The methylecgonine to cocaine ratio in blood samples and the effectiveness of preservation with 0.4 % sodium fluoride

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Abstract

Aim: The aim of this study is to obtain more insight in the role of sodium fluoride (NaF) as preservative of whole blood and to investigate if the methylecgonine/cocaine (ME/COC) concentration ratio or the benzoylecgonine/cocaine (BE/COC) concentration ratio is indicative for hydrolysis of cocaine.

Methods: Electronic data files of the Netherlands Forensic Institute (NFI) were searched for concentrations of COC, BE and ME in blood samples from 1999 through 2010. The BE/COC and ME/COC ratios were calculated and statistically evaluated. Cases of driving under the influence (DUI) as well as autopsies were investigated.

Results: The median ME/COC concentration ratio increased over the years after 2006 in cases of driving under the influence (DUI). This increase coincided with a gradual change from blood tubes containing 0.8% NaF to tubes containing 0.4% NaF from 2006 until mid-2007. A trend was not observed in the BE/COC ratios or in autopsy cases over the years. The median ME/COC ratio increased for the categories DUI (0.8% NaF) < DUI (0.4% NaF) < autopsies. This is the order of decreasing preservation. In autopsy cases, the median BE/COC ratio was significantly lower than in DUI cases.

Discussion: according to the literature, COC will hydrolyze to ME by the action of cholinesterase and to BE by pH-dependent chemical hydrolysis. Fluoride inhibits cholinesterase. The duration of action of NaF depends on its concentration and on the temperature.

Conclusions: The results show that the ME/COC ratio is probably a useful indicator of enzymatic cocaine hydrolysis in cases of driving under the influence as well as in autopsy cases. A ME/COC ratio > 2.1 is indicative of (enzymatic) cocaine hydrolysis. This information will improve the interpretation of forensic results. A concentration of 0.4 % NaF was shown to be insufficient to prevent decomposition of cocaine in blood during more than 1 or 2 days under the practical circumstances in The Netherlands. The relatively low BE/COC ratio in autopsy cases points to the protective role of post-mortem acidification in the chemical hydrolysis of cocaine.

1. Introduction

The limited stability of cocaine in forensic samples has been a problem for several decades. In unpreserved whole blood, plasma or serum, cocaine (COC) will hydrolyze to methylecgonine (ME) by the action of cholinesterase and to benzoylecgonine (BE) by pH-dependent chemical hydrolysis [1]. Methylecgonine and benzoylecgonine are further converted into ecgonine (ECG). Fluoride inhibits plasma cholinesterase and as a result the conversions of COC→ME and of BE→ECG. The conversions COC→BE and ME→ECG can be inhibited by acidification. All conversions are slowed by cooling. Addition of NaF is generally recommended to prevent cocaine hydrolysis. Although the use of fluoride stabilized blood sampling systems is advised, this is not common practice [2].

In postmortem blood, acidification may occur, which renders the conversions COC→BE and ME→ECG less important. However, enzymatic conversions continue after death. As a result, a large part of the methylecgonine measured in postmortem blood may originate from post-mortem hydrolysis of cocaine [3].

In The Netherlands, whole blood samples of car drivers, who are suspected of driving under the influence (DUI) of alcohol and/or drugs, are collected in glass tubes containing sodium heparin and sodium fluoride (NaF). The samples are then sent to the Netherlands Forensic Institute (NFI) by regular mail, which generally takes 1-2 days, without cooling. After delivery handling at the NFI, blood samples are kept at 4ºC for a maximum of 2 weeks and at -18ºC thereafter. Until the end of 2005, blood tubes contained 0.8 % NaF and 700 IU/mL sodium heparin. From 2006 until mid-2007, these tubes were gradually replaced by tubes containing 0.4 % NaF and 143 IU/mL sodium heparin, for commercial reasons.
In autopsy cases, the interval between the finding of the body and the autopsy is generally 1-2 days. Preservation of (femoral) blood samples only takes place after the autopsy, by using the same tubes as in DUI cases and by freezing at -18ºC.

In this paper, we compare the ME/COC and BE/COC ratios in whole blood samples of: DUI cases with 0.8% NaF, DUI cases with 0.4% NaF and autopsy cases. The aim of this study is: to obtain more insight in the role of NaF as preservative and to investigate if the ME/COC ratio or the BE/COC is indicative for hydrolysis of cocaine.

2. Material and Methods

2.1. Chemical analysis

Cocaine, benzoylecgonine and methylecgonine were analysed by using GC-MS, after solid phase extraction (SPE) and derivatisation, or by liquid chromatography-tandem mass spectrometry (LC-MS/MS), after protein precipitation. More specifically, the following methods were used through the years. From 2001 through 2006, cocaine, benzoylecgonine and methylecgonine were identified and quantified using gas chromatography-mass spectrometry (GC-MS) after SPE and derivatization with hexafluoroisopropanol / pentafluoropropionicacidanhydride (HFIP/PFPA). The limit of quantitation (LOQ) for cocaine and metabolites was 25 ng/ml [4]. From 2006 onward, a method for the simultaneous analysis of various drugs, including cocaine and benzoylecgonine was used instead. With this method, drugs of abuse were identified and quantified using GC-MS after SPE and derivatization with hexafluoroisopropanol / trifluoroaceticacidanhydride (HFIP/TFAA). The LOQ for all tested drugs was 50 ng/mL in this assay. After 2007, the presence and concentrations of 57 drugs of abuse, including cocaine and metabolites, was investigated after protein precipitation, by using a validated LC-MS/MS method [5]. LOQs were 0.005 mg/L for cocaine, 0.015 mg/L for benzoylecgonine and 0.005 mg/L for methylecgonine.

2.2. Data analysis

Electronic data files of the Netherlands Forensic Institute (NFI) were searched for concentrations of cocaine, benzoylecgonine and methylecgonine in blood from 1999 through 2010. The BE/COC and ME/COC ratios were calculated and statistically evaluated. Median values and 95% intervals (one-sided) were obtained after log-transformation of the ratios, as their distribution was skewed. Outliers were removed after log transformation. After log-transformation, data were normally distributed and the mean value after log-transformation was in agreement with the median value obtained without data transformation.

3. Results and Discussion

3.1. DUI blood

The time course of the median ME/COC concentration ratios and the median BE/COC concentration ratios (per half year) in the DUI blood samples are graphically presented in Figures 1 and 2.

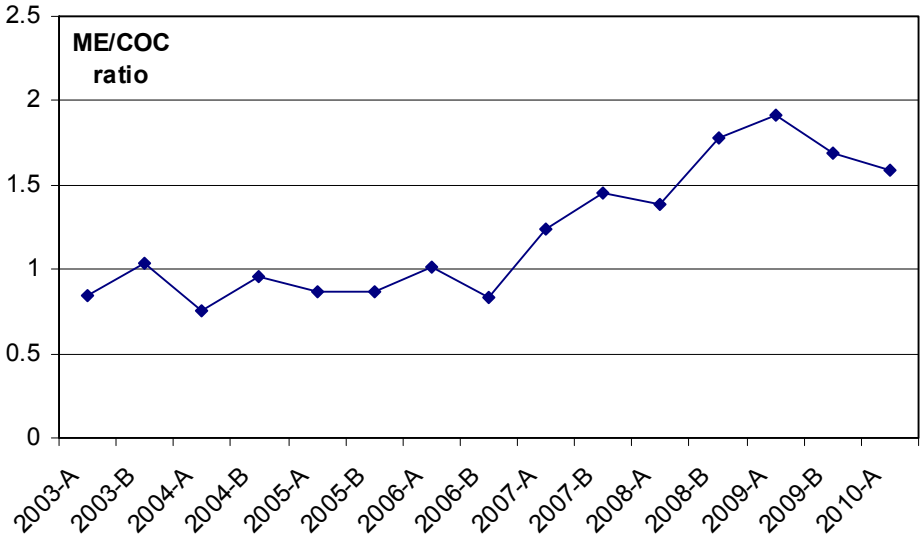

Fig. 1. Median ME/COC concentration ratios concentrations (whole blood) in DUI cases over the years (A= first semester, B= second semester). From 2006 until mid-2007, tubes containing 0.8 % NaF were gradually replaced by tubes containing 0.4 % NaF.

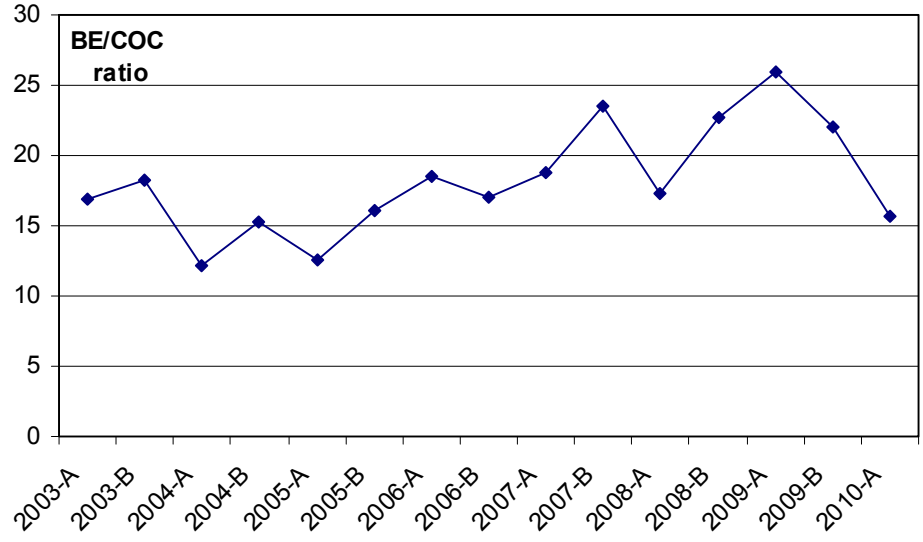

Fig. 2. Median BE/COC concentration ratios (whole blood) in DUI cases over the years (A= first semester, B= second semester). From 2006 until mid-2007, tubes containing 0.8 % NaF were gradually replaced by tubes containing 0.4 % NaF.

Because of an apparent increase in the median ME/COC concentration ratios after 2006 in DUI cases, the ME/COC and BE/COC ratios were grouped into cases from 1999-2005 and

from 2007-2010. Median values and 95% intervals are given in Table 1 for ME/COC ratios and in Table 2 for BE/COC ratios.

3.2. Autopsy blood

Data from autopsies were available from 2003-2010. Median values of the BE/COC and the ME/COC ratios in autopsy cases, with 95% interval, are given in Table 1 for ME/COC ratios and in Table 2 for BE/COC ratios. There was no trend in the half year's median ratios of ME/COC or BE/COC, as seen with the ME/COC ratio in the DUI cases.

Femoral blood was analyzed in most cases. In only 44 cases, heart blood was analyzed.
A statistical difference between ME/COC ratios in heart blood and femoral blood could not be demonstrated, possibly as a result of the small number of heart blood samples.

Tab. 1. ME/COC concentration ratios in various blood samples.

	median ME/COC ratio [95% range]*	**number of data**
DUI cases 1999-2005 (0.8 % NaF)	0.8 [0-2.1]	n=1032
DUI cases 2007-2010 (0.4 % NaF)	1.5 [0-4.6]	n=438
Autopsies 2003-2010	2.1 [0-7.8]	n=269
(* all data: back-calculated after log-transformation)		

Tab. 2. BE/COC concentration ratios in various blood samples.

	median BE/COC ratio [95% range]*	**number of data**
DUI cases 1999-2005 (0.8 % NaF)	16 [0-49]	n=1144
DUI cases 2007-2010 (0.4 % NaF)	20 [9-90]	n=438
Autopsies 2003-2010	7.1 [0-62]	n=286
(* all data: back-calculated after log-transformation)		

3.3. Discussion

As benzoylecgonine concentrations are generally higher than methylecgonine concentrations in blood, hydrolysis of cocaine will affect the ME/COC ratio relatively more than the BE/COC ratio. This makes the ME/COC ratio a more suitable candidate for indicating cocaine hydrolysis. This was confirmed by the observation in this study, that the median ME/COC ratio increased for the categories DUI (0.8% NaF) < DUI (0.4% NaF) < autopsies. This is the order of decreasing preservation of these samples. For the BE/COC ratios, this was not the case. The results indicate that the ME/COC ratio is a useful indicator of enzymatic cocaine hydrolysis.

The lower BE/COC ratio in autopsy cases as compared to DUI cases may point to the protective role of post-mortem acidification in the hydrolysis of cocaine, as postulated by e.g. Isenschmid et al[1]. The possible role of sodium fluoride in preventing benzoylecgonine decomposition, as postulated by Toennes et al [6], is unclear, because in our study the influence of the NaF concentration on the BE/COC ratio in DUI cases was unclear (see table 2).
Figure 1 shows that in DUI cases, the ME/COC ratio was relatively stable over the years 2003-2006 and that this ratio increased from 2007. Figure 2 shows that the BE/COC ratios tend to increase slightly over the years, but a time course as seen for the ME/COC ratios is not

present. The increase in the ME/COC ratio from 2007 coincides with a change from blood tubes containing 0.8% NaF to tubes containing 0.4% NaF. This change took place gradually from 2006 until mid-2007. The results indicate that 0.4% NaF is insufficient for the preservation of whole blood samples under the circumstances of blood sampling and transport in The Netherlands (transport by regular mail, no cooling until delivery at the NFI). This is even more so, as concentrations of NaF are often higher than the nominal value, because the blood tubes are not always filled completely. The other fluctuations between 2007 and 2010 in Fig. 1 may be explained by fluctuating backlogs and changes in handling procedures at the NFI.

The preservative capacity of sodium fluoride has been discussed in the literature. The publications show that the duration of action of sodium fluoride depends on its concentration and the temperature. Baselt et al [7] found that blood containing 0.5% NaF considerably lost cocaine after 3 days at 25°C (about 70% cocaine remaining). In a later study, Baselt [8] demonstrated that NaF inhibits blood cholinesterase in a dose-dependent way, but that cholinesterase activity was never completely inhibited. Brogan et al [9] showed that 0.25% NaF (in combination with potassium oxalate) inhibited cocaine degradation for 48 h at room temperature (91% cocaine remaining after 48h). in contrast, Skopp et al [10] found that neither chemical nor enzymatic hydrolysis of COC, BE or ME could be fully stopped in vitro by 0.25% fluoride. In our study, samples are generally received from the police by regular mail within two days. In view of the results of Brogan et al [9], 0.4% NaF should be enough for an adequate preservation of our DUI whole blood samples. However, longer delivery times than two days do occur, and no record is kept of the temperature. Due to transportation by regular mail, there is an increased risk of haemolysis of blood cells, which could lead to the inactivation of fluoride.

Isenschmid et al [3] postulated that ME is a minor cocaine metabolite in vivo and is produced mainly by post-mortem or post-sampling hydrolysis of cocaine. This hypothesis was discussed by others [11,12]. In 1992, Isenschmid et al [3] suggested that the cocaine concentration at the time of death could be estimated by adding the molar concentrations of ME and COC. However, this study was performed with only 10 blood samples and hydrolysis of BE and ME was not taken into account. Our study supports the idea of Isenschmid et al that the ME concentration will be helpful in the interpretation of cocaine concentrations in blood. Our data show that in well-preserved samples (containing 0.8% NaF), the ME/COC concentration ratio is lower than 2.1 in 95% of the cases. Even in these cases, cocaine hydrolysis may have occurred. Therefore, a ME/COC ratio higher than 2.1 is indicative of (enzymatic) cocaine hydrolysis.

4. Conclusions

The ME/COC ratio is probably a useful indicator of enzymatic cocaine hydrolysis in cases of driving under the influence as well as in autopsy cases.

In autopsy cases, the median BE/COC ratio was significantly lower than in DUI cases. This points to the protective role of post-mortem acidification in the chemical hydrolysis of cocaine.

The results indicate that under the circumstances of blood sampling and transport in The Netherlands (transport by regular mail, no cooling until delivery at the NFI), 0.4 % sodium fluoride is insufficient to prevent decomposition of cocaine in blood during more than 1 or 2 days.

A ME/COC ratio > 2.1 is indicative of (enzymatic) cocaine hydrolysis. This information will improve the interpretation of forensic results.

5. References

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