



Time course of cocaine in rabbit hair

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Abstract

The accurate interpretation of analytical results from hair testing for drugs of abuse continues to be a complex and difficult problem since many questions still remain unanswered. In this paper an animal model was developed to ascertain the time course for the appearance and disappearance of cocaine and its metabolite benzoylecgonine (BE) in hair. Female Fauve Bourgogne red-haired rabbits ($n = 6$) were intraperitoneally administered a single dose of cocaine at 5 mg/kg. Animal hair was shaved just before drug administration and the newly grown back hair was subsequently shaved and collected daily over a period of two weeks. Samples were analyzed for cocaine and BE by gas chromatography–mass spectrometry (GC–MS). The profiles were quite similar for parent drug and metabolite. Cocaine and BE appeared in the first sampling (day 1), with peak concentration appearing that same day: 1.01 ng/mg and 0.51 ng/mg for cocaine and BE, respectively. Levels declined rapidly on day 2, remaining detectable for ten days after drug administration. This study demonstrates that the initial incorporation of cocaine compounds in rabbit hair is very rapid (24 h). A small fraction of the drug is detected ten days after exposure, at a time when concentrations in other biological specimens (blood or urine) are not detectable. © 1997 Elsevier Science Ireland Ltd. All rights reserved

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1. Introduction

The analysis of hair for drugs of abuse has been increasingly utilized over the past several years, and is being progressively admitted by the Courts to support evidence in forensic and drug related cases.

Up to now, the majority of drugs of abuse (like opiates, cocaine, cannabinoids, amphetamines, benzodiazepines, etc.) and their respective metabolites have been

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detected and quantified in hair samples by means of previously developed and validated analytical methods. However, an accurate interpretation of analytical results continues to be complex and difficult because of the many factors that still remain unclear. This includes, but is not limited to, the following: influence of cosmetic treatment on drug concentrations, relationship dose-response, influence of external contamination or diffusion along the hair shaft. Moreover, one of the most frequent questions for the toxicologist concerns the point in time when drugs first appear in hair as well as the length of time they remain in it after drug administration.

The rate of appearance and disappearance of cocaine compounds in hair was examined by Ferko [1] after multiple-dose administration to rats; cocaine was present in the first sampling (day 4) and the mean half-life for its disappearance was 3.1–3.5 days. Henderson et al. [2] found, in their dose-response studies with d_3 -cocaine, that cocaine may be detected in hair within hours (8 h) after drug administration. In addition to cocaine, other classes of drugs, including opiates [3–6] and amphetamines [7,8], have been studied.

Although some of these studies have been performed on human hair [2–5,8], human studies are often difficult to arrange because of the possible ethical problems involved in drug administration. Consequently, animal studies are helpful in understanding the mechanisms involved in the incorporation of drugs into hair.

The present study was designed to establish the time period for the appearance of cocaine and its major metabolite benzoylecgonine (BE) and the long-term stability of the parent drug and metabolite in rabbit hair after a single-dose administration.

2. Materials and methods

2.1. Reagents and standards

All chemicals were of analytical grade (Merck, Darmstadt, Germany). Cocaine, d_3 -cocaine, BE and d_3 -BE standard solutions (100 $\mu\text{g}/\text{ml}$ in ethanol) were purchased from Radian (Austin, TX, USA) for the preparation of standards and other quality control materials. Heptafluorobutyric anhydride and hexafluoropropanol were obtained from Sigma (Madrid, Spain).

2.2. Animal experiments

Female Fauve Bourgogne red-haired rabbits (Granja Cunicula Pi Petit, Barcelona, Spain) weighing 3.10–3.69 kg were used. Animals ($n = 6$) were individually housed in metabolism cages with food and water ad libitum.

Before drug administration the back hair of the rabbits was cut with a scissors and then shaved with an electric shaver, to be used as a control.

Cocaine hydrochloride dissolved in normal saline was intraperitoneally (i.p.) administered in a single dose of 5 mg/kg. As a quality-control procedure, samples were assayed to verify concentration. The newly grown back hair was collected at

daily intervals over a two week period after drug administration. At least 50 mg of hair were collected in each sampling. Hair samples were placed in separate vials and stored at room temperature until analysis.

The whole experiment was carried out in compliance with the regulations relative to the housing and care of experimental animals; the premises are registered with the Ministry of Agriculture, Fishing and Food with the number 41091-4A. In all the tests Good Laboratory Practice (GLP) rules were followed (Directive 87/18 CEE).

2.3. Analytical method

A sensitive and specific GC–MS method for the simultaneous quantification of cocaine and its major metabolite BE was used. Details of the method have been published previously [9], but the essentials of the method are described below.

From 40 to 50 mg of hair were decontaminated twice with methylene chloride for 15 min at 37°C. Then they were incubated in 1 ml of 0.1 M HCl at 50°C overnight in the presence of 250 μ l of the deuterated internal standard methanolic solutions (containing d_3 -cocaine and d_3 -BE at 1 μ g/ml). After neutralization with 1 ml of 0.1 M NaOH, the drugs were extracted with 2 \times 5 ml of chloroform–isopropanol–*n*-heptane (50:17:33, v/v) at pH 9.2 (phosphate buffer). After agitation and centrifugation, the organic layer was removed and evaporated to dryness. The residue obtained was derivatized with 100 μ l heptafluorobutyric anhydride and 70 μ l of hexafluoropropanol for 30 min at 60°C. The samples were again evaporated and then reconstituted in 100 μ l of ethyl acetate. One microliter was subsequently injected onto a Hewlett–Packard 5890 gas chromatograph coupled to a 5971A mass selective detector fitted with an HP-Ultra 1 capillary column (crosslinked methyl-silicone, 25 m \times 0.2 mm i.d. \times 0.33 μ m film thickness). The temperature was programmed from 60°C (3-min hold) to 280°C (12-min hold) at 12°C/min. The injector temperature was 250°C and the GC–MS interface temperature was 280°C. The helium carrier flow was 1 ml/min. The mass spectrometer was operated in the selected ion monitoring mode (SIM).

3. Results and discussion

After drug administration, the concentrations of cocaine and its major metabolite BE were measured daily over a two-week period.

The time course of appearance and disappearance of these two compounds in rabbit hair after i.p. injection of 5 mg/kg of cocaine are illustrated in Fig. 1. In this figure the amount of drug is expressed as the mean concentration obtained from the six rabbits.

Cocaine concentrations rose from non-detectable levels before administration (day 0) to peak concentrations of 1.01 ng/mg on day 1. Levels declined rapidly to 0.68 ng/mg on day 2. Thereafter, cocaine concentrations declined somewhat more slowly and remained detectable until day 9. On day 10, cocaine concentrations were below our quantification limit (0.13 ng/mg).

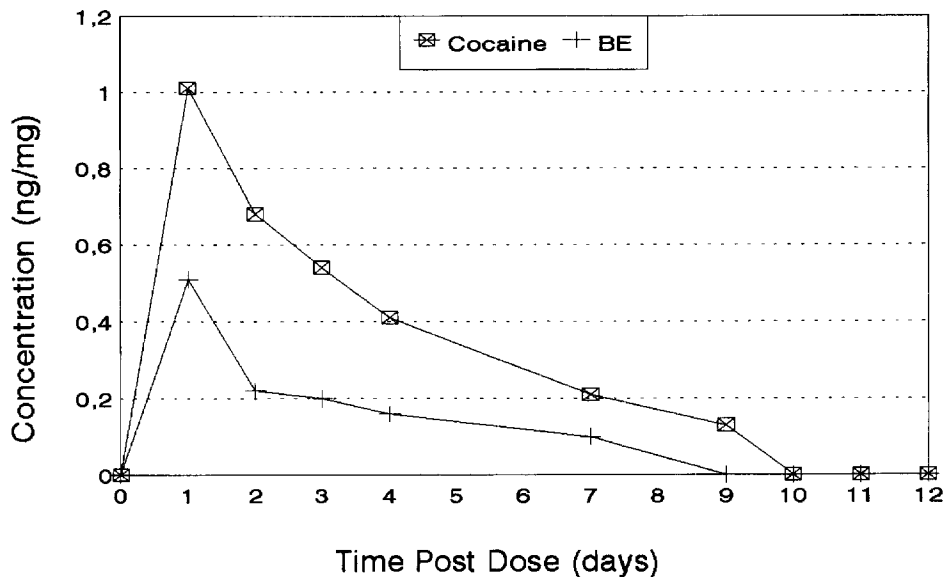


Fig. 1. Time course of cocaine and benzoylecgonine in rabbit hair following a single dose administration of 5 mg/kg of cocaine. The amount of drug is expressed as the mean concentration obtained from the six rabbits.

The BE profile shown in Fig. 1 is quite similar to that of the parent drug, cocaine. The highest mean concentration of BE, 0.51 ng/mg, was detected on day 1. It declined over the period of the test, with 0.10 ng/mg remaining detectable on day 7.

In the majority of the previous papers where animals were used to study drugs of abuse in hair, rats have been the predominant subjects of analysis. Monkeys and guinea-pigs have also been utilized in some studies; but to our knowledge this is the first paper on rabbit hair. We chose rabbits because they are easier to control than monkeys and because their size enables us to obtain sufficient hair for analysis from only one animal, thereby eliminating pharmacokinetic differences between strains of animals as a factor in data interpretation.

Because of the low incorporation rate of drugs in hair, most earlier studies on the appearance and disappearance of drugs of abuse in hair included repeated rather than single doses. In spite of this, in our experiment, which is the first in a series of studies that we plan to perform on the time course of drug incorporation into animal hair, we preferred the administration of a single dose of cocaine. In future experiments we will study behavior after multiple doses.

In the present study cocaine and its metabolite BE started to appear in the first sampling, one day after drug administration. This is very likely due to the arterial capillary bed which surrounds the hair follicle and nourishes the growing hair bulb via the papilla. After drug administration, lipid-soluble substances, like cocaine, diffuse from the blood through the papilla and into the hair bulb very quickly.

On the one hand, our data agree with those reported in earlier publications. Ferko et al. [1] and Gygy et al. [6] showed that cocaine and codeine, respectively, injected in rats for 20 days, started to appear in the first sampling and accumulated until dosage cessation, before beginning to fall. Similarly, Henderson et al. [2] in their dose-response studies with d_5 -cocaine in human hair found that cocaine may be detected in hair within hours (8 h) of drug administration. Niwaguchi et al. [7] reported that methamphetamine in hair collected from rats after oral administration of a single-dose of the drug was detected 8 h after the administration and remained detectable 8 days later. Finally, Nakahara et al. [8] detected metoprolol in the beard from one day after oral administration to days 10–12, with peak concentration on day 3.

On the other hand, our data are different from those reported by Cone [3] in a time-profile study of morphine and codeine in two heroin abuse subjects. He suggests a time lag between dose administration and first appearance in hair of approximately 7–8 days. Our data, however, showed that cocaine started to appear one day after drug exposure. The differences in our data may be due mainly to the fact that Cone's study was performed on humans, while our study was done on rabbits; secondly, different drugs of abuse were used in each study. Cone [3] administered opiates (morphine and codeine), whereas our study was performed with cocaine. Another explanation may be that Cone's study was done on two subjects with a history of intravenous heroin abuse. For this reason, he attributed the elevated opiate levels that appeared in the early samples to the subjects' probable consumption of heroin within 1–2 weeks before the experiment; while these early concentrations could be due, as well, to morphine or codeine administered in the study.

In summary, this study demonstrated that the initial incorporation of cocaine into the hair is very rapid (24 h). Only a fraction of the drug that entered the hair bulb was able to be detected throughout the ten days after initial drug exposure at a time when drug levels in other biological specimens (blood or urine) were not detectable. More detailed studies are required at both higher single doses and multiple doses to establish the relationship (if any) between the different drug dosages administered and the length of time for which they remain detectable.

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