COCAETHYLENE AS A HAIR BIOMARKER TO PREDICT HEAVY ALCOHOL EXPOSURE AMONG COCAINE USERS

Aniket Natekar[1,2], Ilan Matok[1,3], Paula Walasek[1], Chitra Rao[1], Georgina Clare-Fasullo[1], Gideon Koren[1,2,4,]

[1]The Motherisk Program, Division of Clinical Pharmacology and Toxicology, The Hospital for Sick Children, Toronto, Canada; [2]Department of Pharmacology &Toxicology, University of Toronto, Toronto, Canada; [3]Department of Epidemiology, Biostatistics, and Occupational Health Lady Davis Research Institute – The Jewish General Hospital, McGill University, Montreal, Canada; [4]Department of Physiology & Pharmacology, University of Western Ontario, London, Canada

ABSTRACT

Background
Cocaethylene (CE) is a cocaine metabolite formed during alcohol and cocaine co-consumption. There are no previous studies to assess the effectiveness of hair CE as a biomarker indicating chronic alcohol consumption among individuals who have consumed cocaine.

Objectives
To establish the ability of CE to predict chronic alcohol use among individuals testing positive for cocaine.

Methods
We studied all cases referred to our laboratory where both chronic cocaine and alcohol consumption were sought, and values of hair cocaine, benzoylegconine (BE), CE, and FAEEs (as marker of chronic alcohol consumption) were available. Cocaine, BE and CE were screened by ELISA and confirmed using headspace-solid phase microextraction (HS-SPME) and GC-MS. FAEE were analyzed using HS-SPME and GC-MS/EI. Sensitivity, specificity, and predictive values of CE as a marker of alcohol consumption among cocaine users were calculated using different FAEE cutoffs.

Results
Cocaine ($P<0.001$) and BE ($P<0.001$) concentrations were associated with increased FAEE. The positive predictive value of CE to identify alcohol consumption was 0.66 for excessive drinking and 0.76 for chronic drinking among positive cocaine users. Negative CE ruled out almost completely excessive alcohol consumption.

Conclusion
Positive hair CE results had high specificity for chronic excessive alcohol consumption among cocaine users. With no established safe level of alcohol in pregnancy, identification of CE in hair of pregnant women who have used cocaine can serve as a biomarker for fetal alcohol spectrum disorder.

Key Words: *Cocaethylene, hair testing, high-risk population, biomarker, cocaine, benzoylegconine, FAEE*

Cocaine and alcohol are widely abused, with the incidence of cocaine users in Canada decreasing from 1.9% to 0.7% between 2004 and 2010. In parallel, lifetime alcohol use slightly decreased from 92.8% to 88.9%, while alcohol use within the last 12 months decreased from 79.3% to 77%. The proportion of heavy frequent drinkers decreased from 7.1% to 4.3%, while the proportion of light frequent drinkers increased from 27.7% to 32.2%.[1] In the United States, estimated proportion of students from grades 9 to 12 who used any form of cocaine one or more

times significantly decreased from 9.5% to 6.4% between 1999 and 2009[2], while 2.8% of students surveyed in 2009 admitted to using some form of cocaine within 30 days of the survey.[3] In the 2009 National Survey on Drug Use and Health, approximately 30.2 million (12%) people aged 12 or older admitted to driving under the influence of alcohol.[4] It has been estimated that 50% to 90% of cocaine users also co-abuse alcohol.[5]

Cocaine abusers tend to also abuse alcohol as it reduces anxiety, uneasiness, and other adverse psychological effects associated with the cocaine "crash".[6-8] Prolonging these effects has been attributed at least in part to CE, an active metabolite of cocaine formed in the presence of alcohol. Usually cocaine is hydrolyzed by carboxylesterase 1 (hCE1) to BE, an inactive metabolite. In the presence of alcohol, up to 17% of the cocaine-BE hydrolysis pathway is shifted to transesterification, producing CE. CE, like cocaine, blocks the re-uptake of dopamine[9], thus contributing to the reinforcement of subjective responses and acute physiological responses of combined alcohol and cocaine use.[10,11] With CE having a longer elimination half-life ($t_{1/2}$ = ~2h) than cocaine ($t_{1/2}$ = ~1h), this provides a plausible explanation why many cocaine users abuse the drug with alcohol.[12,13] There are potential fatal side effects that may occur with concomitant alcohol and cocaine use. Clinical studies in human volunteers have found that there are differences in heart rate with cocaine vs. cocaine with alcohol. One study found that while cocaine increased heart rates by 18 beats per minute (BPM), the combination of cocaine and alcohol increased heart rates by as much as 41 BPM compared to placebo.[14]

Normally, testing for alcohol consumption is conducted with traditional matrices such as blood, urine, saliva, and expired air for drug licensing and substance abuse monitoring programs.[15-18] However, there are some disadvantages to using these matrices for alcohol testing. Firstly, ethanol is eliminated rapidly, hence multiple samples are required to determine amount and timing of exposure. Secondly, the body metabolizes about 7 g of alcohol, equivalent to one drink per hour.[15] This equates to a rate of 0.015% of blood alcohol concentration (BAC) per hour. A person with a BAC of 0.08% (above legal limit in Canada) will not have any measurable alcohol within 5.5 hours of the last drink, and this applies to breathe testing as well.[19]

Fatty acid ethyl esters (FAEE) are non-oxidative ethanol metabolites produced from the conjugation of ethanol with free fatty acids by enzymes containing FAEE synthetic activity or microsomal acyl-coA-ethanol O-acetyltransferase.[20] With hair growing on average 1 cm per month[21], this matrix, when segmented, can reflect both short-term and chronic exposure. This is due in part to the fact that hair FAEE remains relatively stable for many months.[22]

Cocaine and alcohol consumption have been studied extensively in adult human hair and appropriate concentration cut-offs have been established for each. The International Society for Hair Testing determined that BE/cocaine ratio must be at least 0.05 or greater to differentiate between use and external contamination.[23] An FAEE level above 0.5 ng/mg scalp hair to has high sensitivity and specificity for chronic excessive alcohol consumption. The consensus of the Society of Hair Testing uses the World Health Organization definition of chronic excessive alcohol consumption, which is defined as consuming more than 60 g of pure ethanol per day for several months.[17] This is the definition which is used by most laboratories today in hair testing for chronic excessive alcohol consumption.

To our knowledge, there have been no previous attempts to use hair CE as a marker of ethanol consumption among individual tested positive for cocaine. Such a biomarker may be practically important, as relatively few laboratories worldwide measure hair FAEE or the other alcohol biomarker, ethyl glucuronide, while numerous laboratories measure cocaine, and hence can measure CE.

We hypothesized that CE could be a useful biomarker to predict the level of alcohol consumption among individuals using cocaine. The primary objective was to determine the value of positive/ negative CE levels in predicting positive/negative FAEE levels.

METHODS

We used hair samples of individuals consenting to testing at the Motherisk Clinic at the Hospital for Sick Children, Toronto, Canada, on the advice of children's aid societies, legal or medical

professionals. Hair was cut from the posterior vortex as close the scalp as possible and the samples were submitted to the Motherisk Drug Testing Laboratory (MDTL), for cocaine, BE, CE, and FAEE using established methods.[24,25]

We identified all 588 samples cases where cocaine, BE, CE, and FAEE were measured between September 1, 2010 and May 24, 2011.

Sample Preparation

For cocaine, CE, and BE testing, 10 mg of scalp hair was washed with dichloromethane, dried, and cut before being transferred to a vial (Kimble Chase, Vineland, NJ) with methanol. After intubation for 18 h, the methanol was transferred to a clean glass test tube (Fisher Scientific, Ottawa, ON) and dried with nitrogen. PBS was added to the sample and half was given for ELISA screening with the Cocaine Direct Elisa Kit (Immunalysis, Pomona, CA) and confirmation by headspace solid-phase microextraction (HS-SPME) and GC-MS (Mandel Shimadzu GC-MS QP2010 Plus, Guelph, ON) using a method established in our laboratory.[24]

For alcohol testing, samples were pre-washed with heptanes to remove external contamination and 20 mg were weighed out for extraction and analysis. Extraction and analysis of four FAEE species (ethyl oleate, ethyl myristate, ethyl palmitate, ethyl stearate) was conducted by liquid-liquid extraction with a heptane and dimethyl sulfoxide mixture followed by automated headspace solid-phase microextraction and GC-MS/EI (Mandel Shimadzu GC-MS QP2010 Plus, Guelph, ON) analysis as previously reported.[25]

Statistical Analysis

The total population was stratified into two groups, one representing individuals who were excessive alcohol users (based on FAEE ≥0.5 ng/mg of hair), and the other being social/non-drinkers (FAEE 0.2-0.49 ng/mg of hair). Within the excessive alcohol use cohort, individuals were stratified into cocaine users (based on BE≥5% of cocaine), cocaine-exposed (BE<5% of cocaine), or negative for both. To assess whether cocaine, BE, and CE concentrations correlated with FAEE concentrations ≥0.5 ng/mg indicating chronic excessive alcohol consumption, we used the Mann-Whitney Test. A Chi-Squared Test was used to compare proportions of cocaine users who were identified as chronic excessive alcohol abusers within the study population. Logistic regression was used to identify whetherFAEE≥0.5 ng/mg and cocaine use (BE≥5% of cocaine) could predict a positive CE result. Lastly, we calculated the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of using CE to predict chronic excessive alcohol consumption (FAEE ≥0.5 ng/mg) or alcohol consumption in general (FAEE above 0.2ngémg).

RESULTS

Out of 588 hair samples included in the study, in which both FAEE and cocaine were measured, 45 were found to have quantifiable CE concentrations and an additional 5 had measurable traces (above LOD but below LOQ). (Table 1). Of the quantifiable samples, 28 were also found to have FAEE levels greater than 0.5 ng/mg. CE concentrations ranged from 3.5% to 104% of cocaine concentrations.

TABLE 1 Distribution of results for FAEE positive/negative for excessive alcohol consumption with CE positive/negative values.

	FAEE Positive (≥0.5 ng/mg)	FAEE Negative (<0.5 ng/mg)
CE Positive (including 5 with only traces)	33	17
CE Negative	203	336

A total of 353 non/social drinker (FAEE 0-0.49 ng/mg of hair) and 235 chronic excessive alcohol abusers (≥0.5 ng/mg) were identified. The median level for cocaine in the social drinking vs. in the chronic alcohol abuse population was 273ng/mg and 326 ng/ mg (P<0.001) respectively. For BE, the median level in the same groups were 277ng/mg and 320 ng/mg. respectively (P<0.001).

Analysis of the social drinking/non-drinking group (FAEE o.2-0.49ng/mg), found that 250 were identified as only cocaine-exposed (BE less than 5% of cocaine), while 103 were identified as positive cocaine users (BE above 5% of cocaine). Among the excessive alcohol users (FAEE above 0.5ng/mg), 57% were not cocaine users. In contrast, among the cocaine users, 42.1% were also alcohol abusers. (P=0.002, OR 95% CI 1.76, 1.25-2.49).

In logistic regression, cocaine use was associated with a positive CE result (OR =15.56, 95% CI 5.95-40.67, P<0.001), as were positive FAEE results (OR =2.437, 95% CI 1.21-4.87, P=0.012).

FAEE samples negative for excessive drinking (<0.5 ng/mg) were 95.18% of the time also negative for CE, indicating a very low rate of false positivity. Specificity increased when including social drinking/ non drinking population, indicating that 97.28% of FAEE values <0.2 ng/mg were also negative for CE. This indicates that the rate of false positivity decreases even further for non-drinking individual. In addition, with a PPV of 0.66, if a positive CE is detected, then the sample is 66% likely to be positive for FAEE (≥0.5 ng/mg) (Table 2).

TABLE 2 The sensitivity and specificity of CE in predicting FAEE >=0.5 ng/mg vs. a social/non-drinking population (FAEE <0.5 ng/mg). The sensitivity and specificity of CE for FAEE as a gold standard was also assessed in the non-drinking (<0.2 ng/mg FAEE) versus social drinking population (0.2-0.49 ng/mg FAEE). The PPV and NPV for each condition were also calculated.

CE Value	FAEE (ng/mg)	Sensitivity (%)	Specificity (%)	PPV	NPV
>**0.065**	<0.5 or ≥0.5	13.98	95.18	0.66	0.6234
>**0.065**	<0.200 or 0.2≤x≤0.49	6.311	97.28	0.76	0.42

DISCUSSION

Studies conducted on volunteers administered alcohol and cocaine found blood CE levels to reach 17±6% (mean ± SD) [10] -23.57%[26] of cocaine concentrations. However, these studies had volunteers intoxicated by alcohol before cocaine was given. Perez-Reyes (1994)[27] found that blood CE levels ranged from 4.3%-20.1% of cocaine concentrations whereas we found hair CE concentrations ranging from 3.5% to 104% of cocaine concentrations. These differences can be expected due to different distributions into hair.[28-30]

Recently, CE C_{max} was shown to reach about 2% of cocaine $C_{max.}$[31] Although, it was proposed that CE may not be dose- proportional to ethanol dose[10], the above study demonstrated that CE may be formed even at low levels of alcohol. This agrees with our results, where we identified 17 samples positive for CE, but with FAEE <0.5 mg/mg.

In pigmented hair, cocaine can be incorporated two times more than BE[33], and since cocaine and CE have similar structures, it is theoretically possible that hair pigment could also affect CE distribution in hair. In the present study cocaine and BE concentrations were significantly higher among individual with excessive alcohol use (FAEE <0.5 mg/mg), confirming previous studies where individuals using more cocaine exhibited increased alcohol consumption.[34] An important result of our study was that CE was only found to be positive when both cocaine and BE were present, indicating cocaine use. This is

important because CE is not thought to be present when cocaine is not consumed, thus our results corroborate the accepted understanding of CE detection.

There were a total of 50 positive CE results, of which 44 were quantifiable. The addition of the five trace values improved the sensitivity of our analysis in both FAEE≥ 0.5 vs. <0.5 ng/mg and FAEE≥0.2 vs. FAEE<0.2 ng/mg conditions. Similarly, the PPV and NPV in both conditions also improved. This demonstrates the usefulness of including positive trace CE results in our analysis. In our study FAEE samples negative for excessive alcohol consumption (<0.5 ng/mg) were almost always (95.18%) also negative for CE, indicating a very low rate of false positivity. This strongly suggests that an individual testing positive for cocaine, but negative for CE is highly unlikely to be an excessive user of alcohol. In addition the PPV of 0.66 indicates that if a positive CE is detected, then the sample is 66% likely to be positive for excessive alcohol use (FAEE ≥0.5 ng/mg). These values support our hypothesis that hair CE can predict excessive alcohol consumption among cocaine users. Furthermore, in every case the presence of CE indicates that some amounts of alcohol had been chronically consumed. While this may not be of major clinical significance for the general population, in the context of pregnancy it should indicate to health and social professionals that the mother has consumed alcohol continuously in pregnancy. Even if these levels are not excessive in non pregnant individual, they may be sufficient to cause fetal damage along the fetal alcohol spectrum disorder.

In the future, it will be important to validate hair CE also against ethyl glucuronide, as a second biomarker of chronic alcohol use.

To our knowledge, this is the first study to examine the predictive value of hair CE as a biomarker to predict chronic alcohol use among individuals consuming cocaine. Our data documents high specificity of negative CE to rule out chronic excessive alcohol consumption (evidenced FAEE measurements) and the high predictive value of positive hair CE in identifying chronic excessive drinkers or social drinkers among cocaine users). We also demonstrated that cocaine and BE concentrations are significantly increased with increased alcohol consumption, corroborating results from previous studies. As there is still no safe alcohol level identified in pregnancy, a positive hair CE in a pregnant woman consuming cocaine is a potential biomarker to identify fetuses at risk for fetal alcohol spectrum disorder. Further studies are needed to corroborate these results and validate the effectiveness of using CE as a biomarker to indicate chronic excessive alcohol consumption among cocaine users.

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Corresponding Author: gkoren@sickkids.ca

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