



Some Pitfalls in Forensic Toxicological Analysis of Autopsy Materials in Drug Related Deaths



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Abstract

The role of forensic toxicologist is constantly challenged by analytical, methodological, circumstantial problems in autopsy material. Poison/Drug responsible to cause death can only be identified by a professional person au fait with the field. Complete and proper tissue sampling for analysis is mandatory and simultaneously synergy with the pathologist as well as report of medical examiner is beneficent to forensic toxicologist to avoid mistakes and inferences. Identification of a toxic substance; whether it is a new compound or drug or it is present in traces is a great challenge in forensic toxicology and following these traces might lead to the answer of the case. Constant emerging of the new drugs in the market are provoking a toxicologist to remain up to date. Development of new analytical methods is indispensable task for these advanced drugs. This study highlights some pitfalls in forensic toxicological analysis and results related to drugs in autopsy material.

Keywords: Pitfalls; Drug quantity; Forensic aspects; Autopsy material analysis

Introduction

A forensic toxicologist has to deal with different questions every day. Finding every important detail, however small it could be, is a great challenge in forensic toxicology. There might be a small amount of a toxic compound or a new substance or sometimes there are only traces of it, but following these traces might lead to the answer of the case. Being up to date is important, because constantly new drugs are conquering the market. These new drugs have to be covered, making the development of new analytical methods an indispensable task in forensic toxicology. The class of drugs of abuse is growing constantly. Revolution of new psychoactive substances of different groups has made the task easy for use in clubs or at music events. Besides, the portfolios of the analyzing laboratories often do not contain new psychoactive substances. This results in a high number of unreported cases. Consequently, development of new analytical methods for their determination is one of the pressing tasks of a forensic toxicologist. In fact the interpretation of toxicological data is an important part of any suspicious death investigation involving drugs or poisons. The disposition of a drug includes the processes of absorption, distribution into and out of all tissues, and the metabolism and excretion from the individual [1-6].

Post-mortem toxicological specimens are often seriously influenced by postmortem degradation, redistribution, matrix, temperature etc., therefore, interpretation of the analytical results

becomes difficult. The circumstances of the case, use of therapeutic drugs and autopsy findings are essential for the interpretation, too. This study highlights some pitfalls in forensic toxicological and results related to drugs analysis of autopsy material.

Types of specimen

Depending on the case history and preferences of the submitter; the specimens available for analysis in postmortem cases may be numerous, or limited to blood or a single tissue. Often the limited volumes of blood for analysis are sent to the laboratory for analysis and the collection and preservation of blood has not been done by a professional expert; which is a major drawback. Urine has a great advantage for that it can be obtained non-invasively and high volumes are often available. Unfortunately, all the compounds cannot be identified in urine, because they have been metabolized in the body. In order to analyze the metabolites of a specific compound, the metabolic pathways must be either known or established to assure which structure is of interest. Additionally, metabolites are partly excreted in conjugated form. These conjugates must either be cleaved or included into the analytical procedure. In hair drugs are incorporated and stored for a long time. Hair act like a recorder, it plots the consumption behavior depending on hair length. Therefore, retrospective investigation over months to years is possible but weakness of hair as analytical matrix is the risk of external contamination with drug powder or smoke. A fluid can be

obtained non-invasively like urine, but much more easily and less intimate. Oral fluid testing is also used in controlled trials in other fields, e.g. smoking cessation.

Absorption

Drug absorption is an important process of drug pharmacokinetics [7-9]. The route of administration is an important factor in determining the rate and extent of absorption; these routes have different rates and extents of absorption. Drug absorption is an important process of drug pharmacokinetics. The route of administration is an important factor in determining the rate and extent of absorption. Routes of administration can include oral, rectal, ocular, inhalation through the nose or mouth, absorption through the skin and other body surfaces, and by injection into muscle or veins, etc out of them most of the drugs given orally therefore this route is most important for toxicologist. All of these routes have different rates and extents of absorption. Drugs are usually absorbed either by passive diffusion of the unionized drug or by active transport. Passive diffusion is by far the most common mechanism. Due to large surface area of gastro-intestinal tract, long contact time, high peristalsis and optimal pH results higher absorption rate of most drugs. Tetrahydrocannabinol (THC) from cannabis, cocaine, heroin, drugs such as salbutamol from inhalers and volatile substance abuse absorb mainly by lungs, when they are either smoked or inhaled and some drugs absorb through mucous membranes and by skin. The example of sub-lingual and buccal absorption is vasodilators nitroglycerine and buprenorphine whereas drugs absorbed through skin patches are oestrogens, fentanyl, nicotine, etc. If a drug was distributed instantaneously throughout the body, then the volume of distribution would be constant at all times and the decrease in plasma concentration could be attributed solely to elimination of the drug. However, in practice there are time-dependent changes in tissue concentration, which include absorption and distribution. In a drug overdose, non-linear pharmacokinetics may occur, that is the plasma concentration does not increase in proportion to the dose since one or more of the pharmacokinetic processes reaches saturation. Hence, calculation of dose from the volume of distribution can be substantially wrong and misleading.

Elimination, Clearance and Excretion of Drugs

Most drugs are eliminated from the body by metabolism in the liver and/or by excretion of the drug and its metabolites by the kidneys. Other mechanisms for drug metabolism and excretion also apply for some drugs and poisons. Clearance is the sum total of the elimination process of metabolism, renal excretion and other minor processes. The other major factor that controls the overall ability of an organ to remove drug from the body is the rate of delivery of the drug (i.e. blood flow) to the organ. Drug elimination can be represented as the product of this rate of delivery and the extraction ratio. Renal clearance is often measured with creatinine, which is a metabolic by-product of protein metabolism that is neither reabsorbed nor secreted by the tubules. Its concentration can therefore be used to measure the degree of concentration of

urine from the glomerular filtrate. The diseased kidneys operate less efficiently, and the net change in clearance is proportional to the extent of renal impairment. The concept of clearance has certain limitations for the forensic toxicologist because it does not give an immediate indication of the persistence of a drug in the body e.g. gentamicin and digoxin have similar clearances (about 100mL/min), digoxin stays in the body much longer than gentamicin. This is because the volume of distribution of digoxin is several times that of gentamicin, which requires more fluid for clearance. It is therefore of some advantage to the toxicologist to be able to relate clearance to the persistence of a drug in the body.

Drugs and metabolites are excreted mainly by the kidneys into urine. The rate of excretion of drug in urine depends on the pH. The, acidic drugs like barbiturates, salicylates are excreted more rapidly at high pH than basic drugs (e.g. amfetamines). Conversely, basic drugs are excreted more rapidly at low pH. For example, about 85% of a dose of aspirin is excreted as free salicylic acid in alkaline urine, but only about 5% is excreted when the urine is acidic. Conversely, about 75% of a dose of amphetamine is excreted unchanged in acidic urine, but less than 5% if the urine is alkaline.

Persons who abuse amfetamines have used the effect of urinary pH on excretion to advantage by simultaneously ingesting bicarbonate. This produces alkaline urine, which delays elimination of the amfetamine and therefore prolongs its stimulant effect. Conversely, substances that acidify urine have been taken to enhance the elimination of amfetamine-like stimulants in the hope of avoiding detection in routine dope-screening procedures. Exercise in itself can also decrease urinary pH and thus increase the renal clearance of basic drugs. The quantity of drug in a urine sample is the product of the renal clearance of the drug. Dose from urinary data is not advised because many drugs also show non-linear pharmacokinetics, that is their excretion rate and degree of metabolism are dose dependent.

Drug Metabolism and Active Metabolites

Metabolism is an integral part of drug elimination [10,11]. Metabolites may be pharmacologically inactive or they may be active. This is the case with many drugs of toxicological interest. For example, glucuronidation of morphine on the 6-hydroxyl moiety yields an opioid with more activity than morphine itself. The hydroxylation of THC to the 11-hydroxy form yields an active cannabinoid. Hydroxylation and demethylation of the benzodiazepine diazepam gives the metabolites temazepam and oxazepam, both of which are also available as drugs. Similarly, amitriptyline, a tricyclic antidepressant, is demethylated to yield another antidepressant, nortriptyline. Heroin is deacetylated to 6-acetylmorphine and morphine, both are potent opioids. Active metabolites may also have different modes of action and different potencies; thus dealkylation of the antidepressant drug iproniazid gives the tuberculostatic drug isoniazid, while the anticonvulsants primidone and methylphenobarbital are both metabolised to phenobarbital, another anticonvulsant with a much longer duration of action. Clearly, the formation of active

metabolites changes the profile of drug action. When an active metabolite makes an important contribution to the overall pharmacological response, the interpretation of toxicological data is further complicated. Toxicological situations that involve such metabolites (e.g. oxazepam, nortriptyline, desipramine and phenobarbital, derived from diazepam, amitriptyline, imipramine and methylphenobarbital, respectively) can be misinterpreted if only the parent drugs are assayed.

Drug interactions

In the majority of cases in clinical and forensic toxicology, more than one drug is involved [12-14]. Multi drug therapy and abuse is prevalent and this, together with the added problems of self-medication with over-the-counter drugs and the widespread use of alcohol, makes interpretation of data even more complicated. Drugs with opposite pharmacological activities (e.g. barbiturates and amphetamines) may have an antagonistic effect. Conversely, the additive effects or side-effects of two drugs with the same pharmacological action (e.g. central nervous system depressants) may prove fatal even though the individual drug concentrations are not toxic themselves. Further, a drug with a high affinity for tissue proteins might displace a second drug from binding sites, while a drug that changes urinary pH or that competes for the same active transport system in the proximal tubules of the kidney might inhibit renal excretion.

Changed Physiological State and Pharmacological Response

The role of altered physiological status on drug pharmacokinetics and drug actions plays important role in adverse reactions. Neonates and elderly people generally have a lower metabolic capacity compared with subjects between these extremes of age due to microsomal enzymes. It is important that the known pharmacokinetics of a drug in question be examined when neonates and even children generally, are a focus of an investigation relating to drug effects, since some drugs may behave differently than in adults. In elderly subjects (over 65 years old) there appears to be a decreasing capacity for drug metabolism as a consequence of a gradual decline in overall physiology. This includes effects on volume of distribution, protein binding and both hepatic and renal clearance. The change in the pharmacokinetics is an explanation of the increased sensitivity to drug effects in the elderly. For example, doses of benzodiazepines are reduced in the elderly to avoid excessive sedation and adverse effects on cognition.

Diseases can also affect the processes of drug is absorption, distribution and elimination from the body. A drug may be absorbed poorly during gastro-intestinal disturbance. The rate of uptake of drugs that rapidly cross tissue membranes may be altered in cardiovascular diseases that alter blood flow to critical organs such as the liver, kidney, lungs and heart. Diseases that fundamentally affect metabolic and excretory pathways of drugs also alter their pharmacokinetics. Diseases that affect the liver or kidneys probably

have the greatest effect on drug concentrations because normal functioning of these organs is essential for efficient metabolism and excretion. The liver has a large metabolic reserve. However, severe disease, such as cirrhosis or drug-induced necrosis, causes the pharmacokinetic terminal elimination half-life to increase dramatically. Renal disease leads to a decreased ability to excrete drugs and/or their metabolites. A drug accumulates in the plasma or tissues due to organ disease which causes potentially serious interactions with the accumulated drug or metabolite.

The rate of uptake of drugs that rapidly cross tissue membranes may be altered in cardiovascular diseases that alter blood flow to critical organs such as the liver, kidney, lungs and heart. Incorporation of drugs or chemicals into endogenous metabolic cycles may result in a toxicity (lethal synthesis) that is not related to blood concentrations of the drug. The measured drug concentration in the blood accurately represents the concentration of drug at the receptor site, it must also be established that the clinical response is a primary consequence of the presence of the drug. For example, drugs with an irreversible biochemical effect, such as reserpine and some monoamine oxidase inhibitors still have clinical effects long after drug administration has stopped, and when plasma concentrations of the drug are negligible.

Similarly, unless the time of ingestion is known with reasonable accuracy, it is almost impossible to relate drug concentrations with the secondary and potentially fatal responses to substances such as paracetamol (liver damage) and paraquat (lung necrosis). Incorporation of drugs or chemicals into endogenous metabolic cycles may result in a toxicity (lethal synthesis) that is not related to blood concentrations of the drug. Finally, interpretation is made difficult or impossible when underlying disease alters the pharmacological action of the drug, or when a patient has died from complications associated with inhalation of vomit.

Postmortem Redistribution

The unequal distribution of drugs in tissues leads to changes in the blood concentration of drugs after death. This is called postmortem redistribution and occurs primarily by diffusion of drug from neighboring tissue sites, and organs such as stomach contents. This process is particularly significant for drugs with high lipid solubility, since these drugs tend to show concentration differences in tissues and blood. Blood collected from the heart and other thoracic or abdominal sites may be similarly affected, and should be avoided wherever possible.

Interpretation and Estimating the Dose

The term 'therapeutic' is used in this text as concentrations of drugs normally expected following recommended doses of the substance. Clearly, the term 'therapeutic' has no application for non-drug substances, i.e. illicit drugs and poisons such as organophosphates. Persons often develop a tolerance to drugs with repeated administration compared to their first use; hence some background knowledge on the use of drugs will assist in determining

if this is a likely event. This is relevant in understanding the effects of many opioids, a potentially toxic concentration in a single dose may be easily tolerated with repeated use.

The route of drug administration, together with the nature of the dosage form, determines the rate and extent of absorption. Administration by inhalation, intravenous or intramuscular injection leads to a high bioavailability and quick and often intense response, while oral administration produces lower concentrations of longer duration. Thus, a fatal drug dose given intravenously is often much smaller than a fatal dosing given by mouth because the injected drug is able to reach the site of action very rapidly. A number of factors can influence bioavailability. These include the motility of the stomach and bowel, pH and (for some drugs) activity of gut enzymes that metabolize the drug before it is even absorbed. This issue also applies in situations when coexisting natural disease or injuries may affect the nature of the response to the drug, or when the very young or the elderly are being treated with drugs. Tolerance invariably extends the upper limit of the therapeutic range of drugs, and there is a more marked overlap between concentrations associated with different clinical responses.

When tolerance is suspected, some of the problems of interpreting data can best be resolved by reference to previous results from the same patient (e.g. results of a therapeutic drug monitoring programme). Unfortunately, in most forensic cases such background information is not available, and in these instances blood concentrations alone are of little value. A more reliable interpretation of analytical data can only be made by comparison of blood concentrations with those measured in urine, bile or liver (where concentrations can be much higher in addicts), and/or by measuring the relative amounts of unchanged drug and its metabolites. The estimation of dose is often helpful to confirm other pieces of evidence or to indicate the possibility of an accidental or suicidal death.

Estimating the Time after Administration and Identifying the Route of Administration

The presence of substantial drug relevant to the dosage form probably indicates oral consumption and, if present in the gastric contents, relatively recent ingestion, i.e. a few hours before death. This is a useful test in cases of diamorphine overdose when death has occurred soon after injection (presence of morphine in blood) and little or no morphine is present in urine (<1mg/L of total morphine). This indicates death has occurred within several minutes of injection. It is important to realize that the absence of the drug in urine also indicates that there was no use of this drug in the day or two prior to the most recent dose similarly Cannabis provides a similar example. The detection of Δ^9 -tetrahydrocannabinol in blood (>2ng/mL) indicates very recent use of the drug (within 8h).

Further, the concentrations of 11-nor- Δ^9 -tetrahydrocannabinol-9-carboxylic acid and its glucuronide increase with time, the ratio of the acid to Δ^9 -tetrahydrocannabinol increasing to over 50 after about 3h. This metabolite can be

present in blood for several days, whereas the pharmacological effects only persist for some few hours. If acute use of cannabis is known, then pharmacokinetic modelling can be used to estimate time of ingestion. Care is needed to avoid over-interpreting these data, since coma and certain other physiological states can lead to reduced gut motility which substantially delays gastric emptying time and drug absorption from the bowel. Furthermore, most drugs are excreted into bile and may be present in measurable amounts in gastric and bowel contents even following intravenous injection.

The rate at which a drug reaches its site of action is a critical factor governing the duration and the severity of the pharmacological response [15,16]. When analytical findings are to be interpreted correctly, the route by which a drug is given should, therefore, always be considered. In a case of criminal poisoning it may be essential to establish the route of drug administration in order to corroborate evidence. In some cases, simple facts give a clear indication of the route. The residual drug in the stomach contents or gastro-intestinal tract may point to oral ingestion, a needle mark in the arm indicates intravenous injection, and high concentrations in muscle tissue point to intramuscular injection. However, in the majority of cases it is not reasonably possible to determine with any certainty the route of administration from toxicological data.

Interpretation

An experienced forensic toxicologist is expected not only to provide valid analytical data, but also to assist the investigating officers in relating the findings to a particular case of poisoning. This may be quite straightforward when the presence of a high concentration of a drug or poison is consistent with the patient's symptoms and the circumstantial evidence. In other cases, factors such as the patient's age, sex, health and previous exposure must be taken into account. For example, addicted patients may have developed a tolerance to extremely high concentrations of opiates, benzodiazepines and ethanol, and exhibit relatively mild toxicity. The route of administration (inhalation, oral ingestion, intravenous injection, etc.) can have a very significant effect on the subsequent toxicity, which must also be taken into account when interpreting plasma concentrations. Mixed overdoses of drugs and alcohol are common, and synergistic reactions may confuse the clinical picture. Variable results may be from mistakes in sample collection, for example when blood samples taken from an arm being used to infuse a therapeutic agent may have very high concentrations of that agent because of contamination.

Screening and Detection and Quantification of Drugs and Other Toxins

Postmortem toxicological analysis usually starts with a drug screen. This approach has often been called a search for the 'general unknown'. Targeted testing is sometimes justified where the case history strongly indicates a specific substance is involved, particularly where that substance is not detected by the methods usually employed in the 'general unknown' approach. However,

most experienced toxicologists have encountered instances in which the suspected drug was not found, with an entirely different substance detected in a clearly fatal amount. The forensic toxicology profession and the courts have increasingly demanded that the identification of a substance be beyond reasonable scientific doubt.

The principle has long been established that forensic identification of an analyte requires the use of two techniques that employ different physical and chemical principles. This approach has the advantage that two completely different scientific techniques are used, which is supportive in arriving at a positive result. Therefore, at a minimum, the drug should be detected using two different extracts of the same specimen or two different specimens. This is often accomplished incidentally, because separate extracts may be prepared for the initial drug screen, and for a subsequent quantitative analysis. This is of particular importance in postmortem work when sophisticated hyphenated techniques were used, in which endogenous lipids and putrefactive products can produce significant interference.

Pharmacokinetics

Pharmacokinetics is an invaluable tool to help understand the time course of drugs in the body. In the living, it can be used to determine duration of action, inter-individual differences in peak plasma concentrations and clearance, and the likely effectiveness of different pharmaceutical formulations. However, rarely can pharmacokinetics be applied successfully to postmortem toxicology. Forensic toxicologists have occasionally used analysis of multiple tissue samples from various organs in the body in attempt to overcome the errors inherent in the use of calculations.

Therapeutic and Toxic Concentrations

There is a great temptation for forensic toxicologists and others to refer to tables of therapeutic, toxic and fatal concentrations. While these reference tables may be of some use in clinical toxicology, they are of very limited value for the interpretation of postmortem toxicology results and can be very misleading. The inappropriate use of tables can result in over and under estimation of the potential toxicity of a drug depending on the degree of tolerance developed, natural disease and whether other substances are present. The toxic levels are expressed as a range, which means that the toxic effects may start somewhere in this ranges, depending on the patient and his or her clinical history. It should be taken into account that these values are never static and may change with advancing knowledge or with other (therapeutic) uses of the drug. It is emphasized that these data are intended merely as guidelines and that there is wide individual susceptibility towards the effects of drugs and poisons.

Conclusion

Despite the progress and the increased sensibility and sensitivity in laboratory technology and methods, more often the whole interpretative process of results relies only on pathologist and forensic toxicologist's experience. Actually it should be kept in mind that quantitative data are mostly derived from classical

pharmacology concepts, which are largely invalid, because the pharmacokinetics and pharmacodynamics in living people are quite different from the same parameters in post mortem. To understand this condition forensic toxicologist must be aware of post mortem circulation, wide pH variations, so called reservoir organs and early and late stages of decomposition. The correct approach should include the analysis of the aforementioned circumstantial data, the thoroughly analysis of medical history and, most of all, the keen observation and elaborate report of the medical examiner.

A careful and complete sampling is mandatory, because it is the only way to obtain a lot of information as to complete the "puzzle" to solve the dilemma. So it is better to sample more than less, it is better to collect different tissue samples and body fluids; so that the forensic toxicologists will be able to get more information, even if they are otherwise well aware that data results are to relate to pathologist's own experience and to clinical data.

The relevance of synergy between pathologist and forensic toxicologist emerges from the final discussion and evaluation of the case under study. Some cases are clear cut for the pathologist because the cause and manner of death is straightforward from a pathological point of view, without the presence of significant quantities of substances able to exert a causal effect. Other cases have no problems because the explanations reside in the work of the forensic toxicologist, who finds substances, who explains everything, and the autopsy findings are negative. Finally there are the most difficult cases in which only a deep discussion between forensic toxicologist and pathologist can explain some controversial points. On the basis of this study it can be very well understand that for the forensic toxicologists it is quite challenging to interpret, analyze and quantity of drugs in visceral tissues and these pitfalls affects postmortem toxicological results.

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