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Exploration of the metabolism of dihydrocodeine via determination of its metabolites in human urine using micellar electrokinetic capillary chromatography

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Abstract

After single-dose administration of 40 or 60 mg of dihydrocodeine (DHC, in a slow-release tablet) to four healthy individuals known to be extensive metabolizers of debrisoquine, the urinary excretion of DHC and its four major metabolites, dihydrocodeine-6-glucuronide, nordihydrocodeine, dihydromorphine and nordihydromorphine, was assessed using micellar electrokinetic capillary chromatography (MECC). DHC and two of its metabolites (dihydrocodeine-6-glucuronide and nordihydrocodeine) could be analyzed by direct urine injection, whereas the metabolic pattern was obtained by copolymeric bonded-phase extraction of the solutes from both plain and hydrolyzed urine specimens prior to analysis. The total DHC equivalents excreted within 8 and 24 h were determined to be $30.4 \pm 7.7\%$ ($n = 5$) and $63.8 \pm 6.1\%$ ($n = 2$), respectively, and only about 4% of the excreted DHC equivalents were identified as morphinoids. Furthermore, almost no morphinoid metabolites of DHC could be found after administration of quinidine (200 mg of quinidine sulfate) 2 h prior to DHC intake.

1. Introduction

Although dihydrocodeine (DHC) is a widely used analgesic and antitussive agent, its metabolic fate has been incompletely investigated thus far. In analogy to codeine, the metabolism as depicted in Fig. 1A has been proposed [1]. In that scheme, the metabolites of DHC include nordihydrocodeine (NDHC), dihydromorphine (DHM) and nordihydromorphine (NDHM), as well as conjugates with glucuronic acid and sulfate. To our knowledge, this metabolic pathway in man has never been fully confirmed. For DHC, urinary concentrations have been determined using gas chromatography (GC) [2] or high-performance liquid chromatography (HPLC) [3,4], whereas serum levels have been monitored via GC [4], HPLC [3–5] and radioimmunoassay [1]. Furthermore, Rowell et al. [1] have measured the total amount of acid metabolites including DHM and glucuronides, but have not been able to differentiate the various compounds. More recently, Hofmann et al. [6] and Mikus et al. [7] have confirmed the presence of DHM in plasma using gas chromatography–mass spectrometry. Also, Ohno et al. [8] have determined DHC, dihydrocodeine-6-glucuronide (DHCG), NDHC and DHM in dog urine by

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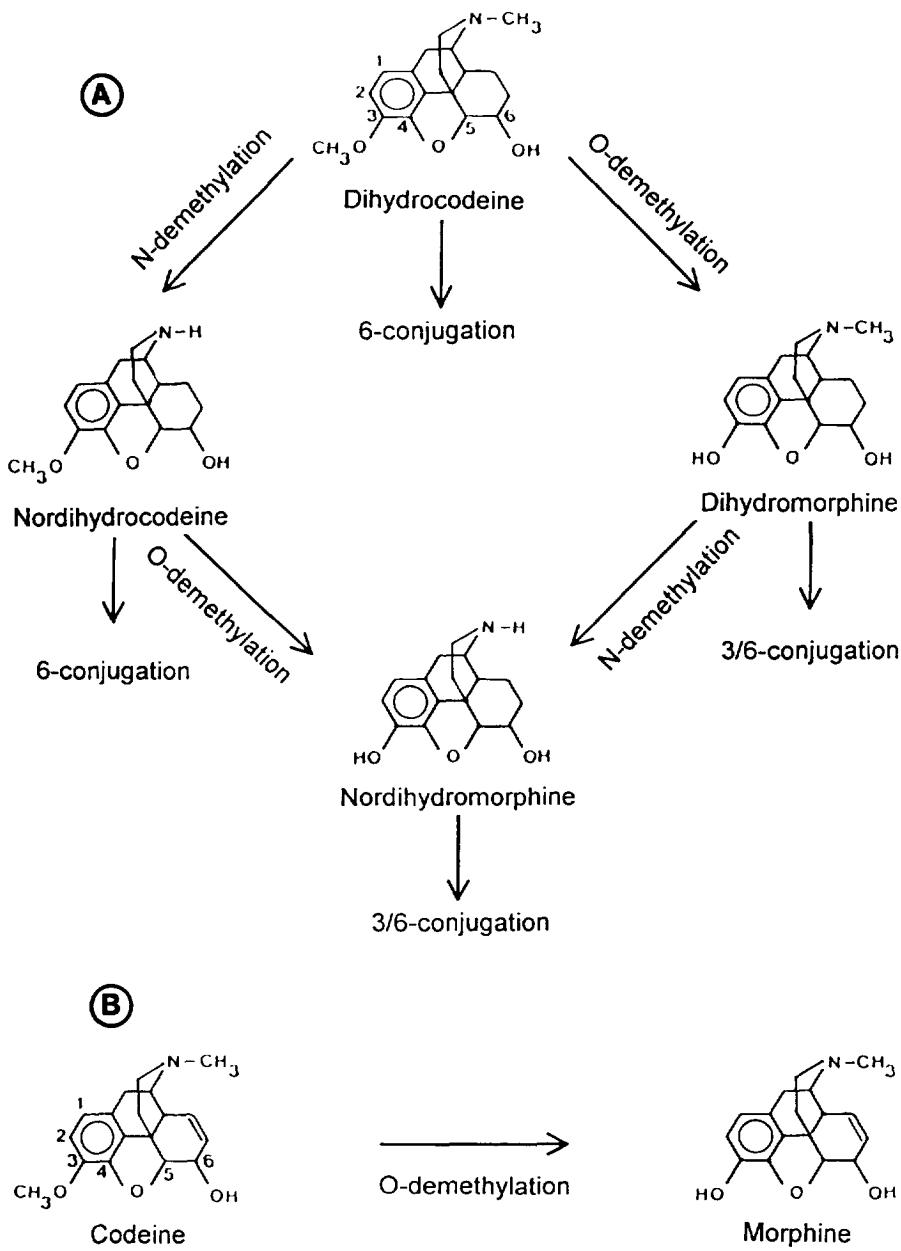

Fig. 1. (A) Chemical structures and metabolic pathway of DHC and its metabolites, and (B) O-demethylation of COD to MOR.

HPLC with electrochemical and UV absorbance detection. In a separate approach, pharmacokinetic properties of DHM and metabolites have been determined after parenteral administration of DHM [9].

In analogy to codeine [10–12], the morphinoid metabolites of DHC could be the analgesic active moieties. Recently, the liver enzyme CYP2D6 (Cyt P-450 db1) has been identified as being responsible for the O-demethylation of DHC to DHM [7]; this corresponds to the genetic oxidative polymorphism observed with debrisoquine, sparteine and dextromethorphan [13]. Thus, it is very well possible that people

lacking CYP2D6 do not show any or at least a much reduced analgesic effect after intake of DHC. Thus, screening for the urinary morphinoid metabolites of DHC could play a central role in the assessment of the analgesic action of this drug. Furthermore, monitoring of DHM and NDHM in relation to DHC and NDHC should permit straightforward differentiation between extensive and poor metabolizers for DHC.

Recently, micellar electrokinetic capillary chromatography (MECC, a combination between electrophoresis and chromatography) was found to be an attractive approach for the analysis of drugs in body fluids [14], including opioids [15,16]. MECC is particularly well suited for the separation of similarly structured compounds [17]. The goals of the work discussed in this paper were (i) to monitor urinary levels of DHC and its metabolites by MECC after single-dose administration of DHC, and (ii) to determine the urinary metabolic pattern after administration of quinidine 2 h prior to DHC in order to elucidate whether quinidine is blocking the O-demethylation of DHC as was described for codeine [12].

2. Experimental

2.1. Drugs, chemicals and standard solutions

All chemicals were of analytical or research grade. The standard substances codeine (COD) and morphine (MOR) were a gift of the Institute of Pharmacy, University of Berne, Berne, Switzerland. Dihydrocodeine (DHC) and its synthesized metabolites dihydrocodeine-6-glucuronide (DHCG), nordihydrocodeine (NDHC), dihydromorphine (DHM) and nordihydromorphine (NDHM) were received from Mundipharma (Basel, Switzerland). Sodium dodecyl sulfate (SDS) was from Sigma (St. Louis, MO, USA), and methanol (p.a.), ammonia (p.a., 25%), acetic acid, KH_2PO_4, Na_2HPO_4, $Na_2B_4O_7$, NaOH and KOH were from Merck (Darmstadt, Germany). β-Glucuronidase–arylsulfatase (from *Helix pomatia*) was purchased from Boehringer Mannheim (Rotkreuz, Switzerland) and β-glucuronidase (from bovine liver with an activity of 5000 units/ml) was from Sigma. Standard solutions of all drugs and metabolites were prepared in methanol at concentrations of ca. 2 mg/ml and stored at −20°C. Spiking of urines was performed by addition of known aliquots of the standard solutions prior to sample injection, sample hydrolysis or extraction.

2.2. Urines and immunological screening of urines for opioids

Urine samples of four test persons with normal urine flow (Table 1) and collected after p.o. administration of DHC in a slow-release form (DHC Continus tablets containing 60 or 90 mg DHC hydrogentartrate, corresponding to 40 and 60 mg DHC, respectively) (Napp Laboratories, Cambridge, UK) were obtained from Mundipharma. All four subjects gave their informed consent to the study. Three of these healthy volunteers (samples A, B, C; Table 1) ingested 40, 40 and 60 mg DHC, respectively, and collected their 0–8 h urines. In another approach, two individuals (samples D1–D3 and E1–E3) took 40 and 60 mg DHC, respectively, and collected their 0–24 h urines over 3 time periods (0–8, 8–16, 16–24 h; Table 1). In a separate effort, test person III also ingested 200 mg of quinidine sulfate (1 tablet Kinidin-Duriles, Astra Pharmaceutica, Dietikon, Switzerland) 2 h prior to swallowing 60 mg of DHC and 0–8, 8–16 and 16–24 h urine collection. The urines were stored at −20°C until analysis.

For the assessment of compliance, the specimens were screened for the presence of opiates by an automated enzyme immunoassay technique (EMIT-dau, Syva, Palo Alto, CA, USA) on a Cobas Fara centrifugal analyzer (Hoffmann-La Roche, Diagnostica, Basel, Switzerland). The EMIT-dau test contains morphine as calibrator with a cut-off level of 300 ng/ml and a positive control of 1000 ng/ml. With this test, all urine samples collected after administration of DHC were found to be markedly positive for opioids with the responses being larger than that of the commercial positive control.

Table 1
Test persons, DHC dosage and urine collection

Test person[a]	Sex	Dosage (mg)	Urine collection			Sample
			Time interval (h)	Amount (ml)	Flow (ml/min)	
I	m	60	0–8	834	1.74	A
II	f	60	0–8	491	1.02	B
III	m	90	0–8	430	0.90	C
III	m	90	0–8	509	1.06	D1
			8–16	415	0.86	D2
			16–24	253	0.53	D3
IV	m	60	0–8	580	1.21	E1
			8–16	256	0.53	E2
			16–24	703	1.46	E3
III	m	90[b]	0–8	650	1.35	DC1
			8–16	530	1.10	DC2
			16–24	614	1.28	DC3

[a] All test persons were determined to be extensive metabolizers for debrisoquine.
[b] Two hours after administration of 200 mg quinidine sulfate.

2.3. Hydrolysis of conjugates

For enzymatic hydrolysis, 5 ml of urine, 4.9 ml of acetate buffer (0.1 *M*, pH 5) and 100 μl of glucuronidase–arylsulfatase or 500 μl β-glucuronidase were incubated overnight at 37°C while gently shaking the 25-ml Erlenmeyer flask covered with parafilm. For acid hydrolysis, 5 ml of urine and 1 ml of concentrated hydrochloric acid were incubated for 30 min at 120°C using a screw-capped Sovirel test tube. Thereafter, the pH of the hydrolysate was adjusted to pH 7 through addition of about 1.25 ml of 10 *M* KOH. The sample was then centrifuged for 3 min at ca. 1500 *g* prior to extraction.

2.4. Extraction of dihydrocodeine and metabolites

If not stated otherwise, extractions were made with 5 ml of urine (or 5 ml of urine which was hydrolyzed according to the procedures described above) and using disposable solid-phase cartridges which contain a proprietary copolymeric material (Bond Elut Certify 1211-3050; sorbent amount, 130 mg; reservoir volume, 10 ml) and a Vac-Elut setup (all from Analytichem International, Harbor City, CA, USA). The extraction procedure previously described for confirmation of urinary opioids and other drugs of abuse [15] was applied. The cartridges were conditioned by passing sequentially 2 ml of methanol and an equal volume of deionized water through the columns. The urine specimen, adjusted to pH 7, was slowly aspirated through the column. Then, the cartridge was sequentially rinsed with 2 ml of deionized water, 1 ml of 0.1 *M* acetate buffer (pH 4), as well as 2 ml of methanol. The column was not dried under full vacuum (vacuum applied never exceeded 16.9 kPa). Elution was effected either with 2 ml of freshly prepared methylene chloride–isopropanol (80:20, v/v) containing 2–5% concentrated ammonium hydroxide solution, or with 1.5 ml methanol (application of two times 0.75 ml) containing 30% concentrated ammonium hy-

droxide solution. If not otherwise stated, eluates were evaporated to dryness under a gentle stream of air at room temperature and the residues were redissolved in 100 μl of running buffer (MECC).

The recovery after sample pretreatment was determined by comparing electrophoretic peak heights after extraction with peak heights obtained by direct injection of equal amounts of the drugs in buffer. Using methylene chloride–isopropanol (80:20, v/v) with 2% concentrated ammonium hydroxide solution as eluent, recoveries for DHC, NDHC and DHM were found to be about 60%, whereas those for NDHM and DHCG were 30% and <10%, respectively. Employing methanol with 30% (v/v) concentrated ammonium hydroxide solution as eluent, provided good extraction efficiency (>80%) for all compounds.

2.5. Instrumentation for MECC and running conditions

Throughout the work a buffer containing 6 m*M* sodium tetraborate, 10 m*M* disodium hydrogenphosphate and 75 m*M* SDS (pH ca. 9.2) was employed. Electrokinetic measurements were made in three instruments featuring 75 μm I.D. fused-silica capillaries of 70 cm effective length; a homemade setup described previously [15,16], the Europhor Prime Vision IV (Europhor, Toulouse, France) and the automated model 270A-HT capillary electrophoresis system (Applied Biosystems, San Jose, CA, USA). The two manual instruments comprised the same on-column detector, the fast forward scanning UVIS 206 PHD (Linear Instruments, Reno, Nevada, USA) which was operated in the high speed polychrome mode as reported previously [15,16]. In the homemade device, sample application occurred manually via gravity through lifting the anodic capillary end which was dipped into the sample vial some 34 cm for about 5 s. The applied power was a constant 20 kV (current about 75 μA). Sample application in the Prime Vision occurred by vacuum (1 s). Applied voltage (current) was 25 kV (about 65 μA). For each experiment, the capillary was conditioned by rinsing with 0.1 *M* sodium hydroxide for 3 min followed by running buffer for 5 min.

For quantitation, the 270A-HT capillary electrophoresis system which features automated capillary rinsing, sampling and execution of the electrophoretic run was used. Injection of the sample occurred via vacuum (applied for typically 0.5 s) combined with electrokinetic injection by application of 30 kV during the injection period. If not otherwise stated, a constant voltage of 30 kV was applied (current 112–115 μA). The temperature was set at 35°C and detection was effected at 213 nm. The PC Integration Pack (version 3.0, Kontron Instruments, Zürich, Switzerland) together with a Mandax AT 286 computer system was used for data acquisition, storage and evaluation. Quantitation was based on peak-area measurements and employing multilevel internal calibration. Four calibrators covering the concentration range 3.8–42 μg/ml were employed together with codeine (8 μg/ml) as internal standard. Before each run capillaries were rinsed with 0.1 *M* NaOH (2 min) and with running buffer (6 min).

Employing 5 ml of urine, extraction with methanol containing 30% concentrated ammonia solution and reconstitution with 100 μl of running buffer, an identification limit (with spectral data) of about 0.1 μg/ml was obtained. Quantitation limits (signal-to-noise ratio of 3) were 0.03 μg/ml for DHC and NDHC and 0.05 μg/ml for all other opioids investigated. Day-to-day and run-to-run variations were determined to be <7% ($n = 4$). All data were generated by single determinations.

2.6. Phenotyping of volunteers

Each volunteer was screened for his ability to oxidize drugs via administration of a tablet containing debrisoquine (10 mg, Declinax, Hoffmann-La Roche, Basel, Switzerland) and mephenytoin (100 mg, Mesantoin, Sandoz, Basel, Switzerland), collection of the 0–8 h urine and chromatographic determination of the two hydroxylation statuses according to Küpfer and Preisig [18]. Urinary 4-hydroxymephenytoin was determined by HPLC [19], whereas debrisoquine

and 4-hydroxydebrisoquine were monitored by GC using a modification of the method of Dick et al. [20]. With both substrates, all volunteers were determined to be extensive metabolizers.

3. Results and discussion

3.1. MECC separation of DHC and metabolites in model mixtures and by direct urine injection

The three-dimensional electropherogram depicted in Fig. 2A represents the absorbance vs. retention time vs. wavelength relationship for a model mixture comprising DHC and its metabolites (Fig. 1), COD and MOR. Each compound is characterized by its retention/migration behavior with DHCG being the fastest and NDHC the slowest of the investigated components. Despite the similarities of the molecules of these opioids, differential partitioning between the buffer and the micelles is occurring, this providing well separated peaks (Fig. 2B). In this configuration, the void and micelle peaks occur at about 7 and 29 min, respectively (data not shown). All three morphinoids are eluting between 20 and 23 min, i.e. before the non-conjugated codeinoids which are detected after about 26 to 27 min. For each group, the demethylated compounds are detected last, whereas the non-dihydro-analogues reach the detector first. Furthermore, three-dimensional data gathering (Fig. 2A) provides absorbance spectra of each eluting peak, spectra which can be used for peak identification (see below). It was interesting to find, that analysis of a freshly prepared standard solution (made in water or methanol) revealed a double peak for DHM, but never for the other opioids (data not shown). No difference could be elucidated via spectral analysis of the two peaks. However, the first peak gradually disappeared while the other increased in size. After storage of the sample for one to two weeks at 4°C or −20°C, the second peak was detected only. Thus far, no explanation could be found for this phenomenon.

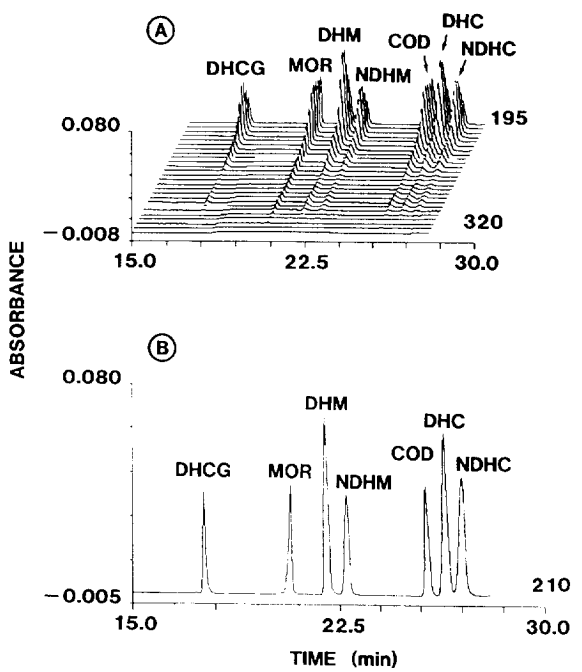

Fig. 2. Multiwavelength (A) and single-wavelength at 210 nm (B) MECC data of a model mixture obtained on the Europhor. Concentrations in sample: DHCG, 35; MOR, 28; DHM, 31; NDHM, 27; COD, 33; DHC, 33; NDHC, 24, all in μg/ml.

Direct injection of urine provides a rather complex electropherogram (panel A of Fig. 3) which hinders the analysis of small amounts of drugs which elute before about 15 min. Our compounds of interest, however, are eluting after 15 min and can therefore be detected by on-column UV absorption provided their concentrations are larger than about 2 μg/ml (> about 7 μM). The electropherogram presented in panel A of Fig. 3 was obtained with direct injection of the 8–16 h urine of test person III (urine D2) who ingested 60 mg DHC. Careful inspection of the data according to detection time and spectral analysis, revealed the peaks eluting after 16.5 min, 24.8 min and 25.3 min as being DHCG, DHC and NDHC, respectively. As is illustrated with the spectral data shown in panels B–D of Fig. 3, good agreement between the normalized spectra of the eluting peaks and those of a computer-stored model run (Fig. 2) are obtained. Peak assignment was further confirmed by rerunning the same sample spiked with the three compounds. Using a calibration graph

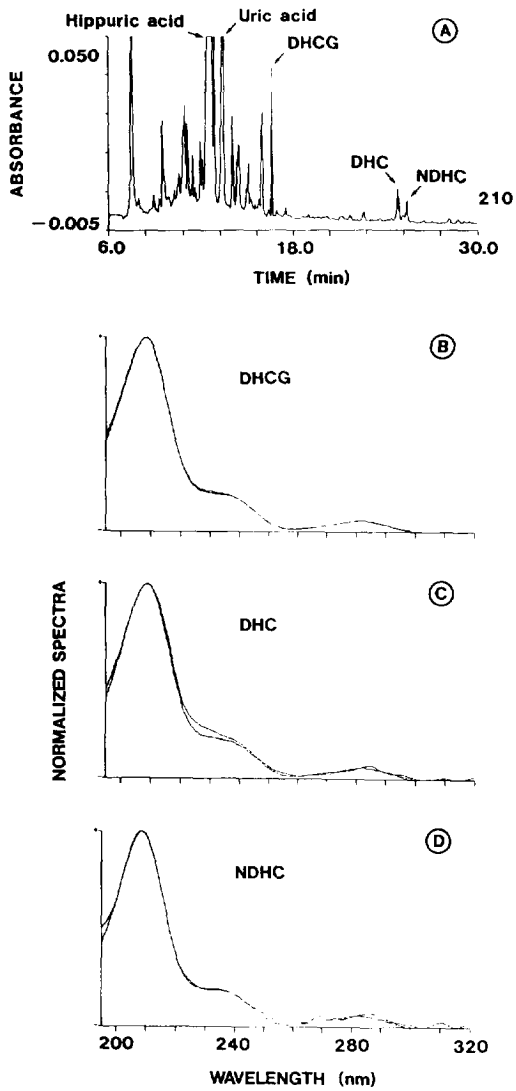

Fig. 3. MECC data (Europhor instrument) obtained after direct injection of urine specimen D2 (panel A). Panels B–D depict spectral identity proofs for DHCG, DHC and NDHC, respectively.

based on peak height (linear, four-level internal calibration with standards between 22 and 88 μg/ml and COD (210 μg/ml) as internal standard, $r = 0.987$), the concentration of DHCG in that urine was estimated to be 32 μg/ml. Concentrations of free DHC and NDHC (as assessed after extraction) were found to be 12.5 and 7.6 μg/ml, respectively.

With direct urine injection, DHCG was found in all specimens, whereas DHC and NDHC could not be determined in samples A and E3, samples in which the solute's concentrations were below the detection limit. The presence of DHM and NDHM, however, could not be identified by direct urine injection. Concentrations of these compounds appear to be too low to be detected by on-column UV absorption. On the other hand, these data obtained with direct urine injection are attractive in the sense that DHCG can be determined without extraction, this being in contrast to the work published with morphine-3-glucuronide [21].

3.2. Extraction of opioids, hydrolysis of conjugates and urinary concentrations

Panel B of Fig. 4 depicts single-wavelength data obtained after Bond Elut Certify extraction of the same urine whose data by direct urine injection are shown in Fig. 3A. A much smaller number of peaks is detected despite that with this approach up to 50-fold concentration of the extracted compounds can be achieved. The two large peaks could unambiguously be assigned to DHC and NDHC. Plotting the same data on expanded absorbance scale also revealed the presence of three smaller peaks which could be assigned to DHCG, DHM and NDHM (see insert). This example reveals that after single-dose administration of 60 mg DHC and 8–16 h urine collection, the parent compound and three metabolites can be found in their unconjugated forms.

Panels C and D of Fig. 4 present data obtained with the same urine as for panel B, but with enzymatic (panel C) and acid hydrolysis (panel D) prior to extraction. The comparison of non-hydrolyzed, enzymatically hydrolyzed and acid hydrolyzed data revealed interesting differences in peak size. Firstly, NDHC and NDHM appear not to be affected much by hydrolysis, this indicating that these metabolites are not or only marginally conjugated and/or are partially decomposing during the acidic treatment. For DHC and DHM, however, the opposite was found. Furthermore, after enzymatic hydrolysis

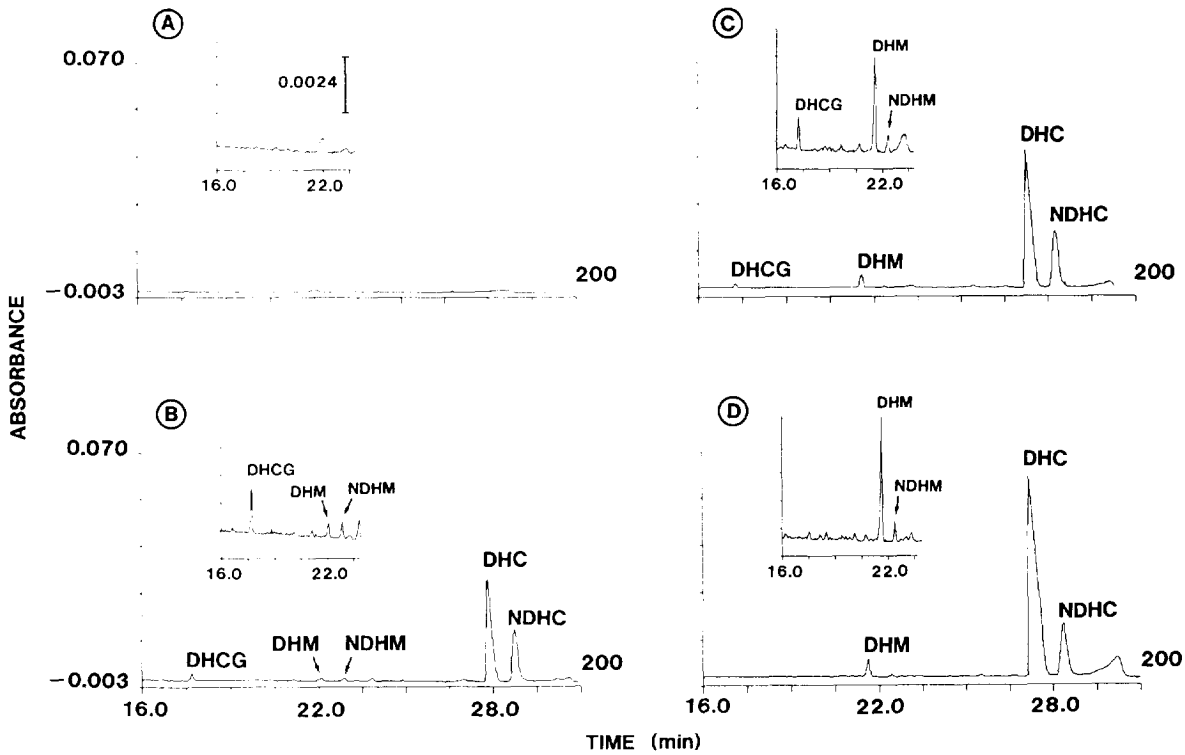

Fig. 4. MECC data for 200 nm absorbance obtained on the homemade apparatus of (A) extracted urine blank, (B) extracted urine D2, (C) enzymatically hydrolyzed and extracted urine D2, and (D) acid hydrolyzed and extracted urine D2. Inserts depict peaks for DHCG, DHM and NDHM on an elongated absorbance scale (for scale refer to panel A).

peak sizes of these two compounds were determined to be intermediate between those of the non-hydrolyzed and acid hydrolyzed cases. Thus, enzymatic hydrolysis with glucuronidase–arylsulfatase as employed here is not cleaving all conjugates present. This is in agreement with previous observations of Hug and Mellett, who reported incomplete hydrolysis of DHM when using β-glucuronidase compared to acid hydrolysis [9], as well as with data obtained with codeine-6-glucuronide [22]. For DHC, incomplete hydrolysis could also be confirmed using two different enzyme reagents, as well as incubation at elevated temperatures (up to 60°C, data not shown). For the two enzyme formulations, no significant differences in the DHC pattern were observed.

3.3. Urinary excretion of DHC and its metabolites

Quantitation was performed on the automated instrument via use of multilevel internal calibration with COD (8.3 μg/ml urine) as internal standard and peak areas as the basis for data evaluation. COD, as potential metabolite of DHC, was not detected in the urines. Thus it could be safely chosen as internal standard (Fig. 5). Two sets of data were produced. First, urines were extracted without any other pretreatment (determination of free drug levels) and second all urines were subjected to acid hydrolysis (determination of total drug) prior to extraction using an eluent composed of methanol with 30% (v/v) concentrated ammonium hydroxide solu-

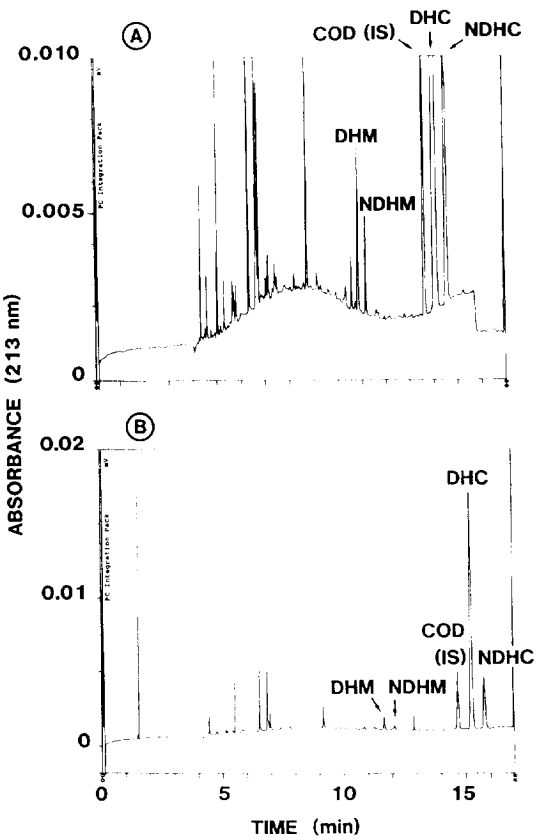

Fig. 5. MECC data for 213 nm absorbance obtained on the ABI 270A-HT apparatus of urine D2 after acid hydrolysis and extraction using methanol with 30% concentrated ammonia as eluent. The data presented in panel A were obtained after reconstitution in 50 μl buffer, whereas those in panel B were measured with a 5-fold diluted extract. Concentrations of DHC, NDHC, DHM and NDHM were determined to be 34.72, 8.59, 1.42 and 1.16 μg/ml, respectively.

tion. The amounts excreted of each compound are presented in Table 2. From these data and those given in Table 1, renal excretion rates could be calculated, and if more time points would be available, time constants for renal elimination kinetics could be extracted. The data of Table 2 confirm that DHC and NDHC excretions are significantly higher than those of their morphinoid analogues (Fig. 1). Insight into the degree of conjugation is obtained by comparison of the free and total amounts. For DHC, it was found that overall about 50% of DHC is conjugated. Furthermore, the data obtained suggest that considerably less DHCG (about 40%) is present in the 0–8 h urine compared to the 60–65% content of DHCG in the 8–16 and 16–24 h specimens. For NDHC, no conjugation in the 0–8 h urine was observed. As a matter of fact, the total concentrations were found to be somewhat smaller than the free drug levels, thus suggesting that part of the drug could be decomposed during acid hydrolysis. On the other hand, the extent of conjugation of NDHC in the second and third time periods was found to be about 15%. Furthermore, based on the low levels of free DHM and NDHM, the extent of conjugation of these compounds was not determined with the employed MECC assay.

The total DHC equivalents excreted within 24 h for individuals III and IV (samples D1 to D3 and E1 to E3, respectively) were determined to be 67.2 and 58.5%, respectively. Assuming that nearly all compounds excreted in urine were monitored, it appears that much less than half of the dose should be found in faeces. After a dose of 40 mg of DHC taken by individuals I, II and IV, total excretion during the first 8 h varied considerably between 27.6 and 42.4% [mean ($n = 3$): 34.2 ± 7.5]. For the same time period and administration of 60 mg DHC, recoveries of 27.1 and 22.2% were obtained (samples C and D1 of test person III). Independent of administrated dose, the mean and standard deviation ($n = 5$) of total excretion within the first 8 h were determined to be 30.4 ± 7.7.

Looking at the data obtained with samples D1 to D3, about 50% of the total dose of DHC was excreted as DHC and conjugates, 13% as NDHC and conjugates, ca. 2.1% as DHM and conjugates, and ca. 2.0% as NDHM and conjugates. The corresponding data for DHC, NDHC and DHM and their conjugates in samples E1 to E3 were found to be 45, 12, and ca. 1.5%, respectively. NDHM could not be determined in specimen E2. Thus, the O-demethylation from DHC to DHM appears to occur on a 1.5–2% level only. This is in contrast to COD, for which

Table 2
Excreted amounts

Sample	DHC				NDHC				DHM		NDHM	
	Free		Total		Free		Total		Total		Total	
	mg	%[a]	mg	%	mg	%	mg	%	mg	%	mg	%
A	4.99	12.5	9.17	22.9	1.66	4.3	1.35	3.5	0.30	0.79	0.16	0.43
B	8.12	20.3	13.6	34.1	1.73	4.5	1.80	4.7	0.90	2.4	0.45	1.23
C	8.87	14.8	13.3	22.2	2.82	4.9	2.31	4.0	0.32	0.57	0.20	0.37
D1	7.42	12.4	10.5	17.5	2.33	4.1	2.00	3.4	0.36	0.66	0.35	0.65
D2	5.17	8.6	14.4	24.0	3.17	5.5	3.57	6.2	0.58	1.01	0.44	0.80
D3	1.72	2.9	5.35	8.9	1.34	2.3	1.91	3.3	0.21	0.38	0.29	0.54
E1	5.30	13.2	10.5	26.2	2.70	7.0	2.07	5.4	0.23	0.61	0.15	0.42
E2	1.41	3.5	5.00	12.5	1.13	3.0	1.26	3.3	0.20	0.52		
E3	1.18	2.9	2.41	6.0	0.88	2.3	1.06	2.8	0.18	0.46	0.12	0.32

[a] % of dose.

a 5–10% conversion to MOR and conjugates was reported [23,24]. Furthermore, for 40 and 60 mg DHC administration, no major difference in excretion is observed.

3.4. Metabolic pattern after quinidine pretreatment

For COD [23], it is known that the O-demethylation to MOR (Fig. 1B) is much reduced in individuals which are poor metabolizers of the debrisoquine–sparteine–dextromethorphan type. Furthermore, a similar effect was found with pre-administration of quinidine [11,12], a compound which blocks the liver enzyme CYP2D6 responsible for the O-demethylation. Thus an extensive metabolizer phenotype can temporarily be converted into a poor metabolizer for COD [25]. All the volunteers which participated in the present study were determined to be extensive metabolizers for debrisoquine. Consequently and not surprisingly, urinary morphinoid metabolites of DHC could be monitored in all urines collected after single-dose administration of DHC. During the course of that study, no poor metabolizer of the debrisoquine–sparteine–dextromethorphan type was available. Thus, for the assessment of the impact of quinidine on the metabolism of DHC, test person III (Table 1) ingested 200 mg of quinidine sulfate 2 h prior to 60-mg administration of DHC. Electropherograms of an acid hydrolyzed urine sample of the same individual collected without (panel A) and in presence of quinidine (panel B) are presented in Fig. 6. The data of panel A essentially correspond to those given previously (Fig. 4D), but were obtained using methanol with 30% concentrated ammonia solution as extraction eluent. Using the spectral identity proof approach, DHM and NDHM are unambiguously detected in front of COD (internal standard, 4.2 μg/ml urine), DHC and NDHC. In case of the quinidine treatment, there is indication of the presence of very small amounts of DHM and NDHM (see peaks marked with arrows in Fig. 6B). However, no identity proof was possible. Thus, the concentrations of these compounds are at or below the detection limit of the MECC assay employed (about 50 ng/ml). Assuming that the small peaks are DHM and NDHM and considering the urine volume collected (Table 1), quinidine appears to reduce DHM production indeed. Similar data were obtained with the urines collected 0–8 and 16–24 h after DHC ingestion. Furthermore, urinary excretion of DHC and its metabolites appears to be some-

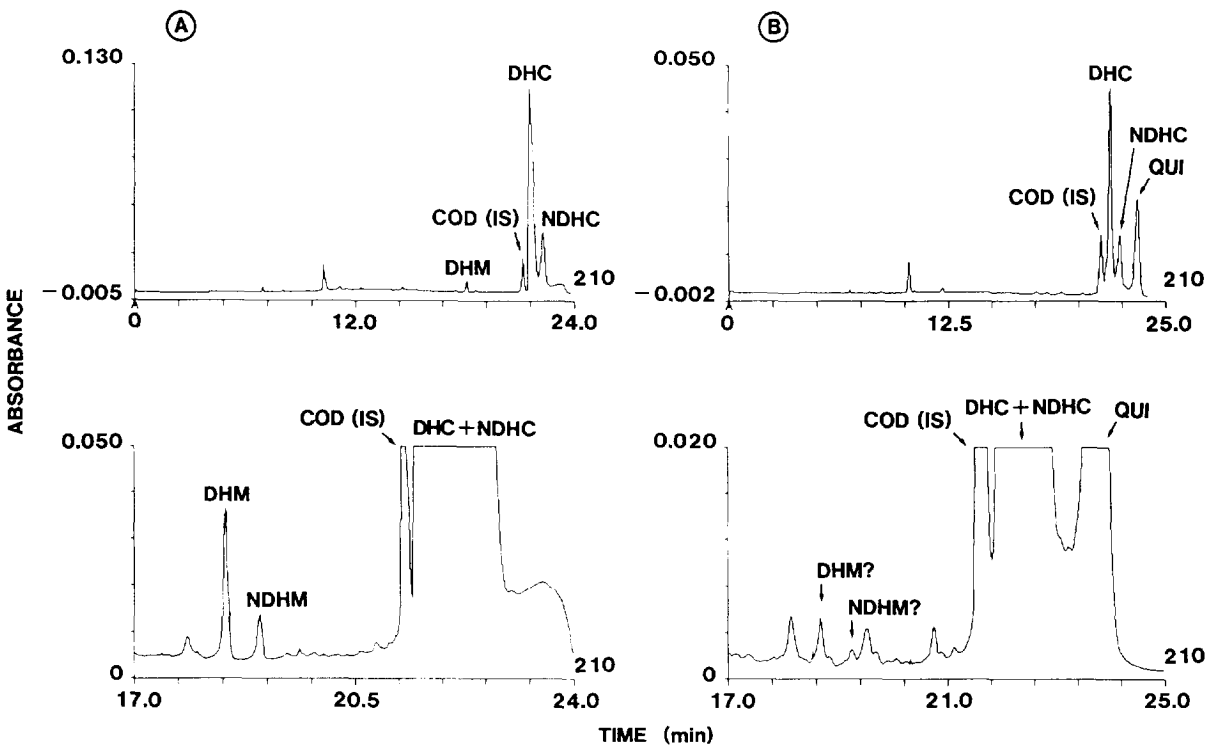

Fig. 6. MECC data for 210 nm absorbance (Europhor) of (A) urine D2 and (B) urine DC2 after acid hydrolysis and extraction using methanol with 30% concentrated ammonia as eluent.

what slower. These experiments (i) reveal the change in metabolism in the presence of quinidine, i.e. a reduced O-demethylation activity, and (ii) suggest that MECC could be a suitable method for differentiation between extensive and poor metabolizers for DHC.

4. Conclusions

MECC is shown to be an attractive methodology for the separation and analysis of DHC and its major urinary metabolites. From the data presented it can be concluded that (i) DHCG, DHC and NDHC are the major urinary compounds found within 24 h after administration of DHC in the form of a slow-release preparation, (ii) DHC and DHM are significantly conjugated and the degree of conjugation is lower in the 0–8 h urine compared to those collected from 8 to 16 and 16 to 24 h after DHC intake, (iii) NDHC and NDHM are little or not conjugated at all, (iv) the extent of the O-demethylation from DHC to DHM is on the 1.5–2% level only, and (v) quinidine appears to block the O-demethylation of DHC. More work is required to clearly characterize this last aspect and its consequences for individuals which are poor metabolizers of the debrisoquine–sparteine type. Furthermore, for a more complete kinetic investigation, more samples collected over a longer period of time would have to be considered and, in order to exclude solute degradation associated with acid hydrolysis, a more effective procedure for enzymatic hydrolysis would have to be found.

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