Cocaine, Cocaine Metabolite, and Ethanol Concentrations in Postmortem Blood and Vitreous Humor

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Abstract

The use of postmortem cocaine and metabolite concentrations is a complex subject. This study was undertaken to determine (1) the usefulness of vitreous humor as a specimen, compared with blood, to quantitate cocaine and cocaine metabolites; (2) whether there is a preferential site of disposition for cocaethylene between vitreous humor and blood; and (3) if the presence of cocaethylene influences the concentration of benzoylecgonine in postmortem specimens. Cocaine, benzoylecgonine, and cocaethylene were quantitated in blood and vitreous humor by gas chromatography–mass spectrometry, and ethanol was quantitated by gas chromatography in 62 medical examiner cases. No differences were found between mean concentrations of vitreous cocaine 0.613 mg/L (standard deviation [SD] 0.994 mg/L), cocaethylene 0.027 mg/L (SD 0.59 mg/L), and ethanol 0.092 g/dL (SD 0.13 g/dL) compared to blood cocaine 0.489 mg/L (SD 1.204 mg/L), cocaethylene 0.022 mg/L (SD 0.055 mg/L), and ethanol 0.058 g/dL (SD 0.91 g/dL), respectively. However, a statistical difference was found between mean benzoylecgonine concentrations in vitreous 0.989 mg/L (SD 1.597 mg/L) and blood 1.941 mg/L (SD 2.912 mg/L) ($p = 0.0004$). Regression analysis demonstrated that linear relationships were present between concentrations of vitreous and blood cocaine ($r = 0.854$) and benzoylecgonine ($r = 0.763$). However, the correlation coefficients were lower for cocaethylene ($r = 0.433$) and ethanol ($r = 0.343$). There were variations between the concentrations of cocaine and metabolites both in terms of magnitude and also direction of change. Mean concentrations of benzoylecgonine in blood and vitreous were higher in cases where ethanol was absent, 2.593 mg/L (SD 3.195 mg/L) and 1.431 mg/L (SD 2.021 mg/L), compared to when ethanol was present, 1.199 mg/L (SD 2.396 mg/L) and 0.469 mg/L (SD 0.553 mg/L). This study demonstrates that vitreous humor may be used to quantitate cocaine and cocaine metabolites; however, because the concentrations of cocaethylene in vitreous humor and blood were not well correlated, vitreous humor may not be a reliable specimen for measuring cocaine and cocaine metabolite concentrations.

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Introduction

The interpretation of cocaine toxicity in postmortem samples is a complicated subject. Previous studies have demonstrated the inability to relate postmortem cocaine concentrations to fatal toxicity (1,2). Cocaine is metabolized by esterases in plasma and liver in ante- and postmortem samples to benzoylecgonine and ecgonine methyl ester, which are both inactive metabolites (3). However, studies have shown that benzoylecgonine does not increase proportionally to the rate of metabolism of cocaine and is therefore unreliable in extrapolating antemortem levels (3). Additionally, there are discrepancies not only in the concentration of cocaine and benzoylecgonine, but also between sites of collection (1,3,4). Hearn et al. (4) showed that subclavian blood tends to be lower than central and lower extremity peripheral levels. Logan et al. (3) showed that cerebrospinal fluid (CSF) levels and blood levels do not always correlate, and the direction and degree of difference cannot be predicted. Beno and Kriewald (2) found vitreous humor postmortem cocaine concentrations to be up to 250% greater than blood concentrations. Although one other study also found this difference (3), a pig-model study did not (5).

The factors hypothesized to account for these differences in postmortem cocaine concentrations are as follows. First, cocaine appears to continue to undergo metabolic activity after death, leading to the formation of benzoylecgonine and ecgonine methyl ester. This in turn results in differences in blood concentrations of cocaine and benzoylecgonine from antemortem concentrations. Second, cocaine has a relatively large volume of distribution (Vd = 2 L/kg), which can result in tissue storage of the drug and metabolites, which may lead to postmortem release of the drug (1,3,4,5). Third, the method of sample collection and preservation influences the rate of metabolism. Elevated temperatures and alkaline pH are known to enhance enzymatic activity. Methods used to minimize further enzymatic activity after collection include NaF, temperatures at or below 4°C, and acidic pH. Spontaneous hydrolysis is also known to continue after death. Hydrolysis can be decreased in collected specimens with acidic pH and cooler temperatures (3,5).

Compounding the issue is the observation that many cocaine users simultaneously ingest ethanol (6). Cocaethylene is formed through the combined metabolism of ethanol and cocaine (7). Cocaethylene is an active metabolite and may actually potentate and prolong the effects of cocaine, thus leading to increased toxicity (8,9). A study in pigs compared vitreous and blood concentrations of cocaine, cocaethylene, and benzoylecgonine, both antemortem and postmortem, to determine if these two sites correlate (5). Although the results demonstrated good correlation between these two sites, there was significant variability in magnitude and direction of change of the concentrations of cocaine and metabolites, especially in blood. Also, vitreous concentrations were found to gradually increase over the postmortem interval. A study by Moriya et al. (10) looked at cocaine and cocaethylene concentrations in decomposed bodies to determine if cocaethylene could be produced exogenously by the ethanol made by bacteria. The results demonstrated that all cocaethylene concentrations were from antemortem concentrations and no exogenous cocaethylene was produced (10).

The purpose of the present study was threefold. First, we evaluated the use of vitreous humor compared to blood, in humans, as a specimen to quantitate cocaine and metabolites. Second, we investigated whether there was a preferential site of disposition for cocaethylene. Third, we determined whether the presence of cocaethylene influenced the concentration of benzoylecgonine.

Materials and Methods

Specimen collection

Over a period of 2.5 years, 62 cases involving cocaine where identified. All specimens were obtained from cases from the Hennepin County Medical Examiners office. As part of routine toxicology evaluation in all cases, urine drug screens were performed by immunoassay and thin-layer chromatography. If cocaine or metabolites were identified by the drug screen, cocaine, benzoylecgonine, and cocaethylene were quantitated by gas chromatography–mass spectrometry (GC–MS). Ecgonine methyl ester, a metabolite of cocaine, is not measured in our laboratory; therefore, concentrations are not reported in this study. Blood and vitreous ethanol concentrations were also performed on all cases by GC. Whole blood samples were collected in gray-top tubes containing 17.5 mg of NaF. Vitreous humor samples were collected in tubes which did not contain any preservatives. All samples were stored at –20°C within 1 h of collection and remained at –20°C until analysis was performed 1–5 days after collection.

Analysis

Cocaine, benzoylecgonine, and cocaethylene were quantitated in vitreous humor and whole blood by GC–MS on a Hewlett-Packard 5972 mass selective detector following chromatography on a 5890 GC equipped with a 30-m DB-5 capillary column. A Unix-based Target Thru-Put operating software computer system was used for data compilation (11,12). All chemicals used were analytical-reagent grade. Standards and deuterated internal standards were obtained from Radian Corp. Briefly, 1 mL of whole blood or vitreous (appropriate standards, controls, and case) was mixed with 50 µL of cocaine-d_3, benzoylecgonine-d_3, and cocaethylene-d_3 (10 µg/dL). The mixture was vortex mixed and sonicated for 15 min at room temperature. The sample was then vortex mixed for 30 min after adding 6 mL of 0.1M phosphate buffer (pH 6.0) and centrifuged for 15 min at 2000 rpm. Supernatant was poured into the extraction column (Bond Elut columns preconditioned sequentially with methanol and phosphate buffer, pH 6.0) and allowed to run through for 2 min. Each column was rinsed with 3 mL water, 3 mL 0.1M HCl, and 9 mL methanol. Two milliliters methylene chloride/isopropanol (80:20) with 2% NH_4OH was subsequently passed through the column and collected. Each tube was evaporated at 30–40°C with nitrogen using a Pierce evaporator. The residue from evaporation was reconstituted with 0.5 mL methylene chloride, vortex mixed, poured into clean tubes, and reevaporated. Fifty microliters BSTFA (with 1% TCMS) and 50 µL DMF were added to the tubes, which were then vortex mixed and capped. Samples were derivatized at 60–70°C for 15–20 min. After cooling, samples were transferred to the autosampler. The MS was operated in the select ion monitoring (SIM) mode, and the following ion were scanned: cocaine quantitating ion 182, qualifier ions 272, 303; cocaine-d_3 quantitating ion 185, qualifier ions 275, 306; benzoylecgonine quantitating ion 240, qualifier ions 256, 361; benzoylecgonine-d_3 quantitating ion 243, qualifier ions 259, 364; cocaethylene quantitating ion 196, qualifier ion 272; cocaethylene-d_3 quantitating ion 197, qualifier ion 275. Standard curves were derived for each analyte. Area ratios for unknowns were used to calculate the corresponding analyte concentrations. Limits of detection (LOD), limits of quantitation (LOQ), and limits of linearity (LOL), respectively, were as follows: cocaine 50, 75, 3000 µg/L; benzoylecgonine 50, 75, 3000 µg/L; and cocaethylene 50, 100, 3000 µg/L. Values < 0.100 µg/L were presented as < 0.1 µg/L. Any value > 5000 µg/L was diluted and re-extracted. At 100 µg/L, CV%s for all three analytes were < 9%.

Ethanol was determined by GC on a Hewlett-Packard model 5890 using headspace sampling and an autosampler. Briefly, 200 µL of a 240 mg/dL *n*-propanol internal standard containing sodium fluoride and 200 µL of a whole blood sample were pipetted into a 20-mL headspace vial and sealed. The vial was then placed into a headspace sampler (Hewlett Packard model 19395A). After 60 s at 60°C, headspace was injected onto GC–MS programmed as follows: oven temperature 55°C, injection temperature 250°C, initial column temperature 55°C, without ramping. A DB-wax 30-m minimum length, 530-µm capillary column was used. The helium carrier gas flow rate was 6 mL/min. A Hewlett Packard 3396 integrator was used to quantitate ethanol concentrations from a pre-established standard curve, which shows linearity from 0.005 g/dL to 1.000 g/dL. The CV% was < 3% from 0.020 to 0.400 g/dL.

Statistics

Correlation coefficients were calculated by the Pearson Product-Moment method. All calculations were performed on

Table I. Biochemical and Demographic Findings for 62 Medical Examiner Cases Involving Cocaine

	Cocaine and metabolites (mg/L)						Ethanol (g/dL)				
	Blood			Vitreous							
Subject	cocaine	benzoylecgonine	cocaethylene	cocaine	benzoylecgonine	cocaethylene	blood	vitreous	Gender	Cause of death	Manner of death
1	.06	.52	.06	.29	.17	.18	.08	.12	M	Shotgun wound	Homicide
2	.03	.35	.06	.13	.18	.09	.25	.30	M	Hanging	Suicide
3	.08	.42	.15	.39	.51	.15	.05	.08	M	GSW*	Homicide
4	.09	3.19	0	.67	2.09	0	0	0	M	Poly drug OD	Accident
5	.14	.31	.10	.29	.25	.09	.09	.05	M	MBFI	Homicide
6	.40	1.40	0	.50	.76	0	.08	.11	M	Shotgun wound	Homicide
7	.36	1.70	0	.40	.99	0	0	0	M	GSW	Homicide
8	2.10	2.29	0	1.54	.96	0	0	0	M	GSW	Suicide
9	0	.10	0	0	.10	0	.30	0	M	CO poisoning	Accident
10	.85	5.40	0	3.70	4.84	0	0	0	M	GSW	Homicide
11	.10	14.2	0	.10	10.7	0	0	0	M	Poly drug OD	Accident
12	0	.23	.06	.17	.15	.16	.21	.31	M	Poly drug OD	Accident
13	.12	.34	.10	.11	.10	.10	.25	ND	M	MBFI	Homicide
14	4.70	12.3	0	4.50	.74	.09	.01	.01	M	Poly drug OD	Accident
15	0	.13	0	.10	.11	.05	0	.42	M	Stabbing	Homicide
16	.12	.77	.10	0	.10	0	0	ND	M	GSW	Homicide
17	.10	.61	0	.52	.45	.31	.35	ND	M	Asphyxia	Homicide
18	0	.65	0	.10	.23	0	.13	ND	M	Hanging	Suicide
19	0	.26	0	.13	.26	0	.25	ND	M	Shotgun wound	Homicide
20	0	.25	0	0	.17	.03	.10	ND	M	Poly drug OD	Suicide
21	.33	2.20	0	.90	1.10	0	ND	ND	M	GSW	Homicide
22	0	2.52	0	.22	2.34	0	0	ND	M	Asthma/Chem. dep.	Natural
23	.08	.55	.05	.07	.50	0	.24	ND	F	MBFI	Accident
24	.46	3.39	0	.81	1.35	0	0	ND	M	Poly drug OD	Accident
25	2.81	5.29	0	1.37	3.22	0	0	0	M	CO poisoning	Suicide
26	0	1.34	0	0	1.06	0	0	ND	M	Poly drug OD	Accident
27	0	1.52	0	.24	.52	0	.01	ND	M	Poly drug OD	Accident
28	0	.57	0	.19	.30	0	.14	ND	M	Shotgun wound	Homicide
29	0	.31	0	.31	.27	0	.05	.06	M	Stabbing	Homicide
30	.30	1.24	.30	.30	ND	.14	.04	.20	F	Hanging	Suicide
31	.26	1.73	0	.29	1.40	0	0	ND	F	Poly drug OD	Accident
32	0	.46	0	0	.45	0	0	ND	M	ASHD/Chem. dep.	Natural
33	0	.17	0	.07	.30	0	.11	ND	M	GSW	Homicide
34	0	.21	0	.08	.11	0	0	ND	M	GSW	Homicide
35	.19	.87	0	1.30	.52	0	.01	ND	M	GSW	Homicide
36	.28	2.41	0	.97	1.87	0	0	0	M	Shotgun wound	Homicide
37	.20	1.14	0	.37	.80	0	0	ND	M	GSW	Homicide
38	0	.62	0	.02	.41	0	.07	ND	M	ASHD/Chem. dep.	Natural
39	.08	.24	.05	.13	.07	0	.09	ND	M	Hanging	Suicide
40	.22	2.24	0	.75	1.05	0	.07	ND	M	GSW	Suicide
41	.34	.93	.21	.61	.45	.06	.16	ND	F	GSW	Homicide
42	0	1.10	0	1.74	1.42	0	.01	ND	M	GSW	Homicide
43	.92	1.23	0	.27	.26	0	0	ND	M	GSW	Homicide
44	0	.35	0	0	.11	0	0	ND	M	Poly drug OD	Accident
45	.26	1.88	0	.19	1.69	.10	0	ND	M	GSW	Suicide
46	.10	.58	0	.54	.33	0	.15	ND	F	Stabbing	Homicide
47	0	.54	0	.10	.87	0	0	ND	M	GSW	Suicide
48	.16	1.54	0	.77	.52	0	0	ND	F	Asphyxia	Homicide
49	.78	2.21	0	.48	.23	0	0	ND	F	GSW	Homicide
50	.81	5.84	0	.87	2.80	0	.01	ND	F	Stabbing	Homicide
51	6.28	11.9	0	4.00	4.03	0	0	ND	F	Poly drug OD	Undetermined
52	0	1.77	0	.10	1.98	0	0	ND	M	Hypertrophic cardiomyopathy/ Chem. dep.	Natural
53	.32	5.07	0	.56	1.51	0	0	ND	F	GSW	Suicide

* Abbreviations: GSW = Gun shot wound; OD = Overdose; MBFI = Multiple blunt force injuries; ASHD = Atherosclerotic heart disease; ND = Not determined.

Table I (continued). Biochemical and Demographic Findings for 62 Medical Examiner Cases Involving Cocaine

	Cocaine and metabolites (mg/L)						Ethanol (g/dL)				
	Blood			Vitreous							
Subject	cocaine	benzoylecgonine	cocaethylene	cocaine	benzoylecgonine	cocaethylene	blood	vitreous	Gender	Cause of death	Manner of death
54	.14	2.33	0	.23	.86	0	0	ND	F	Poly drug OD	Accident
55	0	.10	0	.03	.11	.03	0	ND	M	ASHD/Recent Coc. use	Natural
56	0	.24	0	.10	.23	0	0	ND	M	Keto-acidosis/Chem. dep.	Natural
57	5.00	6.76	0	3.96	.46	0	0	ND	M	Poly drug OD	Accident
58	.10	.25	0	.10	0	0	0	ND	M	Hanging	Suicide
59	0	.26	0	.10	.30	0	0	ND	M	ASHD/Chem. dep.	Natural
60	.14	.12	.10	.10	0	0	.23	ND	M	Hypothermia	Accident
61	.10	.33	0	.10	.28	.10	0	ND	M	GSW	Suicide
62	.41	.53	0	1.01	.41	0	0	ND	M	Poly drug OD	Accident

* Abbreviations: GSW = Gun shot wound; OD = Overdose; MBFI = Multiple blunt force injuries; ASHD = Atherosclerotic heart disease; ND = Not determined.

Table II. Postmortem Cocaine, Cocaine Metabolite, and Ethanol Concentrations in 62 Medical Examiner Cases

	Blood	Vitreous	*p* Value
Cocaine (mg/L)			
(*n* = 62)			
mean	0.489	0.613	0.125
SD*	1.204	0.994	
range	0–6.28	0–4.50	
Benzoylecgonine (mg/L)			
(*n* = 61)			
mean	1.941	0.989	0.0004
SD	2.912	1.597	
range	0.10–14.2	0–10.7	
Cocaethylene (mg/L)			
(*n* = 62)			
mean	0.022	0.027	0.480
SD	0.055	0.059	
range	0–0.30	0–0.21	
Ethanol (g/dL)			
(*n* = 18)			
mean	0.058	0.092	0.384
SD	0.91	0.13	
range	0–0.35	0–0.42	

* SD = standard deviation.

a Power Macintosh 7100/66 computer using StatView 4.1 software. Significant differences between means were evaluated by the paired *t*-test. Probability (p) < 0.05 was considered significant.

Results

The data collected from the 62 cases, including gender, cause of death, and manner of death, are shown in Table I. Blood and vitreous mean concentrations for cocaine (n = 62), benzoylecgonine (n = 61), cocaethylene (n = 62), and ethanol (n = 18) are shown in Table II. The mean concentrations for cocaine were similar between blood and vitreous at 0.489 mg/L (SD 1.204) and 0.613 mg/L (SD 0.994), respectively. Mean concentrations between blood and vitreous cocaethylene 0.002 mg/L (SD 0.055) and 0.027 mg/L (SD 0.059) and ethanol 0.058 g/dL (SD 0.91) and 0.092 g/dL (SD 0.13) were also similar. However, the mean concentrations for benzoylecgonine differed between blood and vitreous at 1.941 mg/L (SD 2.912) and 0.989 mg/L (SD 1.597), respectively. When comparing all patients using the paired *t*-test, there was no significant difference between blood and vitreous cocaine (p = 0.125), cocaethylene (p = 0.480), and ethanol (p = 0.384). However, there was a statistical difference between blood and vitreous benzoylecgonine (p = 0.0004).

Figure 1 shows the following correlations between blood and vitreous for all drugs, including a few outliers where the concentrations were very high in the vitreous but low in the blood: cocaine $y = 0.705x + 0.268$, $r = 0.854$; benzoylecgonine $y = 0.415x + 0.178$, $r = 0.763$; cocaethylene $y = 0.467x + 0.017$, $r = 0.433$; and ethanol $y = 0.476x + 0.062$, $r = 0.343$ (y = vitreous and x = blood concentrations). The correlation coefficients showed good linear relationships between blood and vitreous concentrations for cocaine (r = 0.854) and benzoylecgonine (r = 0.763). The correlation coefficients for cocaethylene (r = 0.433) and ethanol (r = 0.343), however, were not good.

In order to evaluate whether the presence of ethanol and cocaethylene affects the concentration of benzoylecgonine, all cases with ethanol present were separated from cases without ethanol (Table III). Mean concentrations for benzoylecgonine were higher when ethanol was not present (n = 32) compared with when ethanol was present (n = 29). Blood and vitreous benzoylecgonine concentrations were 1.199 mg/L (SD 2.396) and 0.469 mg/L (SD 0.553) with ethanol present versus 2.593 mg/L (SD 3.195) and 1.431 mg/L (SD 2.021) when ethanol was absent. The mean ethanol concentrations for blood and vitreous in cases with ethanol present were 0.122 g/dL (SD 0.099) and 0.151 g/dL (SD 0.138), respectively. Mean blood and vitreous concentrations of benzoylecgonine, cocaine, and cocaethylene in the presence and absence of ethanol were compared by the paired *t*-test. The concentrations of benzoylecgonine were statistically different for blood (p = 0.034) and vitreous (p = 0.013) in the presence of ethanol; however, there was no difference between the concentrations of cocaine in blood (p = 0.157) or vitreous (p = 0.343). The concentrations of cocaethylene were statistically different between blood (p = 0.011) and vitreous (p = 0.003) when ethanol was present, as expected. In addition, there was great variability both in magnitude and direction of change in benzoylecgonine and cocaine concentrations between cases. However, the postmortem time interval and antemortem dosage of cocaine were not known in this study and most likely affected the comparison.

Discussion

In our study, the mean concentrations of cocaine, benzoylecgonine, cocaethylene, and ethanol were compared between postmortem blood and vitreous. Blood and vitreous concentrations of cocaine, cocaethylene, and ethanol were found to be statistically similar with probabilities of 0.125–0.480, but different for benzoylecgonine with p = 0.0004. Regression analysis showed that linear relationships were present between blood and vitreous concentrations for cocaine (r = 0.854) and benzoylecgonine (r = 0.763). However, the correlation coefficients were lower for cocaethylene (r = 0.433) and ethanol (r = 0.343). Even though there was no difference between blood and vitreous cocaine, cocaethylene, and ethanol mean concentrations, when concentrations were compared between cases, there was that great variability in magnitude and direction of change. Similar findings were reported in a pig model (5). A limitation of our study, as in the vast majority of other postmortem studies, was that the antemortem dosages of cocaine and/or ethanol, as well as the postmortem intervals, were not known. Without this information, it is difficult for

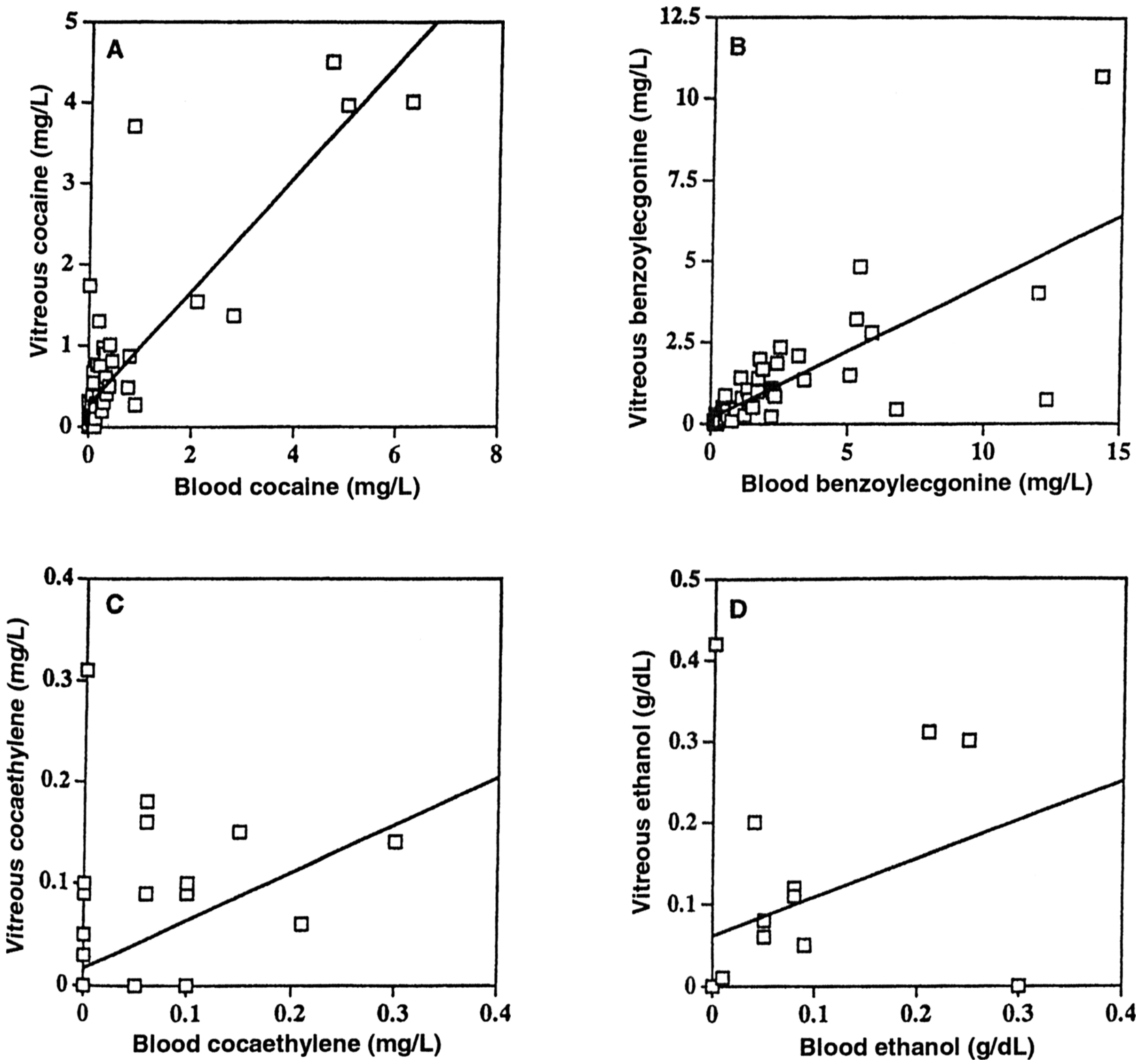

Figure 1. Graphs showing the correlation between blood and vitreous concentrations of A, cocaine (n = 62), $y = 0.705x + 0.268$, r = 0.854; B, benzoylecgonine (n = 61), $y = 0.415x + 0.178$, r = 0.763; C, cocaethylene (n = 62), $y = 0.467x + 0.017$, r = 0.433; and D, ethanol (n = 18), $y = 0.476x + 0.062$, r = 0.343. (y = vitreous, x = blood).

comparisons between blood and vitreous concentrations to be made. The usefulness of this comparison would be to determine if vitreous humor could be used as a source for cocaine and cocaine metabolite quantitation if blood is not available. However, even though comparisons are difficult, trends can still be assessed. Based on our results, there does seem to be a trend of unpredictability of postmortem cocaine, benzoylecgonine, and cocaethylene concentrations both in terms of magnitude and direction of change, which is consistent with previous studies (1,3,4). This may be due to changes in tissue storage and release, as well as environmental factors such as temperature, pH, and the presence of cholinesterase inhibitors (3). Changes are probably not due to postmortem exogenous production of ethanol in decomposed bodies by bacteria (10). Previous studies have reported the variations found between collection sites when evaluating cocaine and cocaine metabolite concentrations (1,3,4), as we have found between vitreous and blood benzoylecgonine and cocaethylene concentrations. Hearn et al. (4) reported higher concentrations in central and lower extremity peripheral blood than in subclavian blood. Logan et al. (3) reported higher concentrations in the central nervous system than in blood. Hearn et al. (4) reported higher concentrations in vitreous than in blood. However, all studies emphasize the caution that must be used in assigning a toxicity to a given concentration, especially in postmortem samples.

McKinney et al. (5) reported a study comparing blood and vitreous cocaine, benzoylecgonine, and cocaethylene concentrations in pigs. The concentrations were determined after a specified dose, immediately postmortem, and 8 h postmortem and were found to be statistically similar. The results from our study also found mean blood and vitreous concentrations of cocaine and cocaethylene to be statistically similar. However, the regression analysis did not show good correlation between vitreous and blood concentrations for cocaethylene, and the mean concentrations of blood and vitreous benzoylecgonine were statistically different, indicating a lack of reliability in using vitreous humor as a specimen for quantitation of cocaine and cocaine metabolites in comparison to blood. McKinney et al. (5) also identified great variability between the concentrations in blood and vitreous, even though all the pigs received the same dose. As in our study, it was noted that the concentrations differed not only in magnitude, but also in direction of change. The conclusion from that study was that vitreous humor cannot be used to predict antemortem toxic concentrations (5). However, documenting the presence of cocaine or metabolites in vitreous humor may be useful in cases where blood is not available.

Table III. Postmortem Cocaine, Benzoylecgonine, and Cocaethylene Concentrations in 62 Medical Examiner Cases With and Without Ethanol

	+ Ethanol (n = 29)		– Ethanol (n = 32)	
	blood	vitreous	blood	vitreous
Cocaine (mg/L)				
mean	0.272	0.499	0.680	0.713
SD*	0.869	0.866	1.422	1.097
range	0–4.70	0–4.50	0–6.28	0–4.00
p value	0.157	0.343		
Benzoylecgonine (mg/L)				
mean	1.199	0.469	2.593	1.431
SD	2.396	0.553	3.195	2.021
range	0.12–12.3	0–2.80	0.10–14.2	0–10.7
p value	0.034	0.013		
Cocaethylene (mg/L)				
mean	0.043	0.05	0.003	0.007
SD	0.073	0.077	0.017	0.025
range	0–0.30	0–0.31	0–0.10	0–0.10
p value	0.011	0.003		

* SD = standard deviation; p values are for comparisons between + ethanol and – ethanol cases.

The formation of cocaethylene has been found to be a product of the metabolism of cocaine and ethanol by liver esterases. The esterases that are involved in the formation of cocaethylene appear to be the same as the esterases that hydrolyze cocaine to benzoylecgonine (13). Although benzoylecgonine is an inactive metabolite, cocaethylene is known to have neurochemical effects, perhaps stronger and longer lasting than cocaine (6). In our study, there was an attempt to determine if the presence of ethanol affected the concentration of benzoylecgonine formed. When the mean concentrations of benzoylecgonine were compared in cases with ethanol present against those without ethanol, the mean concentrations of benzoylecgonine were found to be higher in patients where ethanol was absent (Table III). The fact that cocaethylene was present in a few cases where ethanol was not detected is most likely due to unmeasurable ethanol concentrations due to prior metabolism and excretion or the presence of cocaethylene as a byproduct produced during the extraction of cocaine from coca leaves (14). However, there are limitations in this data, including sample size, unknown drug dosage, and unknown time interval between last dose and specimen collection, all of which are commonly encountered problems in postmortem studies. Although this does not give definitive data regarding the same enzyme being used to produce cocaethylene, it is consistent with prior studies suggesting this finding (13). These data do seem to indicate that ethanol does influence the metabolism of cocaine through the production of cocaethylene and a decrease in the formation of benzoylecgonine, which, in turn, may have ramifications for toxicity.

In conclusion, we found blood and vitreous concentrations of cocaine, cocaethylene, and ethanol to be statistically comparable in postmortem samples. The mean blood and vitreous benzoylecgonine concentrations, however, were found to be statistically different. Regression analysis demonstrated linear relationships between blood and vitreous cocaine and benzoylecgonine concentrations but not between cocaethylene and ethanol. In addition, great intercase variability of concentrations were present both in terms of magnitude and direction of change. We also demonstrated that vitreous humor can be used to quantitate cocaine and its metabolites, but it is not a reliable specimen when compared to blood. However, documenting the presence of cocaine or metabolites in vitreous may be helpful in cases where blood is not available. In addition, the presence of cocaethylene decreased the concentration of benzoylecgonine, suggesting that the same enzyme used to form benzoylecgonine is also involved in the metabolism of cocaine to cocaethylene. Even though the issue of toxicity was not a focus of this study, the data reemphasize the care that should be used when assigning toxic cocaine levels to postmortem samples.

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