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Saliva testing after single and chronic administration of dihydrocodeine

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Abstract In the present study, concentrations of dihydrocodeine and its metabolites in saliva and serum were compared after single low-dose and chronic high-dosage administration of the drug. In the first investigation, blood and saliva were collected periodically from six subjects after oral administration of 60 mg dihydrocodeine. In the second study, 20 subjects on oral dihydrocodeine maintenance provided single samples of blood and saliva simultaneously. Serum protein binding of salivary analytes and their recovery from the adsorbing material of the collection device as well as pH values of saliva samples were determined. The fluids were analyzed for dihydrocodeine and the major metabolites by high-performance liquid chromatography. In the single dose study dihydrocodeine was the only analyte found in saliva for up to 12–24 h post-dose. The half-life of dihydrocodeine in saliva was about twice that found in blood. The ratios of saliva/serum concentrations ranged from 1.2 to 17.0. After chronic high-dosage use, dihydrocodeine was the main salivary analyte and N-nordihydrocodeine was present in a few samples. Saliva/serum concentration ratios of dihydrocodeine were strongly dependent on the pH value of saliva and, to a lesser extent, on serum-protein binding. The saliva/serum ratios were more similar after chronic administration. The data suggest a passive diffusion process as the underlying mechanism for the transport of dihydrocodeine into saliva. After both single and chronic use, the presence of the drug in saliva can be used as evidence of recent substance administration.

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Introduction

The selective barrier function of saliva glands to drug molecules was recognized by Bernard as early as 1856 [2], and to date probably more than 100 drugs have been investigated in saliva [20, 23, 31, 35, 36, 39]. The accessibility of saliva for rapid, non-invasive sampling makes it an attractive biomatrix for detecting drug use. Moreover, saliva can provide information on the drug status of an individual. Saliva has therefore been proposed as a test matrix for non-invasive therapeutic drug monitoring [8, 10, 15, 25, 28, 30, 33] and to detect recent drug exposure when access to laboratory testing is limited either by time or location [19, 32]. In Germany, saliva testing has been applied on a trial basis to monitor the public safety aspects of driving [27, 34]. Although many potential applications have been proposed, there are practical and theoretical reasons for the limited use of saliva specimens in forensic toxicology. In particular, information on variations of drug concentrations with time, possible differences caused by single or chronic use and the occurrence of drug metabolites in saliva is incomplete.

Only a few studies have been performed on salivary excretion of opiates after administration of single low doses [3–5, 14, 22–24]. Therefore, the present study was designed to compare drug findings in saliva after recent single and chronic use. Dihydrocodeine was chosen for practical reasons as well as for its physicochemical similarities to other illicit drugs such as cocaine, showing low protein binding and a pKa value of 8.6 [1].

Materials and methods

Subjects

Informed consent was obtained from 6 healthy volunteers (designated S1–S6, S stands for single dose) and 20 volunteer persons (designated C1–C20, C stands for chronic use) prior to their inpatient treatment for opiate addiction. The times since last drug intake prior to other drug administration under the experimental conditions described, were given by the subjects themselves and are listed in Table 1. The experimental design was approved by the local ethics committee of the University of Heidelberg.

Study protocol of the single dose study

The single dose study was performed under double blind conditions. After medical examination the subjects received either a placebo ($n = 6$) or a single oral dose of 72 mg dihydrocodeine thiocyanate corresponding to 60 mg of the free base in 100 ml of water. Prior to drug administration, saliva and blood samples were taken and used as blank values and collected again at time intervals of 2, 4, 6, 8, 12 and 24 h. During the first 2 h after drug administration neither drinks nor mouth rinsing was allowed.

Saliva samples were obtained on Salivettes (Sarstedt, Nümbrecht, Germany). The cotton roll of the collection device was placed into the buccal cavity for 1 min. Blood was collected by venipuncture using S-monovettes (Sarstedt, Nümbrecht, Germany); subsequently the tubes were centrifuged and serum was separated. Serum and saliva samples were stored at –20 °C until analyzed.

Study design for subjects after chronic use

Patients C1–C20 provided single simultaneous samples of venous blood and saliva at about 8.00 a.m. before starting the detoxification program. Identical collection devices were used in both study groups. Following specimen collection, saliva and serum samples were frozen (–20 °C) until analyzed.

The heroin-dependent subjects had been on dihydrocodeine maintenance for a period of 3 weeks to about 3 years and had been receiving 0.4–2.7 g dihydrocodeine daily (Table 1). The time since last drug intake was given by the subjects themselves (Table 1).

Sample preparation

After thawing, saliva was separated from the cotton roll by centrifugation (4470 g, 10 min), the pH value was measured and 500 µl of ammonium bicarbonate buffer (1 mM, pH 9.2) was added to 0.1 ml of saliva. To 0.2 ml of serum, 500 µl of buffer was added. Solid phase extraction of the diluted samples was carried out with Bond Elut extraction columns (C_8, 50 mg, Varian, Harbor City, Calif.), preconditioned with 3 ml of methanol, 3 ml of distilled water and 3 ml of buffer. After sample application, the column was washed with 1 ml of buffer and dried (30 min in a vacuum). The drugs were eluted with 2×200 µl of 1 M HCl/methanol (1 : 50 v/v). The combined fractions were taken to dryness (nitrogen, 40 °C), the residue was reconstituted with 100 µl of water and 50 µl was injected into the HPLC system. All investigations were performed in duplicate and values given are mean values unless stated otherwise.

Recovery of the analytes from the adsorbing material of the saliva collection device

To determine the recovery of the analytes from the adsorbing material of the saliva collection device, the cotton rolls were incubated for 1 h with 500 µl of drug-free spiked saliva (1000 ng dihydrocodeine and dihydrocodeine-6-glucuronide/ml, 300 ng dihydromorphine and dihydromorphine-6-glucuronide/ml, 500 ng dihydromorphine-3-glucuronide and N-nordihydrocodeine/ml). The rolls were frozen overnight (–20 °C) and processed in exactly the same way as the authentic and spiked samples.

Serum protein binding of dihydrocodeine and N-nordihydrocodeine

The binding of dihydrocodeine and N-nordihydrocodeine to serum protein was measured by ultrafiltration using spiked ($n = 4$, 250 ng dihydrocodeine/ml, $n = 4$, 1000 ng dihydrocodeine/ml, 600 ng

Table 1 Information on dihydrocodeine maintenance of subjects C1–C20 and a summary of dihydrocodeine concentrations detected in saliva and serum samples. Duration of dihydrocodeine maintenance (weeks), daily intake (g) and frequency, time since last intake (h), pH value of saliva, individual concentrations (ng/ml) in saliva and serum and saliva/serum concentration ratios (ratio) (– no information available, *conc.* concentration, *n.d.* not detectable, *n.c.* not calculable)

Subject	Maintenance	Daily intake (frequency)	Time since last intake (h)	pH value	Saliva conc. (ng/ml)	Serum conc. (ng/ml)	Ratio
C1	28	0.58 (2)	10	6.9	8769	1301	6.7
C2	28	1.65 (2–3)	6.5	6.7	66154	9037	7.3
C3	–	0.50 (1–2)	24	6.8	125	n.d.	n.c.
C4	104	1.32 (3)	8	6.4	18708	1194	15.7
C5	52	0.69 (4)	3	6.5	15954	961	16.6
C6	14	0.50 (2)	2.5	6.8	5962	1615	3.7
C7	3	0.83 (1)	1.5	7.2	6958	3340	2.1
C8	14	0.40 (4–5)	3.8	6.8	2254	698	3.2
C9	52	0.66 (3)	6.3	6.7	1291	473	2.7
C10	10	2.65 (2)	8	6.9	20060	3167	6.3
C11	25	1.16 (1)	5.5	7.1	9529	4538	2.1
C12	12	0.66 (3)	24	6.5	n.d.	n.d.	n.c.
C13	87	0.54 (3)	11	7.1	1653	596	2.8
C14	78	0.74 (3)	4	7.0	9901	1448	6.8
C15	16	0.99 (2)	4	6.7	25050	6993	3.6
C16	10	0.83 (3)	4	6.8	6009	2756	2.2
C17	12	0.74 (3)	24	6.9	n.d.	n.d.	n.c.
C18	52	0.50 (2)	20	6.8	2010	649	3.1
C19	14	1.32 (3)	5	6.6	12309	1917	6.3
C20	156	0.66 (–)	10	7.0	4671	1655	2.8

Table 2 Validation data of the analytical method. Linear concentration range, limit of detection (*LOD*) and quantitation (*LOQ*), recovery ($n = 6$), inter- and intraassay variance ($n = 6$). *SD* standard deviation

Analyte	Concentration range (ng/ml)	LOD (ng/ml)	LOQ (ng/ml)	Recovery (% ± SD)	Intraassay variance (%)	Interassay variance (%)
Dihydrocodeine	20–20.000	5.0	19.8	95 ± 4	4.8	8.6
Dihydrocodeine-6-glucuronide	20–20.000	4.9	20.0	91 ± 3	5.0	8.8
Dihydromorphine	15–3.000	4.4	14.8	75 ± 4	8.0	13.5
Dihydromorphine-6-glucuronide	15–3.000	4.5	15.0	71 ± 4	7.5	13.3
Dihydromorphine-3-glucuronide	15–7.000	4.5	15.0	85 ± 6	5.5	9.9
N-Nordihydrocodeine	15–10.000	4.4	14.9	85 ± 4	5.3	9.6

N-nordihydrocodeine/ml) and authentic ($n = 8$) serum samples. The ultrafiltration device (Centrifree, Amicon, Witten, Germany) was centrifuged for 10 min at room temperature and concentrations were measured from the original as well as from the processed samples. Binding of the analytes to the membrane of the device was determined using aqueous solutions of dihydrocodeine and N-nordihydrocodeine.

Chemicals and reagents

Dihydrocodeine thiocyanate and hydromorphone hydrochloride were generously supplied by Knoll (Ludwigshafen, Germany). Dihydromorphine was synthesized from hydromorphone by reduction with $NaBH_4$ [26, 37]. N-nordihydrocodeine and the glucuronides of dihydrocodeine and dihydromorphine were supplied by Lipomed (Arlesheim, Switzerland). Acetonitrile was obtained from Roth (Karlsruhe, Germany), triethylammonium phosphate buffer (1 M TEAP) was from Fluka (Buchs, Switzerland) and diluted in a ratio of 1:40 with double distilled water prior to use. All other chemicals and reagents used were of HPLC or analytical grade.

Instrumentation

The pH values of saliva samples were determined with a pH meter (Portamess, Knick, Berlin, Germany) using an InLab 423 electrode (Mettler-Toledo, Steinbach, Germany).

HPLC analysis was performed with a Hewlett Packard 1050 series LC pump (Hewlett Packard, Waldbronn, Germany), a Shimadzu fluorescence detector (Kyoto, Japan) and a workstation equipped with Hyperdata Chromsoft (Bischoff, Leonberg, Germany). Samples were eluted from a Nucleosil 100 C18 reverse phase column (250 × 4.6 mm, 5 µm; Ziemer, Mannheim, Germany) at a flow rate of 1 ml/min with diluted TEAP/acetonitrile (gradient elution 0–18 min, 2–5% acetonitrile, 18–35 min, 5% acetonitrile) as the mobile phase. For detection, the excitation wavelength was 220 nm and emission was recorded at 340 nm. The linear correlation coefficients were > 0.995 for the 5-point standard curves of spiked serum and saliva samples and the corresponding concentration ranges and validation data are given in Table 2. The limits of quantitation and detection were calculated using SQS software 2.0 demo (Perkin-Elmer, Überlingen, Germany). Interfering peaks from the blank serum or saliva samples (single dose study) were not observed.

Pharmacokinetic parameters

Peak concentrations (c_{max}) in saliva and time to reach c_{max} were taken directly from the concentration-time profiles. The terminal elimination rate constant β was determined from a semilogarithmic plot of the terminal part of the concentration-time curve for mean saliva concentrations ($n = 6$). The half-life was calculated according to the equation

$t_{1/2} = 0.693/\beta$.

Results

Single dose study

In the single dose study, dihydrocodeine was present in saliva in all samples collected from 2 h up to 12 h post-dose and could still be detected in two samples 24 h after drug administration (Table 3). Individual saliva and serum concentrations are summarized in Table 3. Metabolites of dihydrocodeine were not detected in any saliva sample.

Dihydrocodeine levels in saliva were always higher than in plasma (Table 3, Fig. 1) and maximum concentrations were reached 2–4 h post-dose. The drug was eliminated from saliva with a considerably longer half-life of about 8 h compared to blood (Fig. 2), which averaged 4.1 h and is in accordance with the values reported in the literature [12, 26]. The saliva-to-serum distribution ranged from 1.2 to 17.0 and the pH values measured were from 6.5 to 7.1 with small intraindividual variations of ± 0.25.

Results after high-dosage chronic use

The pH values of saliva samples (range 6.2–7.4), concentrations of dihydrocodeine in saliva and serum as well as saliva/serum concentration ratios and time since last drug intake for subjects C1–C20 are summarized in Table 1. Of these, 17 subjects had taken the last dose of dihydrocodeine within a time period of 20 h prior to sampling. In three cases the last dihydrocodeine intake was approximately 24 h prior to collection of the samples and dihydrocodeine intake was confirmed by findings of dihydrocodeine metabolites in urine [26] and positive skin testing [38]. In two persons, dihydrocodeine could not be detected in saliva or in serum. In one of these cases, a relatively small amount of dihydrocodeine was determined in saliva only. In both fluids, dihydrocodeine concentrations were several times higher than the concentrations measured in the single dose study (Tables 1 and 3). The saliva/serum concentration ratios ranged from 2.1 to 16.6 and 70% of the values varied between 2.7 and 7.3 (Table 1). There was a weak linear correlation between the logarithmic saliva/serum concentration ratios and the saliva pH values ($r = 0.68$, $n = 17$). Dihydrocodeine was the main analyte in mixed saliva, and in three samples N-

Table 3 Time profile of dihydrocodeine concentrations in saliva and serum of subjects S1-S6 after single dose administration and saliva/serum concentration ratios. Individual dihydrocodeine levels (ng/ml) in saliva and serum: *Δt* interval after drug administration, *n.d.* not detectable, *Ratio*: mean of saliva/serum concentration ratios ± SD (n = 6)

Δt (h)	Material	Subjects						Ratio
		S1	S2	S3	S4	S5	S6	
2	Saliva	682	416	899	1040	413	1263	3.9 ± 9.3
	Serum	237	163	203	228	125	208	
4	Saliva	941	423	800	1421	584	1202	7.2 ± 15.2
	Serum	153	113	139	84	125	140	
6	Salvia	684	234	406	619	199	675	5.0 ± 18.1
	Serum	106	80	109	82	91	99	
8	Salvia	469	96	398	493	196	648	5.6 ± 13.6
	Serum	85	66	46	69	84	59	
12	Salvia	334	38	166	260	112	1054	7.1 ± 24.6
	Serum	57	33	23	46	54	62	
16	Salvia	200	32	42	75	n.d.	704	
	Serum	n.d.	n.d.	n.d.	n.d.	n.d.	35	
24	Salvia	109	n.d.	n.d.	n.d.	n.d.	316	
	Serum	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	

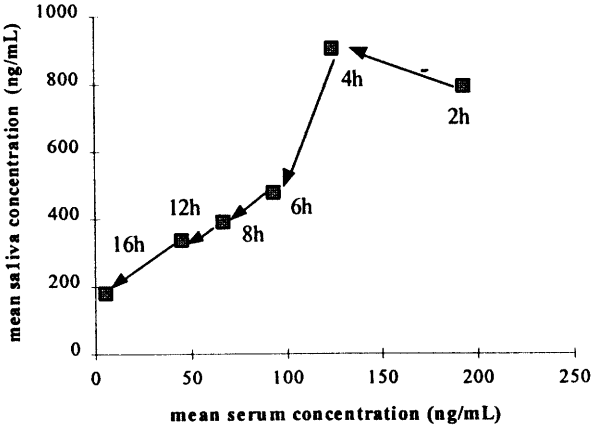

Fig. 1 Time dependence of mean saliva and serum concentrations after administration of a single dose of dihydrocodeine (60 mg) in six persons

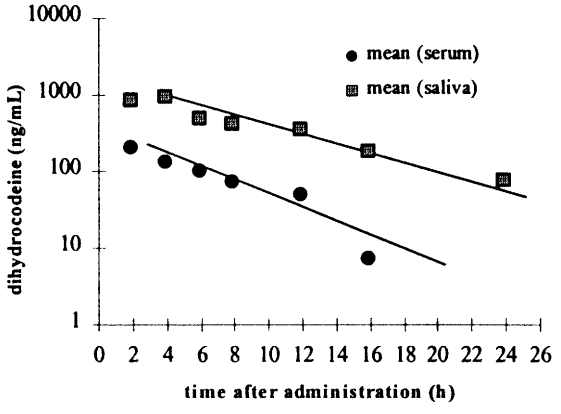

Fig. 2 Semi-logarithmic plot of mean saliva and serum concentrations (terminal phase) and curve fitting (r = 0.98) after a single dose of 60 mg of dihydrocodeine

Table 4 N-nordihydrocodeine concentrations (ng/ml) in biofluids from subjects C2, C4 and C10 and the corresponding saliva/serum concentration ratios

Subject	Saliva concentration (ng/ml)	Serum concentration (ng/ml)	Saliva/serum concentration ratio
C2	556	482	1.2
C4	187	101	1.9
C10	533	295	1.8

nordihydrocodeine was additionally detected (Table 4, Fig. 3), while other metabolites were not present.

Recovery of the analytes from the adsorbing material of the saliva collection device

Dihydrocodeine and its metabolites were adsorbed to a maximum of 14% (Table 5) and recovery from the cotton roll of the collection device appeared to be higher than reported in the literature [20].

Serum protein binding of dihydrocodeine and N-nordihydrocodeine

Binding of dihydrocodeine and N-nordihydrocodeine to serum protein was low (Table 6) ranging from 20% to 25% for spiked and authentic samples. There was no considerable difference whether binding was determined from spiked or from authentic samples. Binding of dihydrocodeine and N-nordihydrocodeine to the membrane of the ultrafiltration device was negligible (≦ 3%).

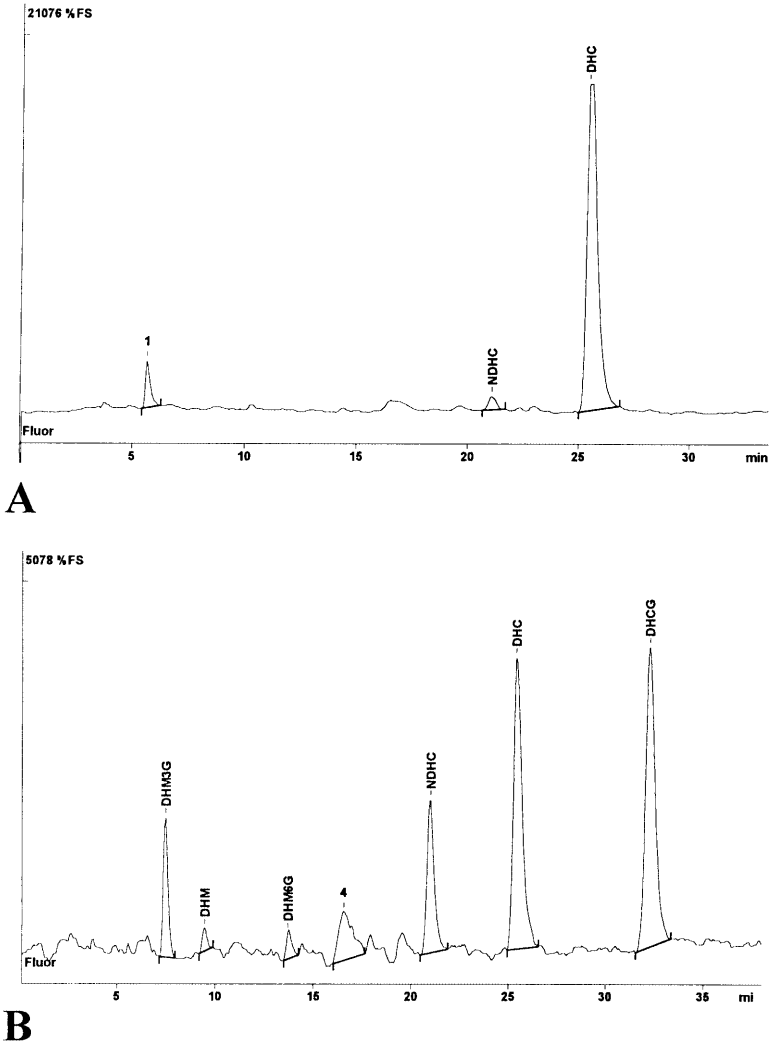

Fig. 3 Chromatogram of **A** an authentic (C10) and **B** a spiked saliva sample, 1000 ng dihydrocodeine (DHC) and dihydrocodeine-6-glucuronide (DHCG)/ml, 600 ng N-nordihydrocodeine (NDHC) and dihydromorphine-3-glucuronide (DHM3G)/ml, 250 ng dihydromorphine (DHM)/ml and 200 ng dihydromorphine-6-glucuronide (DHM6G)/ml

Table 5 Recovery of dihydrocodeine and metabolites from the adsorbing material of the saliva collection device: nominal and measured values (ng/ml) and recovery (%) (n = 3)

Analyte	Spiked value (ng/ml)	Measured values (ng/ml)			Recovery (%)
Dihydrocodeine	1000	936	917	923	92.5
Dihydrocodeine-6-glucuronide	1000	938	952	911	93.4
Dihydromorphine	300	269	246	265	86.7
Dihydromorphine-6-glucuronide	300	262	260	256	86.4
Dihydromorphine-3-glucuronide	500	452	443	451	89.7
N-nordihydrocodeine	500	446	438	449	88.9

Discussion

The apparent volume of distribution of dihydrocodeine exceeds the body volume 1.3-fold, suggesting extensive localization at intracellular sites [16, 20]. According to the pH-partition theory and the generally lower pH value in saliva compared to blood, a higher concentration of dihydrocodeine on the cellular and salivary side than in the intravasal space and a saliva-serum distribution greater than unity was to be expected [16, 21]. The theoretical saliva/serum concentration ratio for dihydrocodeine can be estimated by the equation of Matin et al. [29]. Given a mean pH value of 6.8 for saliva and a mean serum-protein binding of 25%, the saliva/serum concentration ratio was calculated as 2.8 (pKa value of dihydrocodeine 8.8) [1]. In this study most of the ratios calculated from saliva and serum concentrations were higher and differed from the theoretical values in both investigations. Very few drugs obey the pH-partition theory, and for bases pH absorption curves are generally shifted to lower pH values, resulting in higher ratios than the theory predicts [13].

Dihydrocodeine is largely ionized in blood and the free fraction in serum varied from 57% to 88% (Table 6).

Table 6 Serum protein binding (*PB*, %) of dihydrocodeine (*DHC*) and N-nordihydrocodeine (*NDHC*) in spiked and authentic samples (*org* original sample, *fil* filtered sample)

DHC_{org}	DHC_{fil}	PB_{DHC}	$NDHC_{org}$	$NDHC_{fil}$	PB_{NDHC}
Spiked samples					
1000	842	15.8	600	500	16.7
1000	760	24.0	600	452	24.7
1000	830	17.0	600	473	21.2
1000	822	17.8	600	454	24.3
250	237	5.2			
250	169	32.4			
250	211	15.6			
250	180	28.0			
Mean ± SD		19.5 ± 7.9	Mean ± SD		21.7 ± 3.2
Authentic samples					
1300	953	26.7	140	107	23.6
9040	6264	30.7	480	332	30.8
1610	1257	22.0	560	344	38.6
1450	1062	26.7	90	–	–
2760	2104	23.8	260	219	15.8
1920	1616	15.8	180	151	16.1
2170	1236	43.0	310	246	20.6
6990	6135	12.2	390	279	28.5
Mean ± SD		25.1 ± 8.8	Mean ± SD		24.9 ± 7.7

Therefore, differences of the calculated saliva/serum concentration ratio from the experimental value may arise from both the degree of serum protein binding and the particular salivary pH value. Strictly speaking, the equation of Matin et al. [29] applies only after steady state is reached, and explains the closer ranges of saliva/serum concentration ratios that were observed after chronic use of dihydrocodeine. Another reason for the variation of saliva/serum concentration ratios could be differences in saliva flow [17, 18]. For ionic compounds such as dihydrocodeine, the distribution will be influenced by the electrochemical gradient. Although saliva contains electrolytes normal for body fluids, the concentrations show a marked dependency on the flow rate and have a highly variable composition compared to blood [9]. Salivary glands have a high blood supply and are considered as a central compartment. Blood concentrations derived from cubital venous blood represent the peripheral compartment. Therefore, the concentrations in both compartments may not show a strong correlation. In addition to these factors, binding and absorption to the buccal mucosa, oral diseases and a circadian rhythm in the saliva flow rate, also influence the saliva/serum concentration ratio [16–18].

In the single-dose study, the detection time of dihydrocodeine in blood varied from 12 to 16 h, and slightly increased to 24 h in saliva. A similar detection time was found in saliva after 60 mg of orally applied codeine phosphate, although blood concentrations were not reported in this study [24].

Following oral administration of a single dose of 30 mg codeine phosphate Sharp et al. [36] observed saliva/plasma concentration ratios ranging from 2.0 to 6.6. After intramuscular administration of 60 and 120 mg codeine phosphate, a shorter detection time in saliva compared to blood and about the same concentration in saliva and plasma was found by radio immunoassay [4]. The analytical method as well as the route of administration provide an explanation for the differences observed. For cocaine, the effects of three different routes of administration on saliva and blood concentrations were demonstrated [7]. Elevated saliva/serum concentration ratios have been reported after intranasal, oral and inhalative drug administration in the early period after intake due to sequestration in the oral cavity. After 3 h saliva/plasma ratios were identical for the three routes of administration. In the present single dose investigation 2 h after drug administration, sequestration of orally administered dihydrocodeine was not obvious, dihydrocodeine peaked earlier in blood compared to saliva and was not proportionally eliminated from both compartments (Figs. 1, 2). The longer terminal half-life of dihydrocodeine in saliva than in blood suggests that the drug is taken up and retained in the epithelial cells which separate saliva from blood. Under the assumption that dihydrocodeine is transferred from blood to saliva by passive diffusion and that there are different velocities involved in the transport across the capillary wall via the basal membrane of the acinus cell and the luminal cell membranes, the concentration in saliva would primarily depend on the temporary intracellular concentration.

In the chronic high-dosage study, dihydrocodeine levels in saliva and serum were several times higher than the concentrations measured in the single dose study; however, the saliva/serum concentration ratios were comparable. With two exceptions, ratios were in a close range after chronic administration (Table 1). The high saliva concentrations following a high systemic load suggest a passive diffusion process as the underlying mechanisms for the transport for dihydrocodeine into saliva.

After single dose and chronic use, the detection time for dihydrocodeine in saliva went beyond that in blood for

a few hours. Even after chronic use, the drug could only be detected within 20 h after last intake by the method applied (LOD: 5.0 ng dihydrocodeine/ml saliva, Table 2). In contrast, after chronic use cocaine was detected in saliva during abstinence for up to 10 days [6]. However, the authors used a sensitive immunoassay (LOD: ≦ 0.5 ng cocaine/ml saliva) and suggested that cocaine was released from tissue storage sites during withdrawal.

N-nordihydrocodeine was present in a few samples only after chronic use of dihydrocodeine, while other metabolites were not found. Due to their acidic and hydrophilic nature, excretion of the glucuronides of dihydrocodeine, dihydromorphine or N-nordihydrocodeine into saliva seems unlikely. Although predominant in blood, the corresponding glucuronide was not observed in saliva after codeine administration [3]. Accordingly, morphine glucuronides were not detected in saliva after morphine dosing [11]. Dihydromorphine may be present in serum in amounts up to 6% of the dihydrocodeine concentration, depending on the pharmacokinetic phase and the CYP2D6 polymorphism [12, 26]. Its low concentration in blood and reduced lipophilicity compared to dihydrocodeine suggests very small and poor partitioning into saliva. Following single oral doses of up to 120 mg codeine phosphate, morphine was never detected in saliva [5]. There is evidence from the present results that morphine or dihydromorphine will not be detected in saliva even after higher doses of orally administered codeine or dihydrocodeine.

The source of N-nordihydrocodeine in saliva is not clear. It may be attributed to passive diffusion or local enzyme activity. Equal or higher concentrations of N-nordihydrocodeine in serum did not inevitably produce a positive finding in saliva. There is only one report on a demethylated metabolite in saliva which was not detectable in blood [22]. Therefore, a local metabolism in the oral cavity may be involved in N-demethylation of dihydrocodeine. Monooxygenase activity towards foreign substances has been demonstrated in the buccal cavity of the rat [40] but data on drug metabolizing enzymes in human buccal tissue have not yet been reported.

In conclusion, detection of drug use in saliva seems to be promising in general for drugs with physicochemical properties and binding behavior to serum proteins similar to dihydrocodeine. Saliva represents an in vivo model to study permeation of drugs through biological membranes and can be useful in the further evaluation of intracellular drug disposition in humans. However, in pharmacokinetic studies and in any interpretation of results obtained from saliva samples, the strong dependence of saliva/serum concentration ratios on the salivary pH value and the variable serum-protein binding of the drug have to be considered and the results should be confirmed by a specific analytical method.

Saliva samples were tested positive within 12–24 h following both single administration or last intake after chronic use with varying saliva/serum concentration ratios and a window of detection similar to that in blood. Therefore, saliva testing offers a high probability to detect use of those substances with properties similar to those of dihydrocodeine present in the systemic circulation. However, the results obtained by randomly collected saliva samples are qualitative in nature. For further interpretation, information on acute or chronic use, time of last intake, route of administration and in particular dosage form, characterization of the collection device and of the sample is necessary. Nevertheless, saliva seems to be a useful substrate for on-site testing, but compared to blood, a comprehensive interpretation of salivary data is very limited, at least in a forensic context.

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