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Concentrations of cocaine and its major metabolite benzoylecgonine in blood samples from apprehended drivers in Sweden

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Abstract

Cocaine and its major metabolite benzoylecgonine (BZE) were determined in blood samples from people arrested in Sweden for driving under the influence of drugs (DUID) over a 5-year period (2000–2004). Venous blood or urine if available, was subjected to a broad toxicological screening analysis for cannabis, cocaine metabolite, amphetamines, opiates and the major benzodiazepines. Verification and quantitative analysis of cocaine and BZE in blood was done by gas chromatography-mass spectrometry (GC–MS) at limits of quantitation (LOQ) of 0.02 mg/L for both substances. Over the study period 26,567 blood samples were analyzed and cocaine and/or BZE were verified in 795 cases (3%). The motorists using cocaine were predominantly men (>96%) with an average age of 28.3 ± 7.1 years (±standard deviation, S.D.). The concentration of cocaine was below LOQ in 574 cases although BZE was determined at mean, median and highest concentrations of 0.19 mg/L, 0.12 mg/L and 1.3 mg/L, respectively. In 221 cases, cocaine and BZE were together in the blood samples at mean and (median) concentrations of 0.076 mg/L (0.05 mg/L) and 0.859 mg/L (0.70 mg/L), respectively. The concentrations of BZE were always higher than the parent drug; mean BZE/cocaine ratio 14.2 (median 10.9) range 1–55. Cocaine and BZE were the only psychoactive substances reported in $N = 61$ cases at mean (median) and highest concentrations of 0.095 (0.07) and 0.5 mg/L for cocaine and 1.01 (0.70) and 3.1 mg/L for BZE. Typical signs of drug influence noted by the arresting police officers included bloodshot and glossy eyes, agitation, difficulty in sitting still and incoherent speech.

Keywords: Cocaine; Blood; Benzoylecgonine; Concentrations; DUID; Impaired driving

1. Introduction

Cocaine is a highly addictive recreational drug in terms of psychological dependence, craving and the risk of relapse after a period of abstinence [1–3]. Medical complications associated with the use and abuse of cocaine includes hypertension, hyperthermia and risk of cardiac toxicity after intravenous administration of large doses [4,5]. Many deaths have occurred after recreational use and abuse of this powerful central nervous system (CNS) stimulant [6].

Driving under the influence of cocaine has emerged as a traffic safety problem in certain countries owing to the widespread recreational use of this drug along with the increasing popularity of crack cocaine [7–9]. However, available statistics on drug-impaired driving are not always easy to interpret for several reasons. First, some laboratories analyze drugs and/or their metabolites in urine but not in blood. This means that detection times are longer, which tends to increase the prevalence of positive findings for some substances. Second, the urinary metabolites of many drugs are not pharmacologically active so they do not pose a particular problem for traffic safety. Third, the analytical methods used, the cut-off concentrations for screening as well the limits of quantitation (LOQ) might differ between different laboratories [10]. Investigators need to know the concentration of a drug in blood or plasma to permit drawing conclusions about the pharmacological effects on the individual and the potential for drug-related impairment.

The introduction of a zero-tolerance law for driving under the influence of drugs in Sweden (since July 1999) led to an appreciable increase (>10-fold) in the number of blood samples being submitted by the police for toxicological analysis [11]. This prompted us to investigate the prevalence of cocaine use by apprehended drivers in Sweden and to document the concentrations of this stimulant and its metabolite benzoylecgonine (BZE) in blood after recreational use. For comparison, the concentrations of cocaine and BZE were determined in blood

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samples from people arrested by the police for use of illicit drugs (non-traffic cases).

2. Materials and methods

2.1. Collection of body fluids

Blood and urine specimens from all apprehended impaired drivers in Sweden (population 9 million) are sent to one central laboratory for toxicological analysis (Department of Forensic Genetics and Forensic Toxicology, National Board of Forensic Medicine, Linköping). Venous blood is taken from a cubital vein by a physician or registered nurse using 10 mL grey-stopper evacuated glass tubes containing sodium fluoride (100 mg) and potassium oxalate (25 mg) as preservatives. Whenever possible a specimen of urine (10 mL) is taken from the DUID suspect by a police officer and this also contains sodium fluoride (100 mg) as a preservative.

2.2. Police procedures

The first suspicion that a driver is impaired arises after a moving traffic offence, after a collision stopped or in connection with a routine sobriety control. Other road users might also report via mobile phones that they suspect a person might be driving impaired. Each suspect first undergoes a roadside breath-alcohol screening test. If the result is positive (>0.2 mg/g or 0.02 g%) an evidential breath-alcohol test is performed at the nearest police station. A refusal to undergo a roadside breath-alcohol screening test results in a sample of blood being taken and if necessary these can be done by force. If the breath-test result is negative (<0.2 mg/g or <0.02 g%) and the person shows signs of being under the influence of drugs then venous blood is obtained for toxicological analysis. The sampling of body fluids is done at a local police station or in the case of a crash with injuries to the driver blood might be taken at a hospital.

In connection with passing the zero-tolerance law for drugs other than alcohol (1 July 1999) the police were given new powers to examine the appearance of a driver's eyes with a flashlight to record reaction to light and pupil-size was measured with a pupilometer. The existence of any gaze nystagmus was noted as well as any other indications that suggest use or abuse of drugs other than alcohol. The results of these simple impairment tests are recorded on an arrest form and this is sent together with blood and urine samples for toxicological analysis.

2.3. Determination of cocaine and BZE in blood

Blood and/or urine are subjected to a broad screening analysis by enzyme immunoassay methods (EMIT/CEDIA) for five classes of abused drugs (opiates, cannabinoids, amphetamine analogs, cocaine metabolites, and benzodiazepines) with an ADVIA 1650 instrument (Bayer, Health Care Diagnostics, Tarrytown, NY). The screening cut-offs for BZE were 300 ng/mL in urine (EMIT) and 50 ng/mL in blood (CEDIA). All positive results from the screening analysis are verified by more sensitive and specific analytical methods such as GC–MS and LC–MS or GC–NPD in the case of prescription drugs. There was no general GC–MS screening analysis performed on body fluids.

Cocaine and BZE were determined in blood by GC–MS with deuterium labelled internal standards and solid phase extraction. Internal standards d_3-cocaine and d_3-benzoylecgonine (50 μL) were added to an exact quantity of blood (1 g or 1 mL) and acetone (3 mL) was added to precipitate proteins. After centrifugation the supernatant was removed and evaporated under nitrogen at 40 °C to reduce the volume to ~0.5 mL. Phosphate buffer (0.1 M pH 6.0) was added and the contents of the tube mixed well.

Bond elute certify columns were used for solid-phase extraction of cocaine and BZE after activation with methanol and phosphate buffer. The blood extract was added to the columns, which were then washed through with water, HCl (0.1 M) and methanol before being dried under vacuum for 5 min. The cocaine and BZE were eluted with a mixture of dichloromethane and 2-propanol (80:20) and 2% ammonium hydroxide was also added. The extract was then carefully evaporated to dryness under nitrogen (max. 40 °C). For GC–MS analysis the pentafluoropropyl derivatives of BZE was prepared by adding a mixture of PFPA and PFPOH (2:1) and allowing to stand in a warm block at 60 °C for 15 min. The tubes were then cooled to room temperature and the contents were evaporated to dryness under nitrogen. The residue was finally dissolved in ethyl acetate (100 μL), again evaporated to dryness and the residue dissolved in butyl acetate (50 μL) and allowed to stand for 15 min before 1 μL was injected for GC–MS analysis.

The GC–MS equipment was purchased from Hewlett Packard (HP) Agilent Technologies (HP5890A or HP 6890N) and HP 7693 autosampler. The mass spectrometer was HP (Agilent) 5971/5973 with MS Chemstation. A capillary column HP-5MS (Hewlett Packard) 30 m long, 0.25 mm i.d. and film thickness 0.25 μm was used for the chromatographic separations and a three-step temperature program was run; 130 °C (1 min) then 50 °C per min to 240 °C (0.1 min) and 20 °C per min to 280 °C (3 min) with helium as carrier gas. The quantitative analysis of cocaine was done by selected ion monitoring using the mass fragments *m/z* 182/185 and with *m/z* 198 and *m/z* 303 used as qualifier ions. The corresponding ions for BZE were *m/z* 300/303 and *m/z* 316 and *m/z* 421 were the qualifier ions. A seven-point standard curve was prepared for analysis of cocaine and BZE in blood and this was linear between 0.02 and 2 mg/L. The limit of quantitation (LOQ) in routine casework for analysis of both cocaine and BZE was 0.02 mg/L.

2.4. Evaluation of results

This material comprised 795 blood samples from apprehended drivers in Sweden. All specimens contained BZE and hence verified that cocaine had been used some time earlier. Sub-sets of cases were selected depending on whether the blood samples contained parent drug ($N = 160$) indicating fairly recent usage of cocaine. We also formed a sub-set of cases in which cocaine and BZE ($N = 61$) were the only psychoactive substances present and without the confounding influences of other abused drugs. Finally, sub-sets were constructed when the blood samples contained BZE alone or BZE together with other recreational or medicinal drugs. The same case selection criteria was used with a sample of non-traffic cases ($N = 471$) in which cocaine and/or BZE was identified in blood.

The frequency distributions of the concentrations of cocaine and BZE in blood were skewed to the right so mean, median and the highest concentration were used as descriptive statistics. The age of offenders was reported as mean ± standard deviation (S.D.).

Table 1
Number and percent of driving under the influence of drugs cases in which cocaine and/or benzoylecgonine were verified present in blood samples between 2000 and 2004[a]

Year	*N*	Cocaine + BZE *N* (%)	BZE only[b] *N* (%)	Cocaine and/or BZE *N* (%)
2000	3,819	14 (0.37)	76 (2.0)	90 (2.4)
2001	4,653	27 (0.58)	76 (1.6)	103 (2.2)
2002	5,063	39 (0.77)	114 (2.3)	153 (3.0)
2003	5,993	66 (1.1)	136 (2.3)	202 (3.4)
2004	7,039	75 (1.1)	172 (2.4)	247 (3.5)
2000–2004	26,567	221 (0.83)	574 (2.2)	795 (3.0)

[a] Cocaine was not identified in blood without the metabolite benzoylecgonine also being present.
[b] Cocaine reported as negative, below LOQ (0.02 mg/L in blood).