glucuronide, morphine, morphine-3-glucuronide, morphine-6-glucuronide and normorphine by solid phase extraction and h.p.l.c. analysis according to Svensson [15, 16] using an ASPEC robot (Gilson Medical Electronics, France) for automated column extraction, as described previously [17]. Because of a retention time of about 40 min, and thereby a relatively low sensitivity under the described chromatographic conditions, analysis of ethylmorphine-6-glucuronide in serum was done separately using a Supelcosil LC-CN 25.0 cm $\times$ 4.6

mm i.d. column (Supelco Inc, USA). The mobile phase consisted of 5% v/v acetonitrile in 10 mM NaH_2PO_4 , pH 4.6, at a flow rate of 0.7 ml min⁻¹. The extraction recoveries at the concentrations 0.75, 2.0 and 20 µmol l⁻¹ for ethylmorphine-6-glucuronide, and at the concentrations 0.1, 1.0 and 20 µmol l⁻¹ for morphine, morphine-3-glucuronide, morphine-6-glucuronide and normorphine in serum varied from 68 to 90%, with a precision of 1.9 to 7.8% and accuracy from 4.1 to 10.5%. The limits of detection in serum were 0.2, 0.0025, 0.02, 0.002 and 0.005 µmol l⁻¹ for ethylmorphine-6-glucuronide, morphine, morphine-3-glucuronide, morphine-6-glucuronide and normorphine, respectively. The limits of determination for the same compounds were 0.5, 0.0075, 0.05, and 0.01 µmol l⁻¹. Extraction recoveries of ethylmorphine-6-glucuronide at the concentrations 2.0, 20.0 and 50.0 µmol l⁻¹, and morphine, morphine-3-glucuronide, morphine-6-glucuronide and normorphine at the concentrations 0.1, 1.0 and 20 µmol l⁻¹ in urine varied from 73 to 92%, with a precision of 3.8 to 10.1% and accuracy from 4.9 to 12.0%. In urine, normorphine-glucuronides were measured indirectly by the same method after acid hydrolysis as described above. The limits of detection in urine were 1.0, 0.005, 0.1, 0.005, 0.015 and 0.5 µmol l⁻¹ for ethylmorphine-6-glucuronide, morphine, morphine-3-glucuronide, morphine-6-glucuronide, normorphine and normorphine-glucuronides, respectively. The limits of determination in urine for the same compounds were 2.0, 0.01, 0.5, 0.01, 0.05 and 0.5 µmol l⁻¹.

Genotyping

Genomic DNA was isolated according to Gustafson *et al.* [8]. PCR-based allele-specific analysis for *CYP2D6A* and *CYP2D6B* was performed as described by Heim & Mayer [19]. The presence of *CYP2D6C*-alleles was determined by single stranded conformation polymorphism (SSCP) analysis according to Dahl and co-workers [20]. *CYP2D6D* was determined with EcoRI RFLP as previously described [21].

Pharmacokinetic analysis

The AUC(0,8h) of ethylmorphine was calculated using the linear trapezoidal rule. The maximum serum concentration (C_{max}) and the time of its occurrence (t_{max}) were noted directly from the data. The elimination half-life ($t_{1/2,z}$) of ethylmorphine was determined by linear regression of the log values of the last 4–6 data points. Clearance (CL) = $F \cdot \text{dose} / \text{AUC}(0,8\text{h})$, where F is the fractional urinary recovery (0,48h) of ethylmorphine and all measured metabolites. The apparent volume of distribution (V_z) = CL / λ_z .

Urinary recovery and metabolic ratios

Urinary recovery was calculated from the sum of molar amounts of the parent drug and metabolites in the urine samples from 0 to 48 h.

The urinary ratio of ethylmorphine/morphine and

morphine-3-glucuronide plus morphine-6-glucuronide was used as the metabolic ratio (MR_O) determining the patency of the *O*-deethylation pathway [4].

Partial metabolic clearance

Formation clearances (CL_m) to the different ethylmorphine metabolites were calculated from Ae/AUC , where Ae for *O*-deethylation of ethylmorphine is the sum in µmol of urinary recovery of morphine, morphine-3-glucuronide and morphine-6-glucuronide, Ae for the *N*-demethylation of ethylmorphine is the sum in µmol of norethylmorphine and norethylmorphine-6-glucuronide recovery, and Ae for the 6'-glucuronidation of ethylmorphine is the ethylmorphine-6-glucuronide recovery in urine from 0–8 h. AUC is the area under the serum ethylmorphine concentration vs time curve from 0–8 h.

Statistics

Differences between groups were evaluated using the Mann Whitney test.

Results

No major adverse effects were reported. Seven subjects reported some dizziness from 30 to 60 min after ethylmorphine administration, two reported slight drowsiness, and one reported loose stools.

Two subjects had minute concentrations of free morphine, morphine-3 and 6-glucuronide in serum, and only 2.5 and 3.9% of the administered dose of ethylmorphine was recovered in these forms in the urine, compared with a median recovery of 14.7% ($P = 0.0495$) among the remaining eight subjects. Furthermore, the urinary MR_O values for these two subjects were 1.31 and 1.74, compared with 0.09 to 0.61 in the other eight subjects ($P = 0.0502$), and metabolic clearance of *O*-deethylation was 12.8 and 23.0 ml min⁻¹, compared with 88.9–321.8 ml min⁻¹ in the other subjects ($P = 0.0502$). These measures of metabolic capacity were in accordance with those reported by Rane *et al.* [4] who investigated the *O*-deethylation of ethylmorphine to morphine in phenotypically determined (dextromethorphan) poor and extensive metabolisers of the debrisoquine-type.

Genotyping for *CYP2D6A*, *CYP2D6B*, *CYP2D6C* and *CYP2D6D* revealed five subjects with homozygote wild type alleles (wt/wt), one heterozygote *CYP2D6A*/wt, two heterozygote *CYP2D6B* and two heterozygote *CYP2D6D*. No homozygote mutant alleles were detected. The two subjects phenotypically classified as poor metabolisers were *CYP2D6A*/wt and *CYP2D6D*/wt, respectively.

The urinary recoveries of parent drug and metabolites are shown in Table 1. Glucuronidation of ethylmorphine to ethylmorphine-6-glucuronide was the major metabolic pathway constituting 28 to 53% of the dose in 10 subjects. The *O*-deethylation of ethylmorphine to morphine with subsequent glucu-

Table 1 Urinary recovery (in % of μmol given) of ethylmorphine and its metabolites up to 48 h after administration of single oral dose of ethylmorphine (1.5 mg kg^{-1}) to eight subjects with $\text{MR}_0 < 0.7$, and two subjects with $\text{MR}_0 > 1.3$

Interval	1		2		3		4		5		6	
	Ethylmorphine Median	Range	Ethylmorphine-6-glucuronide Median	Range	Morphine Median	Range	Morphine-3-glucuronide Median	Range	Morphine-6-glucuronide Median	Range	3 + 4 + 5 Median	Range
0.4 h ($\text{MR}_0 < 0.7$)	3.6	(0.2-4.0)	21.0	(14.3-26.5)	0.2	(0.1-0.6)	4.1	(1.5-6.0)	1.0	(0.5-2.0)	5.3	(2.3-8.1)
0.8 h ($\text{MR}_0 < 0.7$)	4.1	(1.3-4.9)	32.1	(21.5-44.0)	0.4	(0.2-0.8)	6.8	(2.6-9.5)	1.7	(0.9-2.8)	8.9	(3.9-12)
0.24 h ($\text{MR}_0 < 0.7$)	4.3	(1.8-5.2)	41.1	(27.6-53.4)	0.6	(0.3-1.2)	9.6	(4.0-13.8)	2.5	(1.2-3.9)	12.8	(5.9-18)
0.48 h ($\text{MR}_0 < 0.7$)	4.4	(1.8-5.2)	41.1	(27.6-53.4)	0.8	(0.4-1.4)	12.2	(5.5-16.1)	3.3	(1.5-5.0)	16.7	(7.3-21)
0.48 h ($\text{MR}_0 > 1.3$)	4.7	(4.4-5.1)	47.5	(43.2-51.9)	0.2	(0.1-0.2)	2.3	(1.9-2.7)	0.8	(0.6-1.0)	3.2	(2.5-3.4)
Interval	7		8		9		10		11*		12	
	Norethylmorphine Median	Range	Norethylmorphine-6-glucuronide Median	Range	Morphine Median	Range	Normorphine Median	Range	Normorphine-glucuronides Median	Range	Total Median	Range
0.4 h ($\text{MR}_0 < 0.7$)	2.4	(1.3-4.3)	1.2	(0.5-3.7)	3.7	(2.7-8.0)	1.1	(0.7-2.2)	0.0	(0.0-1.5)	35.2	(28.3-45)
0.8 h ($\text{MR}_0 < 0.7$)	3.4	(3.0-6.8)	2.8	(1.2-5.9)	6.7	(4.2-12.7)	2.0	(1.4-3.4)	0.4	(0.0-3.2)	54.9	(42.0-62)
0.24 h ($\text{MR}_0 < 0.7$)	4.2	(3.6-8.9)	4.5	(2.1-7.9)	8.5	(5.7-16.9)	3.9	(2.7-6.9)	0.8	(0.0-4.7)	74.7	(58.2-84)
0.48 h ($\text{MR}_0 < 0.7$)	4.3	(3.6-9.1)	4.6	(2.2-8.0)	8.7	(5.8-17.1)	4.6	(3.4-8.6)	1.3	(0.0-7.4)	76.9	(64.9-92)
0.48 h ($\text{MR}_0 > 1.3$)	17.1	(15.7-18.5)	2.2	(2.0-2.4)	19.3	(17.7-20.9)	1.7	(1.6-1.9)	0.7	(0.6-0.8)	77.2	(73.2-81)

*Normorphine-glucuronides were detected in all subjects in one or more time intervals.

ronidation was found to be the second most important transformation in eight subjects with at $MR_O < 0.7$, representing about 15% of the given dose. The variation among all 10 subjects was nine-fold with respect to the formation of the pharmacologically active metabolites morphine and morphine-6-glucuronide. Products of the *N*-demethylation pathway (nor-ethylmorphine and norethylmorphine-6-glucuronide) accounted for approximately 9% in eight of 10 subjects ($MR_O < 0.7$), and 17.7–20.9% in the two subjects with $MR_O > 1.3$ ($P = 0.0502$). Normorphine and normorphine-glucuronides which could be formed by both *O*-deethylation and *N*-demethylation together represented about 2–5% of the ethylmorphine dose. The net recovery of ethylmorphine and metabolites varied from 65 to 92%. Ethylmorphine and all measured metabolites were detected in the urine of all 10 subjects in the first three collection intervals (0–4, 4–8 and 8–24 h). Among the eight subjects with $MR_O < 0.7$, ethylmorphine-6-glucuronide was not detected in any of the samples collected between 24 and 48 h, ethylmorphine was detected in three of eight subjects, norethylmorphine was detected in six of eight subjects, and morphine, morphine-3-glucuronide, morphine-6-glucuronide, norethylmorphine-6-glucuronide, normorphine and normorphine-glucuronides were detected in all eight subjects also in the 24 to 48 h interval. In the two subjects with $MR_O > 1.3$, all compounds except morphine in one of the subjects, were detected in the 24–48 h interval. No ethylmorphine or metabolites were detected in serum or urine 7 days after administration in any of the 10 subjects.

A weak positive correlation was found between the glucuronidation and the *N*-demethylation pathways ($r^2 = 0.12$), and a weak negative correlation was found between the *O*-deethylation and both other pathways ($r^2 = 0.33$ and 0.20 for *O*-deethylation vs glucuronidation and *N*-demethylation, respectively).

No correlation ($r^2 = 0.03$) was found between glucuronidation of ethylmorphine in the 6-position and morphine glucuronidation in the 6-position. The ratio of morphine-3-glucuronide/morphine-6-glucuronide in urine varied from 2.5 to 6.3.

Values of $AUC(0,8h)$ of ethylmorphine and the different metabolites (Table 2) were consistent with the urinary recovery data (Table 1). Thus, among the

eight subjects with $MR_O < 0.7$, the highest AUC was found for ethylmorphine-6-glucuronide, followed by that for the *O*-deethylation products, and that for *N*-demethylation products. The median serum concentrations of ethylmorphine and ethylmorphine-6-glucuronide are shown in Figure 2. Ethylmorphine

Figure 2 Serum concentrations of ethylmorphine (a) and ethylmorphine-6-glucuronide (b) after administration of a single oral dose of 1.5 mg kg^{-1} of ethylmorphine to 10 healthy subjects. Median values (and range) in subjects with $MR_O < 0.7$ ($n = 8$) are indicated by solid symbols. Average values in subjects with $MR_O > 1.3$ ($n = 2$) are indicated by open symbols.

Table 2 Serum $AUC(0,8 \text{ h})$ values ($\text{nmol ml}^{-1} \text{ min}$) of ethylmorphine and its metabolites after administration of 1.5 mg kg^{-1} ethylmorphine as a single oral dose to 10 healthy subjects

	Subjects with $MR_O < 0.7$ ($n = 8$)		Subjects with $MR_O > 1.3$ ($n = 2$)	
	Median	Range	Median	Range
Ethylmorphine	222	(93–264)	232	(199–265)
Ethylmorphine-6-glucuronide	979	(1274–1394)	1056	(974–1139)
Morphine	8.5	(4.9–18)	0.6	(0.2–1.1)
Morphine-3-glucuronide	205	(100–303)	35	(22–47)
Morphine-6-glucuronide	45	(26–60)	8.1	(5.8–10)
Norethylmorphine	48	(40–61)	88	(77–99)
Normorphine	34	(25–57)	5.5	(4.1–6.8)

Figure 3 Serum concentrations of morphine (a), morphine-3-glucuronide (b) and morphine-6-glucuronide (c) after administration of a single dose of 1.5 mg kg^{-1} of ethylmorphine to 10 healthy subjects. Median values (and range) in subjects with $\text{MR}_0 < 0.7$ ($n = 8$) are indicated by solid symbols. Average values in subjects with $\text{MR}_0 > 1.3$ ($n = 2$) are indicated by open symbols.

was detected in serum from all 10 subjects for 8 h. Serum concentrations of morphine, morphine-3-glucuronide and morphine-6-glucuronide are shown in Figure 3. Morphine was detectable for at least 8 h after ethylmorphine administration and shortly after administration, morphine-3-glucuronide and morphine-6-glucuronide were present in serum at concentrations substantially higher than those of morphine. Some of the metabolites were detected in serum for a long time after administration of ethylmorphine. Notably, after 48 h, morphine-glucuronides were the only metabolites detected in serum in six of ten subjects.

A summary of the pharmacokinetic parameters for ethylmorphine is given in Table 3.

Discussion

Although we did not phenotype the subjects of this study with regard to the debrisoquine genetic polymorphism, the value of MR_0 and $\text{CL}_{\text{m}0}$ for *O*-deethylation of ethylmorphine agreed well with those reported by others [4], and allowed stratification of the subjects into eight phenotypically extensive and two phenotypically poor metabolisers. The prevalence of poor metabolisers in a group of healthy Caucasians is expected to be 7–10% [22]. However, genotyping for *CYP2D6*-alleles revealed no subjects homozygote for mutant alleles. The *CYP2D6* alleles tested for, would identify approximately 97% of PM of the debrisoquine/sparteine type [23]. The two subjects phenotypically classified as PM by their metabolic ratio for *O*-deethylation of ethylmorphine (MR_0 in these subjects was similar to that reported for ethylmorphine in subjects classified as PM by dextromethorphan [4]), were genotypically found to be *wt/CYP2D6A* and *wt/CYP2D6D*, respectively.

The lack of conformity between phenotyping and genotyping is perhaps not surprising. The formation of morphine from ethylmorphine is reported to be mediated by *CYP2D6* [4], but this is only one of three alternative metabolic pathways for ethylmorphine in humans (Figure 1). Furthermore, this *CYP2D6* pathway is minor to glucuronidation, and about equivalent in importance to the *N*-demethylation which is mediated by a member of the *CYP3A* subfamily [24]. A wide variation in activity exists for both *CYP3A* and *UDPGT* [25–28]. Thus, activity in the alternative metabolic pathways could greatly influence the amount of ethylmorphine available for *O*-deethylation, and thus influence the amount of morphine and morphine-glucuronides formed. This implies, that any difference in *O*-deethylation of ethylmorphine between individuals could be obscured or balanced by a counteracting difference in *N*-demethylation between the same individuals. Further support for this is given by the negative correlation found between the *O*-deethylation vs glucuronidation and *N*-demethylation. Ethylmorphine is, therefore, not a good substrate for studying *CYP2D6* activity. This interpretation is sustained by the relative large variation in MR_0 for ethylmorphine among dextromethorphan phenotyped extensive metabolisers [4].

The total cumulative urinary recovery of ethylmorphine plus metabolites varied between 65 and 92%, with a median of 77%. These values represents minimum figures of urinary recovery of all measured metabolites, since the excretion of all metabolites in urine was not finished after 48 h, since not all possible metabolites were looked for, and since some metabolites, (e.g. glucuronides) might have been recovered from faeces. Our urinary recovery of parent drug plus metabolites was almost twice as high as reported in a previous study [29]. This is probably due to determination of more metabolites and longer observation periods in the present study.

Ethylmorphine-6-glucuronide was unambiguously identified as the major metabolite of ethylmorphine using reference substance synthesised in our laboratory [13]. Previous studies have inferred formation of

Table 3 Pharmacokinetic parameters denoting the fate of ethylmorphine after administration of 1.5 mg kg⁻¹ as a single dose to 10 healthy subjects*

	Subjects with $MR_O < 0.7$ (n = 8)		Subjects with $MR_O > 1.3$ (n = 2)	
	Median	Range	Median	Range
AUC(0,8 h) (nmol min ml ⁻¹)	222.2	(93.1–264.2)	232.2	(199.3–265.1)
$t_{1/2,z}$ (h)	1.97	(1.68–2.36)	2.78	(2.54–3.01)
V_z (l kg ⁻¹)	3.1	(1.9–6.1)	3.8	(3.5–4.1)
t_{max} (h)	0.75	(0.50–1.00)	1.13	(0.25–2.00)
C_{max} (μmol l ⁻¹)	1.09	(0.45–1.70)	1.89	(0.66–3.13)
CL/F (ml min ⁻¹)	1004	(687–2108)	822	(664–979)
CL _R (ml min ⁻¹)	52.9	(18.2–79.0)	44.1	(35.7–52.6)
CL _{NR} (ml min ⁻¹)	954	(634–2029)	777	(628–927)
CL _{MO} (ml min ⁻¹)	113	(88.9–322)	17.9	(12.8–23.0)
CL _{mN} (ml min ⁻¹)	91.0	(56.3–253)	112	(101–123)
CL _{mG} (ml min ⁻¹)	450	(129–698)	391	(323–459)

*CL and V_z were calculated using the fractional urinary recovery of all measured metabolites (0–48 h) as the minimum oral availability (median; 0.74, range; 0.54–0.92, n = 10).

ethylmorphine-6-glucuronide indirectly, based on an increase in ethylmorphine after sample hydrolysis [4, 29]. The finding of ten times more ethylmorphine-6-glucuronide than ethylmorphine in urine in the present study was in accordance with published indirect measurements [4, 29]. In the present study urinary recoveries of morphine plus its 3- and 6-glucuronides constituted about 15%, and norethylmorphine after hydrolysis about 9% of the dose, compared with 24 and 12% of the total urinary excretion products, respectively, reported by Rane *et al.* [4].

The partial metabolic clearances of ethylmorphine *N*-demethylation (CL_{mN}) and 6'-glucuronidation (CL_{mG}) did not differ between subjects with high or low urinary recoveries of morphine and morphine-glucuronides (Table 3), but varied by a factor of five and four times among all the individuals, respectively.

In the two subjects with $MR_O > 1.3$ more of the dose was recovered as *N*-demethylation products than as *O*-deethylation products. The recovery of ethylmorphine-6-glucuronide were similar to those found in the other eight subjects (Table 1). The decreased recovery of normorphine and normorphine-glucuronides probably reflects the facts that these metabolites originate from *N*-demethylation of morphine and/or that the *O*-deethylation of norethylmorphine to normorphine could be catalysed by CYP2D6. Genetic deficiency in these pathways would also account for relatively high recoveries of norethylmorphine plus norethylmorphine-6-glucuronide as a consequence of metabolic shunting.

Recoveries of ethylmorphine and ethylmorphine-6-glucuronide were essentially complete in 24 h, whereas both the *O*-deethylation products morphine, morphine-3-glucuronide and morphine-6-glucuronide, and the *N*-demethylation products norethylmorphine and norethylmorphine-6-glucuronide continued to be excreted up to 48 h.

Rane *et al.* [4] detected no free morphine in serum after administration of 1/4 to 1/5 of the dose that we

gave. However, we detected morphine in eight subjects for at least 4 h, and in two of the subjects up to 24 h.

Since the formation of morphine plus morphine-6-glucuronide varied by a factor of nine in all 10 subjects studied, this suggests that the analgesic and other effects of ethylmorphine might vary substantially among individuals depending on metabolic status, assuming that these metabolites contribute substantially to the net pharmacological response of ethylmorphine.

Morphine-3-glucuronide and morphine-6-glucuronide are reported to be formed by at least two isozymes [30]. No significant correlation was found between the 6-glucuronidation of ethylmorphine and morphine. The ratios of morphine and its glucuronides were similar to those observed after administration of morphine [31, 32]. No difference was found between subjects with high or low urinary recoveries of morphine plus morphine-glucuronides with respect to the morphine-3-glucuronide/morphine-6-glucuronide ratio. The pharmacokinetic parameters observed in this study were compatible with those of Rane *et al.* [4].

In forensic toxicology drugs that are metabolised to morphine represent a special problem. Thus the presence of morphine could indicate administration of morphine itself, heroin (diacetylmorphine) [33], codeine [34, 35], pholcodine [36] or ethylmorphine [3]. Several approaches have been used to elucidate whether the presence of morphine in urine in such cases results from a prodrug or to the intake of morphine itself [33, 37]. The results of this study, demonstrates that serum and urine samples taken more than 8 and 24 h after administration of ethylmorphine, respectively, can contain morphine and morphine-glucuronides, but no ethylmorphine, ethylmorphine-6-glucuronide or (serum only) norethylmorphine. The finding of morphine in serum 24 h after administration of ethylmorphine in two subjects is at first sight somewhat unexpected, given the elimination half-life of ethylmorphine of about 2–3 h.

However, after both intravenous and oral administration of morphine, a slowly declining terminal phase with a $t_{1/2}$ of about 15 h that could be due to enterohepatic cycling, has been reported in addition to a $t_{1/2}$ of about 2 h within 7 h of morphine administration [31]. It is likely that morphine and morphine-glucuronides will behave similarly although initially formed from ethylmorphine. Since morphine and morphine-glucuronides are detected in the absence of ethylmorphine, ratios of morphine to parent drug cannot be used to distinguish the source of morphine

in such cases. On the other hand, norethylmorphine could be detected after hydrolysis of urine samples in all subjects. Accordingly, norethylmorphine should be included in urine assays for opiates in forensic toxicology. No firm conclusions about the source of morphine are possible based on serum samples obtained more than 24 h after drug administration.

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