Alternate Strategies for Postmortem Drug Testing

Steven B. Karch
Assistant Medical Examiner, City and County of San Francisco, San Francisco, California

During the last decade, our ability to extract, and reliably quantify, drugs from blood and tissue has increased drastically. Yet in spite of these advances, it is increasingly apparent that toxicologic measurements, taken in isolation, cannot be used to discriminate between individuals who died as a consequence of drug use and those who did not. The situation is not much better in the living; it is still not possible to relate specific plasma drug concentrations and impairment. Given that subnanogram drug quantitation is already possible, further increases in measurement precision are unlikely to provide the answers needed by pathologists, judges, and juries. Fortunately, the picture is not so bleak as these observations would suggest. Alternate testing strategies employing multiple tissue measurements may provide the needed answers.

Fifteen years ago, at the beginning of the current cocaine pandemic, Smart and Anglin (1) were the first to suggest that the lethal dose of cocaine might never be known. Their doubts were prompted by the observation that reported postmortem blood cocaine concentrations, in both man and animal, could sometimes vary by more than an order of magnitude. Fewer than five years ago, their worries were confirmed when the first large postmortem toxicologic studies of methamphetamine and cocaine users were published; blood concentrations in those clearly dying of drug toxicity completely overlapped concentrations seen in decedents where the presence of drug was an incidental finding (2-4). Although no comparable analyses of heroin/morphine-related deaths have been published, it has been the experience of most Medical Examiners that postmortem blood morphine concentrations measured in drug-related deaths are not significantly different from cases where the presence of morphine is an incidental finding, completely unrelated to the cause of death.

It is tempting to attribute this overlap to postmortem drug redistribution, the movement of drugs between tissues and body fluids that occurs after death. A long list of factors can affect postmortem drug concentration measurements; the more obvious include postmortem interval, method of collection, site sampled, ambient temperature, physical properties of the drug, preservative used, position of the body, and the amount of unabsorbed drug present at the time of death. But even taking all these variables into account, there is still no assurance that drug concentrations measured after death bear any relationship at all to concentrations measured in life.

This long list of confounding variable is acknowledged by pathologists and toxicologists, but until very recently, few realized that even if postmortem measurements were valid indicators of antemortem concentrations, they would still be useless for identifying cases of drug toxicity. The explanation has to do with the phenomenon of drug tolerance. Chronic exposure to opiates and stimulants sets in motion a series of neurochemical and anatomic changes that make drug users less sensitive not only to the drugs psychological effects—with chronic use it takes increasing amounts of drug to produce the same desired mental effect—but also to drug-induced changes in pulse, blood pressure, and respiration (5-7).

The development of tolerance explains why otherwise healthy addicts receiving heroin-maintenance therapy have higher plasma morphine concentrations than others dying of heroin overdoses (8). It also explains why plasma cocaine concentrations cannot be related to symptoms or outcome. A case report published in 1990 described an otherwise healthy cocaine abuser who died instantaneously of a gunshot wound to the head; the blood cocaine concentration was 30 mg/L (9). In a prospective study of 101 cocaine users seeking emergency room treatment, Blaho et al. (10) found there was absolutely no correlation between plasma concentrations of cocaine (and all of its major metabolites) and outcome. In fact, the patient with the highest plasma cocaine concentration (3.4 mg/L) was discharged into police custody just 2 h after arrival at the hospital. Not so long ago, cocaine concentrations of that magnitude were thought to be uniformly fatal.

Fortunately, it is now both possible and relatively simple to identify tolerant opiate users after death. The solution is to measure hair morphine concentrations (11). Morphine concentrations in the hair of active heroin users are much higher than those in abstinent users, and hair concentrations in overdose deaths are comparable to those seen in abstinent users.

Obviously, this situation does not apply in every case, but the observation is consistent with the experience of Medical Examiners everywhere; most heroin overdoses involve chronic heroin users who, for whatever reason, have been abstinent for days or weeks and have lost their opiate tolerance (12). The longer the period of abstinence, the lower the hair morphine concentration is likely to be. As was successfully argued at the trial of serial murderer Dr. Harold Shipman, this inverse relationship can be diagnostic. Shipman killed his victims with lethal injections of heroin. Exhumed victims were found to have high tissue morphine concentrations, but except for one individual with picogram quantities of morphine detected in her hair roots, morphine was not found in the hair of any of the
others. The disparity between hair and tissue testing results conclusively demonstrated that the decedents had not taken morphine previously and could not have been tolerant. This evidence helped to obtain first degree murder convictions in all six cases where bodies had been exhumed.

Although the finding of high postmortem morphine blood concentrations in conjunction with low hair concentration is consistent with, but not necessarily diagnostic for, heroin overdosage, the finding of high postmortem morphine blood concentrations with high hair concentrations is not. The only inference that can be drawn from the combination of high blood morphine and high hair morphine concentrations is that the decedent was a regular drug user. Whether morphine was the cause of death is a question that remains unanswered.

A similar relationship between blood and hair concentrations has not been demonstrated for stimulant drugs, such as cocaine and methamphetamine, and there are reasons for supposing that no such relationship exists. Tolerance to opiates involves changes in the brain stem respiratory centers, with decreased numbers of μ and δ receptors in the medulla (13). Tolerance to cocaine involves a completely different set of alterations, occurring in multiple sites within the brain, the peripheral sympathetic nervous system, and the heart (5,14-17). For those reasons, and because advances in solid-phase extraction have simplified the process so much, measurement of brain cocaine concentrations is becoming an increasingly attractive alternative to the testing of blood.

More than 15 years ago, it was suggested that, at least for cocaine, brain provided a better analytic matrix than blood (18). Even though the suggestion was never widely accepted, the idea is as valid today as when it was first suggested. Cocaine rapidly crosses the blood brain barrier, but its principal metabolite, benzoylecgonine, does not. Because of the brain's isolated position, postmortem redistribution is not an issue.

Some brain regions concentrate more drugs than others, but PET scans of drug users show that drug distribution is much more homogenous within the brain than in the periphery (19). Provided that brain sections are removed and frozen with 24 h of death, and that sampling is confined to regions containing cell bodies and terminals of the mesolimbic dopaminergic system (i.e., the substantia nigra, medial and lateral geniculate bodies, red nucleus, and nucleus accumbens) (20,21), there is good reason to believe that measured concentrations will be an accurate reflection of concentration in the immediate antemortem period.

If the appropriate portions of the brain are tested and cocaine, but little or no benzoylecgonine is detected, it can be concluded that ingestion occurred just prior to death. In the presence of confirmatory anatomic changes, such as pulmonary or cerebral edema, this finding is consistent with acute toxicity. Absent the appropriate anatomic findings, nothing can be concluded except that the decedent was a cocaine user. The detection of benzoylecgonine, but little or no cocaine, suggests chronic use. If the anatomic changes known to be associated with chronic use, such as cardiac enlargement, microvascular disease, and microfocal fibrosis (3), are also identified, then death can reasonably be attributed to chronic cocaine use, even in the absence of cocaine. In the absence of the appropriate anatomic changes, the only permissible conclusion is that the decedent used a moderately large amount of cocaine on the day before death (22).

These inferences cannot be drawn from blood tests. Postmortem redistribution occurs, and measured drug concentrations are site dependent (23). It is generally agreed that drugs with a large volume of distribution (Vd) are those most prone to redistribution (24). Cocaine's Vd is thought to be fairly large, close to 3 mg/L (25). Benzoylecgonine's Vd has never been measured. If the Vd for benzoylecgonine is not the same as for cocaine, then conclusions about the ratio of parent to metabolite cannot be drawn.

If, as seems likely, the Vd for benzoylecgonine is small relative to the Vd of cocaine, then postmortem redistribution of cocaine would alter blood cocaine to benzoylecgonine ratios and provide misleading information about both the timing of ingestion and the amount of cocaine that had been taken. That is not the case when brain is the analyte. Even in the common scenario where both parent and metabolite are present, the ratio of the two compounds can reasonably be assumed to approximate conditions at the time of death; measurements made with postmortem "blood" cannot.

Alternatives to postmortem blood testing are clearly needed. Brain receptor measurements seem to offer the most potential, but the technology is beyond the capabilities of most Medical Examiner laboratories. Until more advanced technologies can be implemented, pathologists should give serious thought to testing vitreous or spinal fluid instead of blood. Even though the diffusion of drugs into the CSF is not well studied, sampling spinal fluid or vitreous can be very helpful, especially when the decedent has been transfused, or when there have been extensive resuscitative efforts. Spinal fluid is also protected by the blood brain barrier, which means that the same inferences can be drawn about cocaine/benzoylecgonine ratios as can be drawn from analysis of brain (26,27).

Brain receptor measurements are also possible and much more likely than blood testing to reveal the cause death. Compared with controls, psychotic stimulant abusers with excited delirium have increased numbers of kappa receptors in the amygdala and decreased numbers of D2 dopamine receptors in the hypothalamus. Kappa receptors stimulation induces psychosis, and changes in hypothalamic dopamine receptors result in abnormal temperature control. With fewer D2 receptors available, D1-mediated temperature increases go unopposed, which is why psychotic stimulant abusers are almost always hyperthermic (5,14-16).

Brain receptor measurements have already proven to be of enormous forensic value, but their use requires viewing the problem of drug toxicity in new and different ways. The familiar concepts of "cutoffs" and concentration ranges are meaningless when applied to neuroreceptors. Neuroreceptor expression is assessed by comparing test subjects to normal controls. For example, receptor density in the striatum of individuals dying of cocaine-associated excited delirium (ED) can be (and has been) compared to density measurements made in the brains of drug-free trauma victims and in non-psychotic cocaine users; dopamine receptor concentrations in the ED patients are comparable to those measured in non-drug users, demonstrating that the brains of ED victims are unable to clear the excess...
dopamine released after cocaine administration (5,14,15). In San Francisco, a recent high-profile death-in-custody case was resolved by making just such comparisons.

Receptor measurements may also prove useful in the investigation of opiate-related deaths. The human opiate receptors have been cloned (28). Their chemical and immunohistochemical localization, as well as their quantitation, is now feasible, even in postmortem material. If brainstem opiate concentrations are measured at the same time, the cause of death can be determined because tolerant opiate users will have down-regulated medullar μ and δ receptors (13). High morphine concentration in the medulla in the presence of normal μ and δ receptor concentration implies that the decedent was not tolerant and that respiratory depression was the cause of death.

Receptor changes can be demonstrated with autoradiographic or immunohistochemical techniques, but they can also be measured in brain tissue extracts, and there is nothing to prevent toxicologists from undertaking such measurements, assuming they have been provided with the proper specimens. Alternatively, immunochemical techniques can be used to demonstrate the presence of morphine within the neurons of the brainstem. When compared to drug-free controls, the brains from victims of heroin overdose exhibit selective staining of ganglion cells, axons, and dendrites (29). Depending on the morphine concentration required to cause staining (which has not been determined), such measurement might be diagnostic, even in the absence of simultaneous receptor measurements.

Whether these new approaches are ever adopted by the forensics community, or whether they will remain a research curiosity, depends partly on industry’s willingness to develop and market reagent-simplified analytic diagnostic kits. It also depends on the willingness of pathologists and toxicologists to consider the introduction of new, and very different, diagnostic techniques. But the greatest impetus for change will come when the forensics community finally comes to grips with the fact that postmortem blood drug concentration measurements are useful for the diagnosis of drug use and nothing more.

References