On buprenorphine findings in hair

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Abstract

Aim: A dose-response relationship has been established in hair analysis for just a few drugs. Drugs such as buprenorphine (bup) used under controlled conditions present an opportunity to approach this problem. Both, bup and norbuprenorphine (nbup) have been detected in hair; however, discrepant results with regard to the concentration ratio bup/nbup have been reported. Therefore, the following questions have been addressed: does a relationship between the daily dose and the respective bup and nbup concentrations in hair exist? Is sample processing using diluted acid a possible cause for the different recoveries of the analytes?

Methods: Bup and nbup in proximal hair segments (≤ 4 cm) from 18 subjects participating in a maintenance program were determined by liquid chromatography/tandem mass spectrometry. Pure substances were incubated in 0.1 M HCl at 60°C for 24 hours; the alleged rearrangement products were simultaneously monitored.

Results and Discussion: Bup (range: LOD-0.238 ng/mg hair) and nbup (range: 0.043-0.961 ng/mg hair) could be determined from all specimens with nbup concentrations being consistently higher than of bup in 17 samples. Degradation of nbup was faster than of bup following incubation in 0.1 M HCl at 60°C.

Conclusion: Recovering of bup and nbup under acidic procedures results in an underestimation of the respective concentration and may invert their concentration ratio in hair. The combined concentration of bup and nbup determined from a hair sample may provide an estimate of bup exposure following long-term administration of the drug.

1. Introduction

Three pathways for incorporation of drugs into hair have been proposed: 1) diffusion of drugs present in the blood into the cells of the hair follicle, 2) diffusion from sweat and sebum, and 3) environmental exposure [6]. The most important route of drug incorporation into hair is considered to be via blood implying that a certain dose-response relationship should exist between individuals. Up to now, a certain correlation between dose and amount of drug in hair could be shown for just a few drugs [11, 12]. Analysis of drugs such as methadone or buprenorphine (bup) used in maintenance programs under controlled conditions present an opportunity for verifying whether a relationship between the daily dosage and the concentration in hair is likely to exist [10, 11].

The main metabolite of bup is formed by N-dealkylation mainly via CYP3A4; both, the parent drug and norbuprenorphine (nbup) are then cleared by glucuronidation [8]. Bup and nbup have been detected in hair following extraction with either diluted HCl, NaOH or buffer pH 7.4 followed in most instances by analysis using high pressure liquid chromatography

tandem mass spectrometry (LC/MS/MS) [3, 15]. Some studies revealed higher concentrations of bup than of nbup, whereas others showed nbup being present in hair in higher concentrations than the parent drug [2, 4, 5, 13, 15].

The main objectives of the present study were to check

- whether a relationship between the daily dose and the concentration of bup and nbup in hair following log-term administration exists,
- whether bup and nbup are susceptible to degradation in diluted hydrochloric acid, and
- whether a degradation of bup equals that of nbup or not.

2. Material and Methods

The study protocol was approved by the Ethics Committee of the Medical Faculty of Mannheim, and subjects (7 females, 11 males) provided informed consent prior to collection of a hair sample from the posterior vortex which was cut as close to the scalp as possible. Subjects were treated with bup by the sublingual route at a constant dose for several months at least; some personal data are summarized in Table 1.

Tab. 1. Gender (G; f: female; m: male), weight (W; kg) and daily dose of buprenorphine (D; mg) of subjects participating in the study.

No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
G	m	m	m	f	f	m	m	m	f	m	f	m	f	f	m	m	m	f
W	97	131	105	74	72	83	75	87	62	88	51	85	47	95	85	60	90	55
D	8	5	11	10	12	8	3	12	12	8	6	8	12	2.2	4	8	8	6

Hair colors ranged from honey blond to brown; the specimen from subject 5 was bleached and that from subject 14 was obviously dyed. The concentration of bup and nbup was determined by LC/MS/MS from specimens with a total length ≤ 4 cm (n=4) or from the proximal 4-cm section (n=14). Pure substances (500 ng bup and nbup, respectively) were incubated in 0.1 M HCl at 60°C for up to 24 hours mimicking procedures that have been published to extract bup and nbup from hair [15].

2.1. Materials

Undeuterated and deuterated bup and nbup (1 or 0.1 mg/mL methanol) were provided by LGC (Wesel, Germany); methanol and acetonitrile (both HPLC grade) were purchased from Roth (Karlsruhe); all other solvents and chemicals were of the highest purity available; drug-free pooled Caucasian hair was obtained from Kerling International (Backnang).

2.2. Sample preparation

Hair samples were washed twice with dichloromethane (2 min, ambient temperature). Methanol (2 mL) and internal standards (4 ng bup-d_4 and nbup-d_3, respectively) were added to pulverized hair samples (ca. 40 mg), placed in an ultrasonic bath (35°C, 4 h) and then left at 40°C overnight. The dried methanolic phase was re-dissolved in 500 µL 0.1 M NaOH; extraction was performed using 1-chlorobutane/acetonitrile (1 mL, 4:1, v/v). The dried organic phase was re-dissolved in 50 µL of the mobile phase. Aliquots from the degradation study

were taken at 0.5, 1, 2, 4, 6, and 24 h; 1.5 mL 0.1 N NaOH and internal standards were added, and analytes were extracted using 1-chlorobutane/acetonitrile (2 mL, 4:1, v/v).

2.3. Instrumentation

Analysis was performed on an API 4000 tandem mass spectrometer (AB Sciex, Darmstadt) with a TurboIon ionization source (positive mode) interfaced to a HPLC pump and an auto-sampler (1100 series, Agilent, Waldbronn). Separation was achieved on a Zorbax Eclipse XDB C8 column (2.1 x 150 mm, 5 µm particle size; Agilent, Waldbronn) with acetonitrile/methanol/4 mM ammonium acetate, pH 3.2 (35:35:30 v/v/v) as the mobile phase at a flow rate of 250 µL/min. Transitions monitored for quantitation were: bup (-d_4) m/z 468→468 (472→472); nbup (-d_3) m/z 414→414 (417→417); alleged rearrangement products of bup and nbup were monitored at m/z 436→436 and 382→382, respectively.

2.4. Evaluation

Calibration curve (hair samples): 0.02, 0.05, 0.1, 0.5, 0.75 and 1.0 ng/mg hair (0.1 ng bup-d_4 and nbup-d_3/mg hair); a blank and a zero sample were also prepared. Calibration curve (degradation study): 100, 300, 500, 1,000 ng bup or nbup (200 ng bup-d_4 and nbup-d_3). The following parameters were checked: linearity, LOD, LOQ, ion suppression, extraction efficiency, imprecision and inaccuracy at 0.1 and 0.75 ng bup or nbup/mg hair [7, 9].

3. Results and Discussion

Bup and its metabolite were well separated (3.15 min, 2.06 min). Calibration lines were linear ($r > 0.998$); LOD/LOQ: 0.003/0.008 ng/mg hair for bup, 0.004/0.010 ng/mg hair for nbup; ion suppression could not be observed; extraction efficiencies varied from 80 to 90% (bup) and from 43 to 47% (nbup); imprecision / inaccuracy was ≤ 5.7% / ≤ 18.3% for bup and nbup at both, the low and high concentration levels.

All hair samples tested positive for bup (mean: 0.069 ng/mg hair, range: LOD-0.238 ng/mg hair) and nbup (mean: 0.233 ng/mg hair; range: 0.043-0.961/mg hair); the molar concentration ratios bup/nbup were highly variable (mean: 0.34, range: 0.015-1.19). The concentration of the metabolite was always higher than that of the parent drug except sample 1 (Fig. 1). This finding is in line with the results reported by Wilkins et al. [14] and Goodwin et al. [4], where digestion was performed using diluted NaOH.

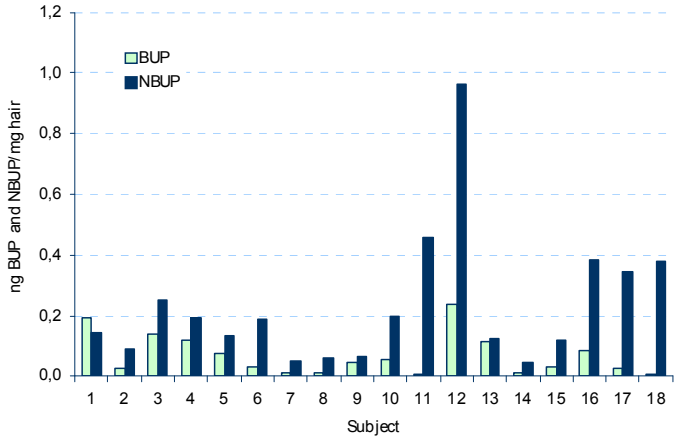

Fig. 1. Concentrations of bup and nbup (ng/mg) in proximal hair segments of subjects (n=18).

It should be noted, that only the proximal sections have been used in the present investigation and that an increasing ratio of bup/nbup distally along the hair shaft has been observed [4].

Both bup and nbup degraded at the acidic condition chosen dependent on time (Fig. 2); simultaneously, peaks of the respective rearrangement products increased at the same time (Fig. 3). Such rearrangement products could not be detected using the present processing for sample preparation. Following incubation in diluted acid often resulted in a lower concentration of nbup compared to that of bup in hair; even negative nbup findings along with positive bup findings have been observed [15]. A likely explanation may be that degradation of nbup occurred faster than of bup.

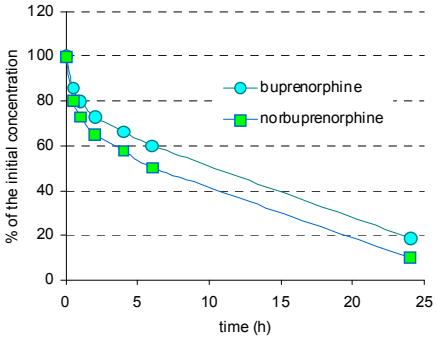

Fig. 2. (on the left): Decrease of bup and nbup with time (0.1 M HCl, 60°C).

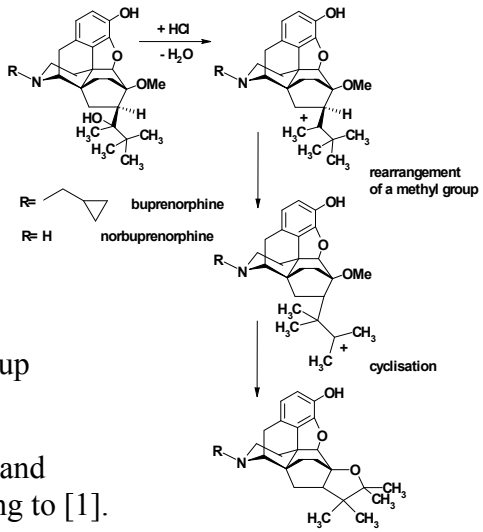

Fig 3. (on the right): Rearrangement of bup and nbup at acidic conditions, modified according to [1].

Results were adequate to establish a relationship (r = 0.851, n = 15) between the weight-related dose and the combined concentration of bup and nbup (referred to the molecular weight of bup) if findings < LOQ (n = 1) and from cosmetically treated hair (n = 2) were excluded from data analysis (Fig. 4).

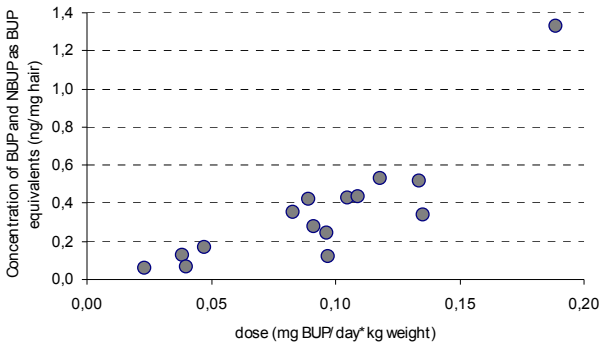

Fig. 4. Relationship between hair assay results (n = 15) and the daily doses of bup referred to the individual weight (r = 0.851).

These data should cautiously be applied to an interpretation of hair results if specimen preparation has not been specified, single or varying doses have been applied or if improper or illegal use of the drug is suspected.

4. Conclusion

Nbup is the major metabolite in hair samples from subjects maintained with bup. The concentration of bup and nbup in hair will be underestimated if analytes are recovered by acidic procedures; in addition, their concentration ratio may be inverted in hair.

Although a highly variable molar concentration ratio bup/nbup could be estimated from individual results, there is evidence that the combined concentrations of bup and nbup in hair increase with increasing dose. A positive relationship could be established between the daily doses relating to weight.

5. References

[1] Blom Y, Bondesson U (1985) Analysis of buprenorphine and its N-dealkylated metabolite in plasma and urine by selected-ion monitoring. J Chromatogr 338:89-98

[2] Cirimele V, Kintz P, Lohner S, Ludes B (2000) Buprenorphine to norbuprenorphine ratio in human hair. J Anal Toxicol :448-9

[3] Favretto D, Frison G, Vogliardi S, Ferrara SD (2006) Potentials of ion trap collisional spectrometry for liquid chromatography/electrospray ionization tandem mass spectrometry determination of buprenorphine and norbuprenorphine in urine, blood and hair samples. Rapid Commun Mass Spectrom 20:1257-68

[4] Goodwin RS, Wilkins DG, Averin O, Choo RE, Schroeder JR, Jasinski DR et al. (2007) Buprenorphine and norbuprenorphine in hair of pregnant women and their infants after controlled buprenorphine administration. Clin Chem 53:2136-43

[5] Kintz P, Villain M, Tracqui A, Cirimele V, Ludes B (2003) Buprenorphine in drug-facilitated sexual abuse: A fatal case involving a 14-year-old boy. J Anal Toxicol 27:527-9

[6] Kronstrand R, Scott S (2007) Drug incorporation into hair. In: Kintz P (ed.) Analytical and practical aspects of drug testing in hair. Taylor & Francis, Boca Raton, FL, USA, p. 1-23

[7] Matuszewski BK, Constanzer ML, Chavez-Eng CM (2003) Strategies for the assessment of matrix effect in bioanalytical methods based on HPLC-MS/MS. Anal Chem 75:3019-30

[8] Oechsler S, Skopp G (2010) An in vitro approach to estimate putative inhibition of buprenorphine and norbuprenorphine glucuronidation. Int J Legal Med 124:187-94

[9] Peters FT, Drummer OH, Musshoff F (2007) Validation of new methods. Forensic Sci Int 165:216-24

[10] Poetsch L, Eser HP, Skopp G, Moeller MR (1998) Zum Suchtmittelnachweis in Haaren. VII: Untersuchungen bei Methadonpatienten unter konstanter Wirkstoffeinnahme. Rechtsmedizin 8:47-50

[11] Poetsch L, Skopp G (2004) Inkorporation von Fremdsubstanzen in Haare. In: Madea B, Mußhoff F (Hrsg) Deutscher Ärzteverlag, Köln, S. 29-98

[12] Skopp G, Strohbeck-Kuehner P, Mann K, Hermann D (2007) Deposition of cannabinoids in hair after long-term use of cannabis. Forensic Sci Int 170:46-50

[13] Vincent F, Bessard J, Vacheron J, Mallaret M, Bessard G (1999) Determination of buprenorphine and norbuprenorphine in urine and hair by gas chromatography-mass spectrometry. J Anal Toxicol 23:270-9

[14] Wilkins DG, Valdez AS, Nagasawa PR, Gygi SP, Rollins DE (1998) Incorporation of drugs for the treatment of substance abuse into pigmented and nonpigmented hair. J Pharm Sci 67:435-40

[15] Yegles M, Wennig R (2007) Opioids testing in hair. In: Kintz P (ed.) Analytical and practical aspects of drug testing in hair. Taylor & Francis, Boca Raton, FL, USA, p. 73-94