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Relationships Between Concentrations of Cocaine and Its Hydrolysates in Peripheral Blood, Heart Blood, Vitreous Humor and Urine

ABSTRACT: Cocaine is known to degrade *in vivo* and *in vitro* by several hydrolytic mechanisms. A previous study found that the initial amount of cocaine added to plasma could be accounted for by summing the molar concentrations of cocaine's hydrolysis products and the cocaine remaining after hydrolysis. The present study was undertaken to investigate whether or not relationships might exist between such molar concentration sums for different postmortem bodily fluids. Determinations of cocaine, benzoylecgonine, ecgonine methyl ester, and ecgonine were performed using liquid chromatography/mass spectrometry (LC/MS/MS) with heart blood, femoral blood, vitreous humor (VH), and urine (UR). The results demonstrate a strong correlation between blood and VH concentrations (correlation coefficients of 0.88–0.94), weak correlation between the UR and blood concentrations (correlation coefficients of 0.61–0.64), and weak correlation between UR and VH concentrations (correlation coefficient of 0.59). The results demonstrate that ecgonine is a significant hydrolysate with concentrations on the same order of magnitude as benzoylecgonine. The results are consistent with rapid distribution of the parent drug and its hydrolysates in the blood and VH. The strong correlation between the blood and VH demonstrates that VH is an important medium for toxicology testing when attempting to make a determination of cocaine intoxication.

KEYWORDS: forensic science, toxicology, cocaine, cocaine metabolites, drug abuse, vitreous humor

Toxicological tests are an essential part of death investigations conducted by medical examiners. The toxicology testing that is routinely performed frequently demonstrates positive levels of cocaine and/or its major metabolites, hydrolysates (1). Studies have shown that in blood and in aqueous solutions in general, cocaine can rapidly degrade (2,3). The degradation of cocaine is known to involve hydrolysis by several mechanisms. Cocaine is converted to ecgonine methyl ester, and benzoylecgonine via pH and temperature-dependent spontaneous hydrolysis in aqueous solutions and also by enzymatic hydrolyses in biological systems (3,4). Enzymatic hydrolysis is affected by plasma and liver esterases in both ante- and postmortem specimens (4). Both benzoylecgonine and ecgonine methyl ester are further hydrolyzed to ecgonine (3). Although in the past, ecgonine has been thought to be a minor cocaine metabolite without much significance, in recent years ecgonine has been found in significant concentrations comparable with those of benzoylecgonine in both blood and urine (UR) (5). Based upon the magnitude of concentrations, the most significant hydrolysates or de-esterified moieties of cocaine after ingestion are benzoylecgonine, ecgonine methyl ester, and ecgonine (5).

Several studies have demonstrated the lack of correlation between cocaine concentrations among postmortem fluids taken from any one autopsy (6,7). Other studies suggest possible correlation for concentrations for cocaine and separately for benzoylecgonine between vitreous humor (VH) and blood (8,9).

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One study found that although the *in vitro* degradation of added cocaine in human plasma was highly dynamic, the molar sum of the products formed by de-esterification accounted for the initial amount of cocaine added (10). The purpose of the current study was to determine whether or not any relationships might exist for such molar sums between various postmortem autopsy specimens. To this end, we have determined the concentrations of cocaine, benzoylecgonine, ecgonine methyl ester and ecgonine in postmortem heart blood (HB), peripheral blood, VH, and UR.

Materials and Methods

Specimen Collection

At the time of autopsy, HB was collected in 10 mm Becton-Dickinson gray- and red-stoppered Vacutainer® tubes (Franklin Lakes, NJ). The gray-stoppered tubes contained 25 mg of sodium fluoride and 20 mg of potassium oxalate. The red-stoppered tubes contained no additives. Femoral blood (FB) was collected only in gray-stoppered Vacutainer® tubes. VH was collected in red-stoppered Vacutainer® tubes. UR was collected in 50 mL polypropylene tubes. All specimens were stored at 4°C after collection. Cadavers were stored in a cold room at 4°C from the time of receipt until the time of autopsy. Such collection and storage procedures have been suggested to slow the hydrolysis of cocaine (2,3). Peripheral blood was obtained by femoral venopuncture and HB was obtained by needle puncture under direct visualization of one or more of the great vessels immediately adjacent to the heart. VH was withdrawn from both eyes by inserting an 18-gauge needle through the lateral aspect of the sclerae. UR was collected by needle puncture of the bladder under direct visualization.

Analytical Methods

Initial screening tests for drugs other than volatiles were performed using enzyme-linked immunoabsorbant assay (ELISA)

tests (ISOTEL, Ithaca, NY). For solid tissues, enzymatic digestion was performed before ELISA (11). For volatile substances a gas chromatographic procedure was used (12). For drugs other than cocaine and its metabolites, a liquid chromatography method with photo diode array detection was used (13). Using a ThermoOrion model 420 pH meter (Beverly, MA), specimens from tubes having no additives were evaluated to determine the pH at 25°C.

For cocaine and its metabolites and degradation products, confirmation and quantitation were performed using liquid chromatography coupled with atmospheric pressure chemical ionization mass spectrometry (LC-APCI-MS/MS). The liquid chromatograph was a Surveyor® (ThermoFinnigan, San Jose, CA) having an autosampler and a photo diode array detector in series with the mass spectrometer. The mass spectrometer was a ThermoFinnigan® LCQ Advantage® with DECA XP® orthogonal APCI. The operating software was XCALIBUR®1.3.

With minor modification of only the specimen extraction procedure, the procedures used were those described by Dams et al. (14,15). The extraction modification involved using 25 μL of specimen instead of the 200 μL used by Dams et al. Accurate pipetting of 25 μL was assured by using a Microman® M25 (Gillman, Middleton, MA). Analyte standards and deuterated internal standards were obtained from Cerillaint (Austin, TX). As previously observed by Dams et al., no medium effects were found; presumably in part because of the small amount of sample used. Cocaine, ecgonine methyl ester, benzoylecgonine, ecgonine, norcocaine, cocaethylene, and ecgonine ethyl ester were included in the LC-APCI-MS/MS analyses. Cases involving any ethanol were excluded from the present investigation. Calibrators routinely covered the range 0.1 to 7 μM. The upper limit of linearity was about 33 μM. Specimens found to be more concentrated than 10 μM in any one analyte were reanalyzed by diluting with de-ionized water prior to reanalysis. Whole blood custom controls containing ecgonine, ecgonine methyl ester, benzoylecgonine, cocaine, and cocaethylene were obtained from UTAK laboratories (Valencia, CA). To evaluate the accuracy of the analyses for some of the analytes, blood from five cases were sent to Wuesthoff Reference Laboratories (Melbourne, FL) for analysis via gas chromatography/mass spectrometry. To convert micromolar concentrations to milligrams per liter, the following conversion factors as multipliers are required: for ecgonine (0.18522), for ecgonine methyl ester (0.19925), for benzoylecgonine (0.28933), and for cocaine (0.30335).

Calculations

Most calculations were performed using Microsoft® Excel® in Microsoft Office XP Professional® (Microsoft, Seattle, WA). Regression and correlation results were confirmed using JMP® version 4 (SAS, Cary, NC). Confidence limits for the correlation coefficients were calculated using equations and tables given by Zar (16). Significant figures were also presented in accordance with Zar (16).

Results

Three types of coefficient of variation (CV%) were determined to assess the imprecision associated with the analytical procedure. These CV%s are presented in Table 1. The within-run CV%s were based on the whole blood controls at the midpoint of the calibrators. The between-run CV%s were determined from the data of runs on different days. The between-laboratory CV%s were determined using postmortem case blood. The concentration of those specimens ranged from 0.1 to 4 μM.

TABLE 1—*Precision and accuracy relating to analytical procedure.*

	Ecgonine	Ecgonine Methyl Ester	Benzoyl-ecgonine	Cocaine	*N*
Within-run CV%	4.3	2.8	5.1	5.3	4
Between-run CV%	11	11	7.7	5.5	4
Between-laboratory CV%	–	5.9	5.6	2.7	5

CV, coefficient of variation.

The ranges, means, and standard deviations of the determined concentrations in micromoles per liter for ecgonine, ecgonine methyl ester, cocaine, and benzoylecgonine in peripheral blood, HB, VH, and UR are presented in Table 2. Ranges of pH for the specimens are also presented. No pHs were determined for the FB specimens as they were only collected in tubes with pH adjusting additives.

For further presentation purposes, the sums of ecgonine, ecgonine methyl ester, benzoylecgonine, and cocaine in micromoles per liter were calculated and represented as a total concentration, T, for each of the specimen types: VH; FB; HB; and UR. Graphs are presented as Figs. 1–6 for T of one specimen vs. T of another specimen for the specimen pairs (VH, FB), (VH, HB), (HB, FB), (UR, HB), (UR, FB), and (UR, VH). The line in each graph corresponds to the linear least squares fit. The correlation coefficients (*r*); slopes (*b*) with standard errors; intercepts (*a*) with standard errors; number of cases (*N*); lower 95% confidence limits (L1) for the correlation coefficients; and upper 95% confidence limit (L2) for the correlation coefficients for each fit are presented in Table 3. No concentrations of norcocaine, cocaethylene, or ecgonine ethyl ester greater than 0.01 μmol/L were detected in any of the specimens presented. Again, cases involving ethanol were excluded from this study.

Discussion

By analyzing the data in Fig. 1 through Fig. 6 and by analyzing the results in Table 3, it is apparent that by using the total sums of micromolar concentrations of cocaine and its hydrosylates, the VH and FB are highly correlated, VH and HB are highly corre-

TABLE 2—*Ranges, means, and standard deviations of concentrations in micromoles per liter and ranges of pH.*

	Ecgonine	Ecgonine Methyl Ester	Benzoyl-ecgonine	Cocaine	pH
Femoral blood	0.5–11.7*	0–5.59	0–15.26	0–4.71	NA§
	4.5†	1.5	5.9	0.7	
	3.1‡	1.5	5.5	1.1	
Heart blood	0.6–10.1	0–12.2	0–19.78	0–9.83	5.99–6.65
	4.2	2.6	6.4	1.2	
	3.0	3.4	6.4	2.3	
Vitreous humor	0–6.77	0–3.70	0–7.86	0–4.77	6.73–7.40
	0.8	1.1	3.7	1.0	
	0.7	1.0	3.8	1.5	
Urine	0–369	0–959	0–2870	0–1370	5.43–7.10
	120.7	343.7	971.2	124.8	
	174.1	540.4	1920.2	333.6	

*Range.
†Mean.
‡Standard deviation.
§NA indicates not analyzed for pH because the tubes had pH-adjusting additives.

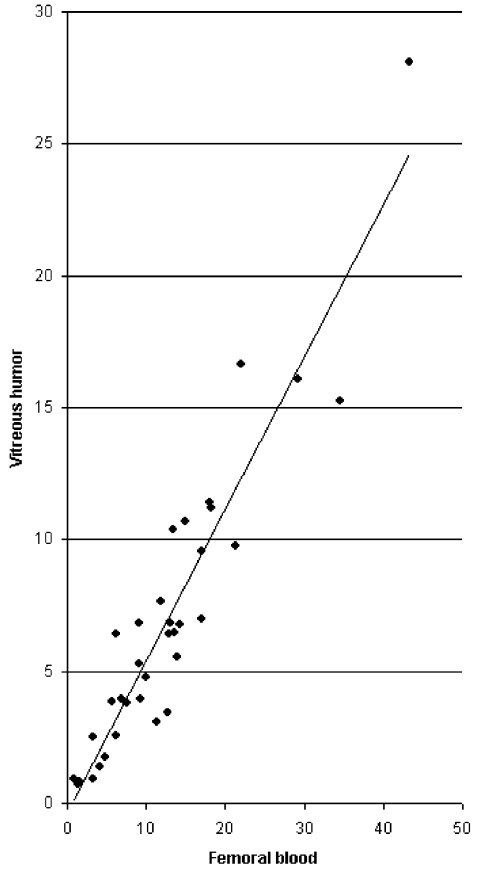

FIG. 1—*Vitreous humor vs. femoral blood; total micromolarities of cocaine and its hydrolysates.*

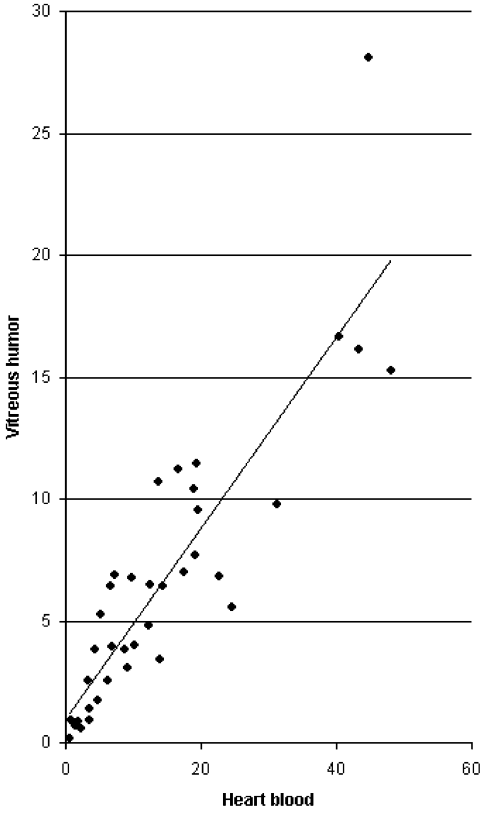

FIG. 2—*Vitreous humor vs. heart blood; total micromolarities of cocaine and its hydrolysates.*

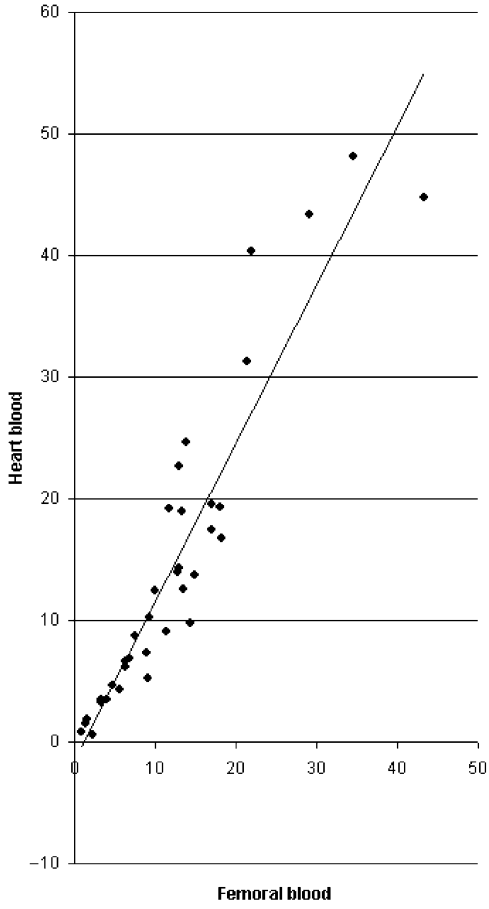

FIG. 3—*Heart blood vs. femoral blood; total micromolarities of cocaine and its hydrolysates.*

lated and HB and FB are highly correlated. The correlations between UR and other specimens appear poorly correlated. The 95% confidence intervals (CIs) for the correlation coefficients for VH vs. FB, VH vs. HB, and HB vs. FB are narrow. Conversely, the 95% CIs for the correlation coefficients for UR vs. HB, UR vs. FB and UR vs. VH are wide. These CIs reflect the relatively small scatter for the points about the least squares lines in Fig. 1 through Fig. 3 and the relatively large scatter for the points in Fig. 4 through Fig. 6.

The high correlations may indicate a rather rapid distribution of cocaine and its hydrolysates among peripheral blood, HB and VH. Rapid distribution of cocaine in the living has been previously suggested (17). Rapid distribution of cocaine into VH has been demonstrated in the swine (18). Within 8 min after administration or 3 min after death, the concentrations of parent cocaine in the VH and the FB were comparable (18). The results in Table 3 of the present study demonstrate comparable mean concentrations for parent cocaine in VH, peripheral blood, and HB.

The strong correlation between total cocaine concentrations in the blood and VH indicates that VH is an important medium for analysis when performing forensic toxicology testing. In the absence of other pathological or health history factors, diagnosis of acute cocaine intoxication is contemporarily based upon isolation of parent cocaine from the heart or peripheral blood; however, our results suggest that when it comes to cocaine testing, VH may be as reliable as blood. This is consistent with previous observations for cocaine and benzoylecgonine, individually (8,9). Furthermore, the strong correlation between femoral and HB indicates that

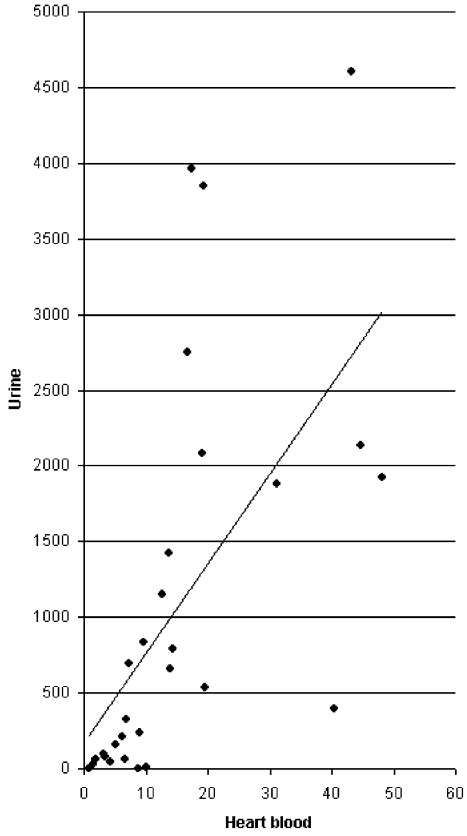

FIG. 4—*Urine vs. heart blood: total micromolarities of cocaine and its hydrolysates.*

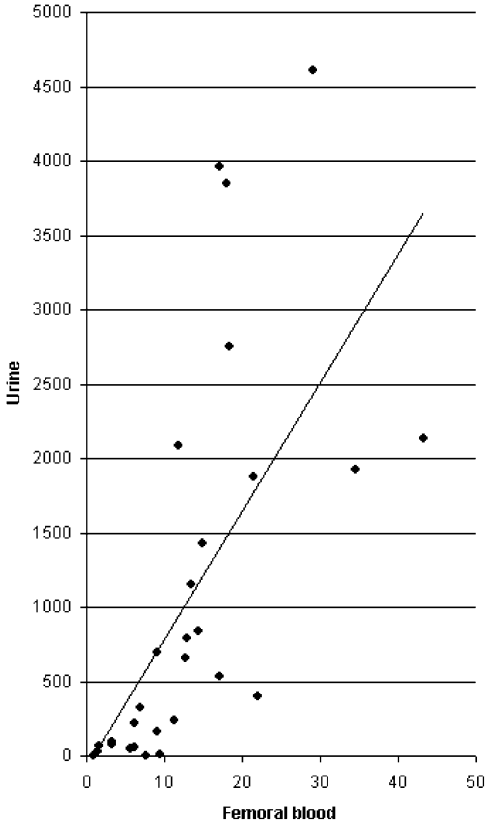

FIG. 5—*Urine vs. femoral blood: total micromolarities of cocaine and its hydrolysates.*

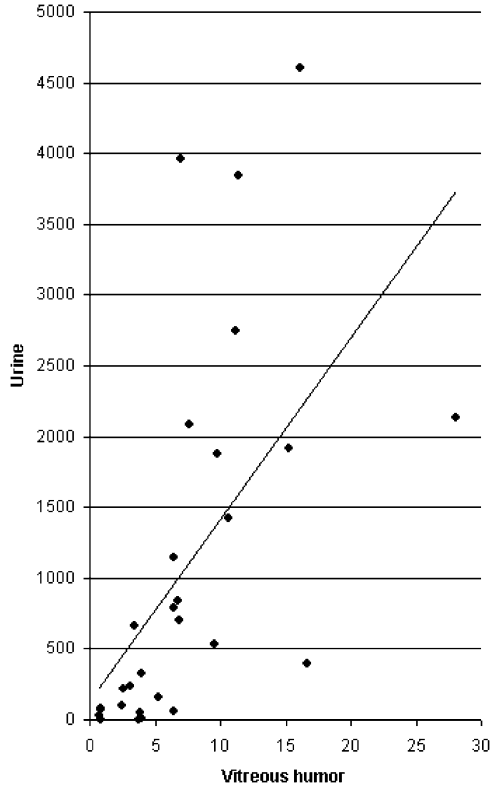

FIG. 6—*Urine vs. vitreous humor: total micromolarities of cocaine and its hydrolysates.*

TABLE 3—*Statistical parameters for specimen correlations.*

	r*	b†	a‡	N§	L1¶	L2‖
VH vs. FB	0.9387	0.5761 ± 0.0368	−0.3397 ± 0.5739	35	0.88	0.97
VH vs. HB	0.8825	0.3918 ± 0.0353	0.9726 ± 0.6754	37	0.78	0.90
HB vs. FB	0.9356	1.3016 ± 0.0842	−1.4224 ± 1.2941	36	0.87	0.97
UR vs. HB	0.6124	59.120 ± 14.69	172.08 ± 297.35	29	0.32	0.80
UR vs. FB	0.6477	86.03 ± 19.47	−67.716 ± 319.69	29	0.37	0.82
UR vs. VH	0.5863	128.23 ± 34.10	131.28 ± 20.74	29	0.28	0.78

*r = correlation coefficient.
†b = slope.
‡a = intercept.
§N = number of data points.
¶L1 = lower 95% confidence limit for correlation coefficient.
‖L2 = upper 95% confidence limit for correlation coefficient.
VH, vitreous humor; FB, femoral blood; HB, heart blood; UR, urine.

postmortem redistribution may not be a significant factor when interpreting positive total cocaine results.

Acknowledgments

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