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The detection of dihydrocodeine and its main metabolites in cases of fatal overdose

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Abstract The levels of dihydrocodeine found in impaired individuals and in fatalities show a wide overlap in the ranges. Among other factors, the genetically controlled metabolism of dihydrocodeine should play an important role in dihydrocodeine toxicity.

For the first time, the most important metabolites of dihydrocodeine were investigated in femoral blood from three fatal cases by simultaneous determination using HPLC and native fluorescence for detection. The amount of parent drug always exceeded dihydrocodeine-glucuronide formation and dihydromorphine concentrations ranged from 0.16–0.21 mg/L. The similar binding affinities of dihydromorphine and morphine to μ-opioid receptors suggest similar pharmacological effects and adverse reactions. The determination of the pharmacologically active metabolites should help to clarify the cause of death in fatal cases especially if a relatively low concentration of the parent drug is found.

Key words Dihydrocodeine · Dihydromorphine · Glucuronide metabolites · Fatal intoxication

Introduction

Dihydrocodeine is a semisynthetic opioid which was first prepared in 1920 by hydrogenation of codeine [18]. Besides its use as an analgesic and antitussive drug, dihydrocodeine was increasingly prescribed to out-patients as a substitute drug in the treatment of heroin addicts.

Unlike methadone, dihydrocodeine syrup preparations containing up to 2.5% of the drug substance or as a slow release preparation were often prescribed over a period of several days and consequently, were widely misused. Dihydrocodeine is now strictly licensed by the German public health authorities since February 1998 [23].

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An increasing number of accidental death cases due to dihydrocodeine overdose has been observed in recent years. In 1993 Penning et al. [14] reported on 36 fatalities involving dihydrocodeine, in which blood concentrations of the drug ranged from 0.6–25.3 mg/L.

An assessment of opiate positive samples from our case work during the last 2 years showed that serum dihydrocodeine concentrations in 54 specimens of conspicious motorists averaged 0.7 mg/L (range 0.1–3.3 mg/L, unpublished results). Although data relating dose and adverse effects of dihydrocodeine are limited [12–14], blood concentrations causing impairment or leading to fatal intoxication seem to widely overlap.

As an explanation for this phenomenon, differences in opiate tolerance were assumed [7] and, in heavy users, pharmacodynamic tolerance may arise from the formation of opiate antibodies [5].

The genetically controlled O-demethylation of dihydrocodeine to dihydromorphine is another possible explanation [4, 15]. Dihydrocodeine is believed to undergo the same series of biotransformation steps as codeine [22], however, data on the biotransformation have not been completely established.

As a preliminary result of a more detailed study on dihydrocodeine metabolism, the corresponding metabolites were simultaneously analysed in three fatal cases using HPLC with fluorescence detection.

Materials and methods

Materials

Blood samples were collected from the femoral vein at autopsy from suspected cases of dihydrocodeine overdose and stored at –20° C until analysis.

All reagents were of HPLC or analytical grade. Dihydrocodeine (DHC) hydrogentartrate and hydromorphone hydrochloride were gifts from Knoll AG (Ludwigshafen, Germany). Dihydromorphine (DHM) was obtained from hydromorphone by reduction with $NaBH_4$ [2]. Nordihydrocodeine (NDHC) and the glucuronide metabolites of dihydrocodeine (DHC6G) and dihydromorphine (DHM3G, DHM6G) were supplied by Lipomed (Arlesheim, Switzerland). Deuterated morphine and codeine were purchased from Radian

(Austin, CA). Triethylammonium phosphate buffer solution (TEAP, 1 M) and pentafluoropropionic anhydride (PFPA) were obtained from Fluka (Buchs, Switzerland).

Extraction

HPLC

From thawed hemolysed blood samples, 200 µL was diluted with 500 µl of ammonium sulfate buffer (32 mM, pH 9.6) prior to solid phase extraction on C_8-cartridges (1 mL, Bond Elut, Varian, Harbour City, Calif.). The analytes were eluted with 400 µL methanol/1 M HCl (50:1 by vol.). The extract was dried under a stream of nitrogen at 40° C, the residue was reconstituted with 100 µL of double distilled water and 40 µL was injected into the HPLC system.

GC/MS

Morphine-d_3 (100 ng/mL) and codeine-d_3 (100 ng/mL) were added as internal standards to 1 mL of blood prior to solid phase extraction on C_{18}-cartridges (3 mL, Chromabond drug, Macherey & Nagel, Düren, Germany). The samples were further processed as recommended by the manufacturer. The eluent (isopropanol/dichloromethane/25% ammonia 80:20:2 by vol.) was evaporated to dryness under a stream of nitrogen at 40° C and the residue was derivatized with 100 µL PFPA for 30 min at 60° C. The mixture was again dried, reconstituted in 30 µL ethyl acetate and 1 µL of the PFPA derivatives was injected into the GC/MS system.

Instrumentation

HPLC

HPLC analysis was performed with a Hewlett Packard 1050 Series quaternary pump (Waldbronn, Germany) equipped with an autosampler and a Shimadzu fluorescence detector (Kyoto, Japan).

Samples were eluted from a Nucleosil 100–5 C_{18} column (250 mm × 4.6 mm i.d., Ziemer, Mannheim, Germany) with 4 vol% acetonitrile in 25 mM TEAP (pH 3.4) at a flow rate of 1 mL/min. The excitation wavelength was set at 220 nm and emission was recorded at 340 nm [19].

GC/MS

The GC/MS system consisted of a Hewlett Packard 5890 gas chromatograph and a Hewlett Packard 5972 mass selective detector (Waldbronn, Germany) operated in the EI-SIM mode. Samples were eluted from a fused silica gel capillary column CP-Sil 5 (12.5 m × 0.25 mm i.d., 0.4 µm film thickness, Chrompack, Middelburg, The Netherlands). The ion masses monitored for dihydrocodeine and dihydromorphine were m/z 284, 432, 447 and m/z 416, 432, 579, respectively. The concentrations of dihydromorphine and dihydrocodeine were calculated with reference to morphine-d_3 (m/z 417) and codeine-d_3 (m/z 285).

Table 1 Concentration ranges (µg/L) used for calibration and concentrations (µg/L) used for recovery, intra- and inter-assay variance

	Concentration ranges (µg/L) used for 5-point calibration	Concentrations (µg/L) used for recovery, intra- and interassay-variance (n = 9)
DHC	20–1500	500
DHC6G	20–1500	500
NDHC	15– 900	300
DHM	5– 300	100
DHM3G	15– 750	250
DHM6G	5– 250	70

DHC: dihydrocodeine, DHCG: dihydrocodeine-6-glucuronide, NDHC: nordihydrocodeine, DHM: dihydromorphine, DHM3G: dihydromorphine-3-glucuronide, DHM6G: dihydromorphine-6-glucuronide

Linearity, reproducibility and recovery of the analytes by HPLC

Standard solutions of each analyte were added to 200 µL of drug-free blood for validation of the HPLC method and the samples were processed as described. The analyte concentrations used to establish validation data are outlined in Table 1.

Case reports

In all cases investigated, morphological and histological findings could not reveal the cause of death and there was no aspiration of gastric contents. Alcohol was not found in any specimen.

Case 1

A 25-year-old male (72 kg, 172 cm) was found dead after a party and attempts at resuscitation failed. There was evidence that the man had consumed DHC syrup and several tablets containing diazepam before death. Almost 4.5 h had passed between administration of the drugs and death. Blood concentrations were 0.2 mg/L and 0.17 mg/L for diazepam and nordiazepam, respectively.

Case 2

A 30-year-old male (66 kg, 178 cm) was seen to have a gait disturbance. He seemed slightly dizzy and claimed to be under the influence of a substitute drug. He was put to bed and soon felt asleep but after 8.5 h he was found dead. The blood-concentration of diazepam was 1.03 mg/L and that of nordiazepam was 0.86 mg/L.

Case 3

A 28-year-old male (65 kg, 179 cm) suffered from headache and vomiting before he collapsed unconscious and attempts at resuscitation failed. In addition to a DHC formulation he had swallowed chloralhydrate capsules and 42 mg trichlorethanol/L was determined from a femoral blood specimen.

Results

Linear correlation coefficients > 0.99 were obtained for all 5-point calibration graphs. The recovery of DHM and its 6-glucuronide was approximately 70% and approximately 80% for all other analytes investigated. After a 9-run validation the inter-assay variance was < 13.5% for DHM and DHM6G and < 10% for DHC, NDHC, DHM3G and DHC6G. The intra-assay variance was determined to be < 8.0% for DHM and DHM6G, and < 5.5% for dihydrocodeine and the other metabolites.

The concentrations of DHC and DHM measured by HPLC were in accordance with those obtained by GC/MS analysis and differences were less than 10% starting from

Table 2 Concentrations of DHC and metabolites in blood (mg/L) from 3 dihydrocodeine-related fatalities

	DHC	DHC6G	NDHC	DHM	DHM3G	DHM6G
Case 1	18.45	7.18	2.21	0.21	1.08	0.08
Case 2	1.92	2.68	0.27	0.18	1.04	0.08
Case 3	2.36	1.61	0.12	0.16	0.31	0.07

the corresponding mean values. The concentrations of parent drug and metabolites are set out in Table 2.

Except for case 2, with a possibly longer survival time, the amount of DHC always exceeded the DHC-glucuronide concentration. Dihydromorphine concentrations ranged from 0.16–0.21 mg/L and the 3- and 6-glucuronides were formed in ratios similar to those established from morphine pharmacokinetics [9, 11].

Discussion

In the present study, the metabolites of dihydrocodeine were determined in three overdose cases to gain a first insight into their possible role in dihydrocodeine toxicity. Adverse reactions to dihydrocodeine were observed at blood concentrations exceeding 1 mg DHC/L [17] including dizziness, drowsiness, nausea and constipation. Blood concentrations above 2 mg DHC/L may result in respiratory depression, convulsions, cardiovascular collapse and death [17]. In case 1 death due to dihydrocodeine overdose could be easily explained by the concentration of the parent drug. Dihydrocodeine concentrations in cases 2 and 3 may be more critical to the diagnosis of a dihydrocodeine-related death, if co-medication is not considered.

The data presented are further discussed with regard to dihydrocodeine metabolism (Fig. 1) and the effects of dihydrocodeine metabolites as far as is known. Glucuronidation of dihydrocodeine has been shown to be the major metabolic pathway.

The O-demethylation of dihydrocodeine to dihydromorphine with subsequent glucuronidation yields the corresponding 3- and 6-glucuronides and was found to be the secondmost important transformation. N-demethylation results in N–nordihydrocodeine which is conjugated at the 6-carbon [15].

O-demethylation is catalysed by a cytochrome P450 (CYP) enzyme, whose expression and activities are regulated by genetic and environmental factors. According to a study of Fromm et al. [4] the polymorphic CYP2D6 seems to be the major enzyme involved in dihydromorphine formation.

Compared to extensive metabolisers, poor metabolisers lacking this enzyme formed only one-seventh of the amount of dihydromorphine [4]. The prevalence of poor metabolizers in a group of healthy Caucasians was expected to be 7–10% [8]. CYP2D6 also catalyses dealkylation of the opioids codeine [3, 21, 22], hydrocodone, oxycodone [10] and ethylmorphine [1].

The dealkylated metabolites of these drugs usually showed a higher binding affinity to the μ-opioid receptor and a higher therapeutic efficacy compared with the parent drug. In 1980 Gillan et al. [6] demonstrated that μ-receptor binding of dihydromorphine and morphine were of the same order of magnitude.

The results of a recent study on the binding affinities of dihydrocodeine, dihydromorphine, nordihydrocodeine and dihydrocodeine-6-glucuronide to the μ-, δ- and κ-opioid receptors in guinea pig brain homogenates [16] and IC_{50}-values for all substances investigated including morphine as a reference are summarized in Table 3.

Glucuronidation of the 6-hydroxyl group of dihydrocodeine and N-demethylation did not affect the affinity

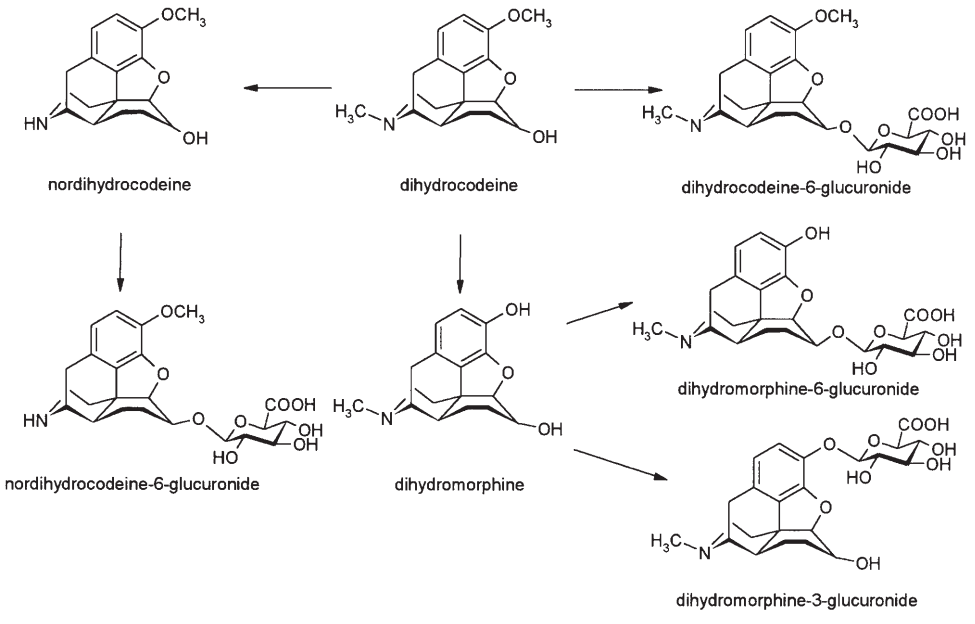

Fig. 1 Metabolic pathway of dihydrocodeine modified according to [4]

Table 3 IC_{50}-values (nmol/L) of DHC and metabolites on μ-, δ- and κ-opoid receptors (according to Schmidt et al. [16])

Metabolite	μ-Opioid-receptor	δ-Opioid-receptor	κ-Opioid-receptor
DHC	35	200	2000
DHC6G	65	400	5000
NDHC	50	1000	2000
DHM		20	25
Morphine		50	25

to μ-receptors, whereas O-demethylation caused a remarkable decrease in the IC_{50}-values for all types of receptors investigated. The affinity of dihydromorphine to the μ-opioid receptor was approximately 100-fold higher than for dihydrocodeine and in a comparable order of magnitude to that of morphine.

Although coupling of receptor occupancy to the ultimate response still needs to be established for dihydrocodeine and metabolites, the binding affinity of dihydromorphine and morphine to the μ-opioid receptor suggests similar effects. Besides analgesia, respiratory depression is the most important adverse effect after morphine dosing is mediated by the μ-opioid receptor. Assuming a similar intrinsic activity of morphine and dihydromorphine, the fatality in two of the cases presented can be explained considering the dihydromorphine concentration.

In conclusion, the concentration of the pharmacologically active metabolites should be determined in dihydrocodeine-related deaths for a reliable interpretation especially if the parent drug is present in a low concentration and a clear cause of death could not be ascertained at autopsy.

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