THE ROLE OF ETHANOL IN COCAINE CONCENTRATION IN HUMAN POST-MORTEM WHOLE BLOOD

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ABSTRACT: Regardless of its form, the coingestion of cocaine (COC) and ethanol is still a very important problem in drug abuse. The formation of the transesterification byproduct, cocaethylene (CE), which has lower LD₅₀ has led to the idea that the combination of the two drugs is more toxic (lethal) than just cocaine individual response and that smaller concentrations of the drug concentration should be present in those related cocaine deaths. The aim of this study was to evaluate the role of ethanol as an agent of interaction in lethal intoxication and to establish the influence of CE in post-mortem whole blood cocaine concentration. Thirty six post-mortem cases, in which cocaine was the only cause of death, were compared to eighteen cases of cocaine/ethanol interaction in terms of COC, benzoylecgonine (BE) and ecgonine methylester (EME) concentrations. Cocaine and cocaethylene, BE, EME concentrations correlated positively, but CE concentrations did not correlate with blood ethanol. When the correlation of each metabolite and the precursor were analyzed statistically by Manova, no differences between the two groups were found. The ratio of BE and COC concentrations (BE/COC) for cocaine as the only drug was statistically greater than BE/COC when ethanol was present. On the other hand, statistics with Manova (Wilks λ test, α = 0.05) showed that there is a significant difference between the two groups when COC and its main products of biotransformation, BE and EME were focused on variables.

KEY WORDS: Cocaine; Post-mortem material; Cocaethylene; Ethanol.

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INTRODUCTION

Cocaine (COC) abuse in its different forms is growing fast not only in Brazil but also in many other countries. This picture of drug abuse is intensified by the concurrent use of alcohol. In fact, it is estimated that 50 to 90% of recreational of drug users drink alcoholic beverages to have a prolonged or intensified effect or to reduce the unpleasant effects that follow COC abuse [9, 25, 26, 27, 31].

The toxic effect of the interaction between alcohol and COC is not simply due to the potentiation but, instead, due to the formation of cocaethylene (CE), a transesterification product with much more intense expression of acute toxicity than its precursor [18, 21, 31, 32].
In about 80% of all forensic analyses which are performed in the Medical Legal Institute in São Paulo, Brazil and were related to morbidity and mortality of COC, ethanol is also involved.

In forensic toxicology post-mortem specimens, the data are not always easy to explain and the interpretation of them constitutes a challenge to forensic pathologists and toxicologists. The discovery of CE, due to its toxic potential, has brought along with it extra elements of intricacy.

In the evaluation of COC blood concentration related to _causa mortis_, some authors have found lower concentrations of COC when ethanol was present than when ethanol and cocaine were in combination [10]. This result should be indicative of the potentiation of COC effects by ethanol [30] or of the toxicity of the CE produced [16].

On the other hand, there are some references in the literature that take in account the possibilities of ethanol inhibiting COC biotransformation, increasing, in this way, its blood concentration. This was observed both in vitro [8, 9, 31] and in vivo [11, 29] and could explain the intensity of toxic effects of COC in the presence of alcohol. So, it is not clear if this increment in COC toxic effects is due to CE _per se_ or if it depends on the increase in cocaine blood concentration.

So, the purpose of this research is to establish the relationship between blood concentrations of COC and its products of biotransformation, benzoylecgonine (BE) and ecgonine methylester (EME) and CE, when present in cases of lethal exposure to COC, as well as to verify the existence of variations in COC concentration related to death when ethanol is present.

**EXPERIMENTAL**

**Casuistic**

Medical examiners in São Paulo City conduct forensic investigations of all cases of sudden deaths or of those unattended by health care services (suspected suicide, drug overdose, violence, injury, drowning, etc.). All cases are routinely subjected to a complete autopsy, and toxicological analyses are performed whenever requested by the pathologists. These analyses are orientated by autopsy findings or by any evidence either from the testimony of the witnesses or from evaluation at the scene and at the circumstances of death (licit or illicit drugs and drug paraphernalia).

Fifty four samples of post-mortem heart-blood of victims, whose cause of death was classified as cocaine overdose were analyzed. We considered only the cases in which there was no evidence of death from trauma, “natural” causes, drugs other than cocaine or “body packing”. The cocaine was detected in the blood, liver, kidney and urine (when available). Post-mortem whole blood samples were obtained from previously non-hospitalized victims and the interval of time not greater than 12 hours between death and delivery of the material to the Forensic Lab. Sample integrity was achieved by way of cold
storage (41°C) and by adding NaF to hinder cholinesterase and carboxiesterase in vitro activities to transform COC to EME and BE respectively.

The samples were distributed accordingly to whether or not there was an indication of the presence of ethanol. Based on this criterion, 2 groups were formed:

Group 1 (G1) – 18 samples in which cocaine and ethanol were present.

Group 2 (G2) – 36 samples which were positive for COC but ethanol was not detected.

This, hypothetically, means respectively, interaction between COC and ethanol for G1 and non-interaction for G2.

All samples were analyzed for COC, BE, EME and CE, utilizing a GC/MS procedure, previously described [6] and for ethanol utilizing a headspace and GC/FID quantification method, currently used in the Lab [24].

STATISTICAL ANALYSIS

Ethanol blood levels, COC, CE, EME, BE, BE/COC (ratio of BE and COC concentration) were the variables studied. The possibility of predicting an estimate of the blood COC concentration at the time of death, using the Isenschmid model [17] was determined. It postulates that the sum of the molar concentrations of EME and COC (COC + EME) present in an unpreserved heart-blood specimen can be used to establish such prediction.

The correlations between the variables were analyzed in G1, using Pearson coefficient ($r = 0.01$). Anova test was used to study the “group effect” on COC, BE, EME and BE/COC individually. The study of “group effect” on COC, BE and EME simultaneously was performed using Manova (Wilks $\lambda$ test). For both tests the level of significance was 5% ($\alpha = 0.05$).

RESULTS AND DISCUSSION

The estimation of the mean value and standard error of the 4 variables for groups G1 and G2 are shown in Table I. The distribution of the variables COC, EME and BE levels are shown in Figures 1 and 2.
TABLE I. ESTIMATED MEAN VALUE OF CONCENTRATIONS AND STANDARD ERROR OF THE 4 VARIABLES SEPARATED IN GROUPS G1 AND G2

<table>
<thead>
<tr>
<th>Group</th>
<th>Variable</th>
<th>Estimative [ng/ml]</th>
<th>Mean value</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>COC</td>
<td>1215.00</td>
<td>248.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EME</td>
<td>1723.90</td>
<td>271.91</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BE</td>
<td>1637.64</td>
<td>274.50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BE/COC</td>
<td>1.80</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>G2</td>
<td>COC</td>
<td>775.87</td>
<td>155.61</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EME</td>
<td>1342.51</td>
<td>131.36</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BE</td>
<td>1641.71</td>
<td>176.51</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BE/COC</td>
<td>3.04</td>
<td>0.23</td>
<td></td>
</tr>
</tbody>
</table>

Table II shows the values of Pearson linear correlation coefficient for the combination of concentrations of ethanol and the variables CE, COC, EME and BE, respectively, for the samples in which the CE was found (G1). The values that rejected the hypothesis of a null correlation ($\alpha = 0.01$) are indicated with an asterisk (*).

TABLE II. PEARSON’S CORRELATION COEFFICIENT FOR THE COMBINATION OF ETHANOL WITH EACH OF THE FOLLOWING: CE, COC, EME AND BE CONCENTRATIONS IN GROUP 1

<table>
<thead>
<tr>
<th></th>
<th>ETHANOL</th>
<th>CE</th>
<th>OC</th>
<th>EME</th>
<th>BE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CE</td>
<td>–0.127</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>COC</td>
<td>0.004</td>
<td>0.858*</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>EME</td>
<td>0.279</td>
<td>0.618*</td>
<td>0.575*</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>BE</td>
<td>–0.083</td>
<td>0.779*</td>
<td>0.734*</td>
<td>0.768*</td>
<td>1</td>
</tr>
</tbody>
</table>

(*) rejected the null hypothesis ($\alpha = 0.01$).

Table III lists the results obtained by the ANOVA ($\alpha = 0.05$). The Tables IV and V show some descriptive statistical data between concentrations of BE and COC (BE/COC) and the sum of the equimolar concentrations between COC and EME (COC + EME), in the presence or not of cocaethylene.

The Manova test with the criteria $\lambda$ of Wilks and a significance level of 0.05 was used and the descriptive level of 0.0163 ($p = 0.0163$) was obtained.

TABLE III. RESULTS OF ANOVA TEST FOR COC, EME, BE, AND BE/COC CONCENTRATIONS ($\alpha = 0.05$)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Descriptive level (p)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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All samples were submitted to the analytical methodologies during laboratory routine, to analyze COC and ethanol. At the beginning, the groups studied (G1 and G2) were in order according to the presence or not of ethanol. Besides COC and ethanol, all samples were submitted to screening for volatile substances, acidic and basic substances and pesticides. However, two samples showed negative for ethanol but positive for CE. This was the basis we had for classifying these cases as belonging to group G1, since the classification of the groups was according to the presence or not of CE, which is the biomarker for the interaction of COC and ethanol.

The study was carried out with samples from subjects of both sexes with ages varying from 14 to 38. The percentage of males was 81.5% and of females was 18.5%. The histogram of distribution of cases in relation to sex and age is shown in Figure 3. Similarly to what happens in other parts of the world, there is a prevalence of males who died from overdose of COC [1, 10, 19].

Although data related to age was not statistically determined, descriptive observation shows that 81.4% of cases were under the age of 30, which implies a prevalence of such pattern of abuse among young males [1, 3, 10, 23, 28]. This fact is in accordance with the investigation of crack abuse, the main form of COC abuse, in the city of São Paulo [28].

Data related to records of peri-mortem observations are very difficult to obtain in our forensic system. Due to the difficulties in establishing the route of administration in all cases, it was not possible to study the relationship between concentration and form of use.

The collection site for blood samples is not noted in records and, in spite of the findings of Hearn et al. [13, 14, 15] it has been shown that there are differences among the different body sites. The blood was obtained from the heart chamber because this is the general practice in Brazil, for forensic cases.

In 33% of the cases studied, high levels (higher than 1,000 ng/ml) could be considered the classic overdose picture, the rest were 50% between 400 and 1000 ng/ml and 50% below the low toxicity level of 400 ng/ml. The latter presented similar levels of recreational use in which cocaine was involved, but the circumstantial peri-mortem evidence and necropsy findings led us to conclude that cocaine was the agent of the \textit{causa mortis}.

Escobedo [10] reported that, in overdose cases, when the exposure was to crack, COC concentrations were lower than those in which all the evidences showed the use of cocaine in the hydrochloride form. In our study, it was not possible to know the form of cocaine the deceased were exposed to, because in 80% of the cases the route of administration was not specified. There were 8 (of 36) cases in G2 and 3 (of 18) in G1 in which

<table>
<thead>
<tr>
<th>Method</th>
<th>Value</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>COC</td>
<td>0.1246</td>
<td>There is no difference</td>
</tr>
<tr>
<td>EME</td>
<td>0.1586</td>
<td>There is no difference</td>
</tr>
<tr>
<td>BE</td>
<td>0.9898</td>
<td>There is no difference</td>
</tr>
<tr>
<td>BE/COC</td>
<td>0.0008</td>
<td>Shows difference</td>
</tr>
</tbody>
</table>
the history of crack was reported. It would be ideal if we could control the administration route in order to establish the concentration related to death. Unfortunately, in our system this information is very difficult to obtain.

According to Isenschmid, in recreational exposure to COC, EME blood concentrations is about 5% of the BE. However, Cone [7] did not detect the presence of this metabolite in plasma using a method with a high limit of detection. This probably explains why there are only a few papers on EME analyses in plasma, serum and whole blood. We had the opportunity to analyze limited number of blood samples from live individuals and could not detected the presence of EME, even in those samples in which COC was present in concentrations above 112 ng/ml.

Isenschmid et al. [17] discussed the possibility of predicting the levels of COC at the moment of death based on blood levels of EME found if one assumes that these levels are resulted from post-mortem hydrolysis since this metabolite occurs in very low levels and is not possible to be detected in vivo [7, 17, 22]. The hydrolysis could occur, however, during post-mortem period up to the time of preservation of the whole blood.

Thus, when we used the algorithm suggested by these authors, we got the “predicted” values obtained by the micromolar sums of COC and EME concentrations, being 3800 and 2790 ng/ml respectively for G1 and G2. This means that the values obtained were compatible with those values classified as classical overdoses. Although this criteria is useful one has to take into account that all EME is derived from unaltered COC at the time of death without considering the EME values in acute intoxication when the kinetic parameters are altered and it could show high concentrations of EME in vivo.

Figure 4 shows the histogram of the concentrations found among the four analytes in both groups G1 and G2.

The comparison with the plasmatic values observed in recreational intoxication [22] shows that the relationships among them are substantially different: the ratio EME/COC is approximately 0.12 in live subjects compared to the ratio of 1.5 in our study.

The ratios EME/COC for groups G1 and G2 were 1.4 and 1.7 respectively which could indicate that the EME was a post-mortem production because one could expect that the ratio EME/COC would be higher in vivo in G1 compared to G2 because of the presence of ethanol which shifts the biotransformation to other pathways with a consequent increase in the resulting products [31].

This fact and the possibility of the changing of the COC concentration (depending on the interval of time from death to sample collection, the temperature, site of collection and redistribution due to the “kinetic post-mortem”, suggest that the proposition of Isenschmid [17] is a possibility in the interpretation of the cause of death.

In our casuistic the average value for CE concentration was 50% of that for COC (696 ng/ml for CE and 1215 ng/ml for COC). These values are in agreement with those reported by Bailey et al. [1], in plasma samples of patients from emergency rooms or trauma units and those reported by Wu et al. [33] in serum from patients suspected of intoxication.
In only one out of 18 cases in group 1, did the concentration of CE exceed that of the COC and in 2 of them, no ethanol was detected. The CE was not detected in 5 cases in which the analyses were positive for ethanol and COC. The explanation for this fact could be the following:

1. the time elapsed between the exposure and the elimination of ethanol occurred before death;
2. ethanol was ingested in small quantities;
3. standard kinetics of elimination were different for the 2 agents;
4. the LOD (limit of detection) of the dosage of ethanol method was not sufficiently low.

On the other hand, the presence of ethanol and the non-detection of CE could be due to the LOD of CE method or due to inter-individual variations of the enzymatic function or some other idiosyncratic reactions. It should be pointed out, however, that the average concentration of ethanol in this cases was 0.5 (0.4–0.7), and that these values were the lowest of group I and considerably lower than the average (1.5 g/l) observed in the group.

**TABLE IV. DESCRIPTIVE STATISTICS FOR VARIABLE BE/COC IN SAMPLES WITH OR WITHOUT THE PRESENCE OF CE**

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Median</th>
<th>Range</th>
<th>CI (α = 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>18</td>
<td>1.80</td>
<td>0.74</td>
<td>1.65</td>
<td>0.71–3.01</td>
<td>1.68–2.45</td>
</tr>
<tr>
<td>G2</td>
<td>36</td>
<td>3.04</td>
<td>1.36</td>
<td>2.77</td>
<td>0.79–7.16</td>
<td>2.79–3.65</td>
</tr>
</tbody>
</table>

CI – confidence interval.

The average concentrations and the related standard error of mean (SEM) for the 3 analytes studied are shown in Table I. The distribution of each of concentrations was done by boxplot method (Figure 1) and showed asymmetric distribution with higher variability in G1.

The variable BE/COC was introduced to measure the influence of the carboxylesterases pathway. This ratio whose descriptive statistics are in Table IV, showed higher variation in G2 (Figure 2). The confidence interval clearly demonstrates the capacity that this variable (BE/COC) has to discriminate the 2 groups.

The variable (COC + EME) represented by the sum of micromolar (mM) of COC and EME, was studied following the model proposed by Isenschmid et al. [17]. The descriptive statistics showed in Table V, demonstrates that the lowest predictive value found in the 2 groups (614 ng/ml) gives more evidence of the occurrence of overdose than the correspondent COC concentration of 94 ng/ml found in this sample. This model could help to elucidate the cause of death and substantially reduce the overlap, that occurs between “recreational” and toxic cocaine blood concentrations. This corroborates the statement of Karch et al. [10], that this “overlap” may be more apparent than real.
The analysis of Table II shows that there is a statistically significant positive correlation between the COC and the CE concentrations ($r = 0.858$, $p < 0.01$). According to Bailey [1], this correlation was to be expected because the CE concentration is ethanol and cocaine dependent, and ethanol is always in excessive molar concentrations. So, the COC concentration actually acts as a limiting agent in the reaction. In the same way there were positive and statistically positive correlation between the concentration of COC and BE ($r = 0.734$, $p < 0.01$) and EME ($r = 0.575$, $p < 0.01$). This means that the pathway saturation delays even during acute intoxication.

Table II also shows that there is no correlation between ethanol concentrations and the aforementioned concentration. The result of Anova test (Table III) shows that the av-
verage of the variables COC, EME and BE is not statistically significant, except for the ratio BE/COC which is significantly higher (p = 0.0008) in samples of G2. This fact could be explained by the competition for the biotransformation pathway of the hepatic carboxylesterases which metabolizes COC to BE and also performs the

![Graph showing age and sex distribution in G1 and G2.](image)

**Fig. 3.** Age and sex distribution in G1 and G2.

![Graph showing disposition of cocaine and its biotransformation products in whole blood with and without an interaction with ethanol.](image)

**Fig. 4.** Disposition of cocaine and its biotransformation products in whole blood with and without an interaction with ethanol.
transesterification of CE [4, 12, 13]. For the variable COC + EME there was no difference (p = 0.104) and therefore this variable did not show any difference between the groups.

### TABLE V. DESCRIPTIVE STATISTICS FOR VARIABLE COC + EME IN SAMPLES WITH OR WITHOUT THE PRESENCE OF CE

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Media</th>
<th>Range</th>
<th>CI ( = 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>18</td>
<td>3800.84</td>
<td>2490.05</td>
<td>3887.21</td>
<td>614.0–7737.61</td>
<td>2650.52–4951.17</td>
</tr>
<tr>
<td>G2</td>
<td>36</td>
<td>2789.63</td>
<td>1911.80</td>
<td>2366.67</td>
<td>643.93–8675.83</td>
<td>2165.12–3414.13</td>
</tr>
</tbody>
</table>

CI – confidence interval.

The Manova test is a technique which considers all the variables simultaneously when comparing the 2 groups. The null hypothesis in this test would consider that the mean concentrations (for COC, BE and EME) analyzed concomitantly for the two groups are the same. This test (λ of Wilks) showed the difference between the two groups (p = 0.0163). So, the discrimination between the two groups could not be demonstrated when the analytes were compared two by two (Anova test), but it was demonstrated when we focused all of them concomitantly, that they acted as a single vector (Manova test).

This could be explained due to the kinetics of COC. Due to its extensive biotransformation with different rate for each pathway and also difference among individuals, it is not possible to show any difference when one analyses each pathway separately. However, the sum of all kinetics factors as a whole phenomenon, can point out the differences of kinetics disposition due to the interaction with ethanol.

In conclusion, the interpretation of the interaction with ethanol can easily be done through the analysis of the ratio BE/COC which is significantly lower when COC and ethanol are used concurrently.

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