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Role of Metabolites in Aviation Forensic Toxicology

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16. Abstract

In aviation accident investigations, specimens from fatal aircraft victims are analyzed for drugs. Their presence indicates exposure to drugs and suggests possible associated medical conditions for which they might have been taken. As drugs are mostly present in therapeutic to subtherapeutic levels in aviation forensic toxicology cases, determination of parent drugs and their metabolites in multispecimens is of significance. Although chemically reactive metabolites are difficult to detect, physiologically active and inactive metabolites can be analyzed. Selective and sensitive techniques are available, but unavailability of metabolite reference standards, endogenous substance interference, and low tissue metabolite levels limit the analyses. However, the majority of primary metabolites can be effectively characterized/quantitated. Demonstrating the presence of drug (e.g., terfenadine, cocaine, THC) metabolites provides a compelling evidence for exposure to the parent drug and facilitates interpretation of results, particularly when the metabolites are active. Such analyses are not as helpful if the metabolites are also available as drugs (e.g., diazepam, temazepam, oxazepam).

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INTRODUCTION

Although drugs are normally taken for medical reasons, exposures to drugs could also be accidental, recreational, suicidal, or even homicidal. Mood altering drugs impair performance and may contribute to accidents, but accidents could also be attributed to medical conditions for which a drug is prescribed. During aeromedical accident investigations, postmortem biological specimens from aircraft victims are analyzed for drugs, volatiles, and primary fire gases (Canfield et al., 1994; White et al., 1992). The presence of drugs in the biological samples indicates exposures to the drugs and suggests possible associated medical conditions for which they might have been taken. Such analytical findings are instrumental in establishing the cause of aviation accidents.

Metabolic studies are essential for evaluating the safety and efficacy of drugs. Such types of studies provide a better understanding of a drug's mode of action, toxicity, and interaction with other medications; they also elucidate the biochemical reaction mechanisms that are involved in the biotransformation and toxicity of drugs (Nelson, 1982). In forensic toxicology, the knowledge of metabolic principles is of significance in making the proper choice of a biological specimen for analysis, in understanding the possible metabolites of a drug, and in interpreting the analytical data for toxicological investigations (Evans and Baselt, 1981). Demonstrating the presence of drug metabolite(s) in postmortem samples becomes *prima facie* evidence for exposure to the parent drug, particularly when those *in vivo* biotransformed metabolic entities are otherwise not available as drugs for common usage. Contrary to general forensic toxicology cases usually encountered in coroner and medical examiner systems, drugs in aviation forensic toxicology cases are mostly found in therapeutic to subtherapeutic levels (White et al., 1992); normal drug pharmacokinetics patterns in the former situations are altered because of the involvement of the toxic/lethal doses of drugs. At therapeutic or subtherapeutic doses, drugs in biological samples are generally present in very low levels, but their primary metabolites might exist in higher concentrations in a suitable sample type. Determination of metabolites becomes more prudent with those drugs which are efficacious at low doses/concentrations and/or undergo extensive first pass biotransformation (diltiazem, terfenadine, and triazolobenzodiazepines). The relative low drug concentrations usually experienced in aviation cases warrant the need for identification and determination of possible drug metabolites. Such qualitative and quantitative analyses of metabolites, along with parent drugs, in multispecimens, further facilitate aeromedical accident investigation and authenticate its conclusion.

DRUG METABOLISM

Once foreign chemicals enter the body by ingestion, injection, inhalation, and/or absorption through skin, they are eliminated into the urine, feces, bile, perspiration, vomitus, milk, hair, and/or expired air. The elimination largely depends upon their hydrophilicity; lipophilic compounds have a tendency to accumulate in adipose tissues. There is a variety of enzymes in the body that metabolize xenobiotics into more water soluble forms, thereby enhancing their elimination and diminishing their biological response (Zimmerman, 1978). The enzymes responsible for metabolizing foreign compounds are primarily found in the liver. However, metabolism in other tissues, such as brain, kidneys, and intestine, also occurs, but to a lesser degree than in the liver. Metabolism is a biological process that alters the chemical structure of parent drugs; there may be one or more metabolites of a drug, and drug metabolites may further be biotransformed into other chemical entities. It is not always true that all metabolites are biologically inactive. Some drugs are biotransformed into active forms, such as primidone to phenobarbital, flurazepam to N-desalkylflurazepam,

and codeine to morphine, exhibiting higher activity than the respective parent drugs; others are at least as active as, or less active than, the parent drugs (diazepam to nordiazepam and temazepam, and then to oxazepam; fluoxetine to norfluoxetine; amitriptyline to nortriptyline). On the other hand, drug metabolites are also physiologically inactive, but some of them may be chemically reactive, e.g., N-acetyl-*p*-benzoquinone imine (NAPQI), imminium ions, arene oxide and aliphatic epoxide intermediates, and free radicals; reactive metabolites have a strong potential to chemically react with macromolecules causing cell death and tumorigenesis. Benzoylecgonine of cocaine, azacyclonol of terfenadine, and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (11-nor-9-carboxy-Δ^9-THC) of Δ^9-THC are examples of physiologically inactive metabolites; they are not considered reactive metabolites.

The enzyme systems responsible for metabolism of foreign compounds are integral parts of the smooth endoplasmic reticulum. The reactions catalyzed by these enzyme systems can be classified into Phase I and Phase II (Williams, 1959). Phase I reactions consist of oxidation, reduction, and hydrolysis, whereas Phase II reactions include conjugations of molecules containing suitable polar groups with endogenous groups or substances, such as glucuronidation, acetylation, glycine, sulfate and glutathione (GSH) conjugations, and transsulfuration. The oxidation reaction is the major pathway for biotransformation of xenobiotics. Almost all xenobiotic oxidations are carried out by the enzymatic machinery mixed function oxygenases system (MFO), also referred to as the cytochrome P-450 containing monooxygenases. During the oxidation reaction, equivalent amounts of O_2, NADPH (nicotinamide adenine dinucleotide phosphate - reduced form), and a drug are utilized (Gillitte and Jollow, 1974; Neal, 1980). There are several agents that can alter the MFO activity, and thereby the metabolism of an agent. Phenobarbital, ethanol, 3-methylcholanthrene, DDT, toxaphene, and 1-phenylcyclohexene (PC) are examples of MFO inducers, while SKF-525A, piperonyl butoxide, cobaltous chloride, CCl_4, and α-naphthylisothiocyanate are of MFO inhibitors. Effects of these agents on the MFO are generally studied by *in vitro* metabolism of drugs/chemicals, like phenacetin, aniline, amidopyrine, and benzo[*a*]pyrene; and by the determination of hepatic cytochrome P-450 levels (Abe and Watanabe, 1982; Chaturvedi and Kuntz, 1988; Chaturvedi et al., 1991; Omura and Sato, 1964a; 1964b; Overton et al., 1985). In experimental animals, alterations in the MFO activity can be measured by changes in the sleep time induced by the short acting barbiturate pentobarbital (Chaturvedi, 1993); the barbiturate is primarily metabolized by the MFO, and its metabolites are weaker in action. The induction in the MFO activity is further supported by the increase in liver/body weight ratio and proliferation, along with dilatation and fragmentation of the endoplasmic reticulum in the liver (Hu et al., 1984; Kuntz et al., 1990). Liver pathological conditions also alter biotransformation of drugs; drug metabolism decreases during hepatic necrosis and cirrhosis, while it increases during the active regeneration of hepatic cells following a toxicant-caused liver injury (Sipes and Gandolfi, 1991).

Chemical structure of a drug plays a key part in biotransformation. A slight change in drug molecules may alter their metabolism—for example, esters are hydrolyzed at a faster rate than the corresponding amides. Procaine, cocaine, and pentobarbital are quickly metabolized in the body. The ester procaine has a lower half-life than the amide procainamide. Determination of benzoylecgonine in the urine will be more feasible than the ester cocaine, since cocaine is extensively biotransformed and excreted unchanged only up to 9% in the urine (Baselt and Cravey, 1989). Different to the termination of pentobarbital action through its hepatic metabolism, effects of barbital are predominantly terminated by its excretion through the kidneys; barbital is excreted in the urine unchanged.

Lipophilic compounds are present in very low concentrations in the urine. The lipophilic Δ^9-THC is metabolized to the hydrophilic 11-nor-9-carboxy-Δ^9-THC via 11-hydroxy-Δ^9-THC, which, like the parent compound, is short-lived in the body. Therefore, the presence of 11-nor-9-carboxy-Δ^9-THC in a suitable sample would indicate a possible abuse of marihuana (Foltz et al., 1980). It is known that

phencyclidine (PCP) is an abused drug (Petersen and Stillman, 1978), and one of its major routes of administration is smoking (Henderson, 1982). During smoking, about 50% PCP pyrolizes into PC (Freeman and Martin, 1981). Therefore, the presence of PCP and PC and their metabolites will suggest the PCP abuse by smoking, as PCP does not metabolize into PC.

DISCUSSION

Demonstrating the presence of drug metabolites in suitable biological samples facilitates toxicological investigations, since this acts as convincing evidence for exposures to the parent drugs. The levels of the parent compound and its active metabolites help in establishing the extent of intoxication, as well. Finding the relative concentration of the parent drug and metabolite can assist in determining the approximate time of parent drug exposure, as demonstrated with Δ^9-THC and 11-nor-9-carboxy-Δ^9-THC (Huestis et al., 1992). Levels of both amitriptyline and nortriptyline, codeine and morphine, ephedrine and phenylpropanolamine, imipramine and desipramine, isopropyl alcohol and acetone, methamphetamine and amphetamine, phenacetin and acetaminophen, or propoxyphene and norpropoxyphene will be indicative of the possible exposure to the respective parent compound. Since metabolites may also be available as medications, their presence may not necessarily suggest exposures to the corresponding parent drugs only; it could also imply exposures to the parent drug and/or the metabolite drug. In spite of this, their concentrations in specific bio-samples will be of significance in establishing the degree of drug intoxication. Certainly, if metabolites–for example, benzoylecgonine of cocaine, azacyclonol of terfenadine, and 11-nor-9-carboxy-Δ^9-THC of Δ^9-THC–are not available as drugs for common usage, exhibiting the metabolite presence in the samples will support exposures to the parent drugs because the metabolites were formed in the body. In the presence of ethanol, the cocaine metabolism is altered leading to the bio-formation of cocaethylene, a pharmacologically active cocaine analog (Bailey, 1994; Dean et al., 1992). Therefore, the presence of cocaine, cocaethylene, and benzoylecgonine along with ethanol would suggest exposure to the stimulant cocaine and ingestion of ethanol; this information will be crucial in forensic accident investigations.

There are some limitations for analysis of metabolites. Major metabolites of drugs are not always feasible to characterize because reference standards are not easily available; the possibility to detect or identify minor metabolites is hardly high. However, detection and characterization of metabolites can effectively be achieved by applying the basic principles of biochemical metabolic reactions and using sensitive and selective analytical techniques. Gas liquid chromatography/mass spectrometry and capillary gas liquid chromatography utilizing flame ionization, nitrogen phosphorus, electron capture, Fourier transform infrared, and atomic emission detectors have been instrumental in the identification and/or quantification of drugs and their metabolites. Derivatization of extracted metabolites is recommended for gas-chromatographic analyses, but sometimes, interferences are reported because of endogenous materials. High pressure liquid chromatography equipped with mass spectrometry has been found to be suitable in the identification of metabolites; this technique has been used for studying metabolism of drugs, particularly those of high relative molecular masses and polar metabolites (Skellern, 1983). Preparative liquid chromatographic methods in drug metabolism studies have been reported (Dieterle and Faigle, 1983). Radioimmunoassays and/or enzyme immunoassays for various drugs, such as Δ^9-THC and diazepam, have routinely been utilized in forensic laboratories. Although lacking specificity and precision, these assays do offer an advantage over other methods. The advantage is due to the cross reactivity between the parent compound (e.g., Δ^9-THC) and its metabolites and related analogs (other cannabinoids; Petersen, 1980). The cross reactivity is a clear-cut indication for the presence of the parent drug and/or structurally related molecule(s) in the sample.

Although isolation of conjugate metabolites of drugs establishes their metabolic routes, it also demonstrates exposures to the parent drug in forensic toxicology situations. Acid or enzymatic hydrolysis of conjugates improves the yield of a parent drug and/or its Phase I metabolites. Therefore, the detection becomes easier. Protein precipitants are used with tissue homogenates. Some precipitants react with chemical entities in question, thus interfering with assay procedures. Consequently, precipitants should be carefully selected. Do levels of a drug and its metabolites after the hydrolysis or deproteinization really correlate with the true drug toxicity or the drug-induced impairment? Such levels could be misleading, as only the free drug contributes to a pharmacological response; bound or conjugated drugs and metabolites are generally considered ineffective. Regardless, their presence would indicate possible exposure to the drug and potentially incapacitating medical conditions. Some analytical procedures do not distinguish metabolites from the parent drugs. The UV spectrophotometric method for propoxyphene (McBay, 1978; McBay et al., 1974) determines both propoxyphene and its metabolite norpropoxyphene (Finkle et al., 1976), and it does not distinguish propoxyphene from norpropoxyphene. Thus, the observed levels reflect the inclusion of the metabolite giving an aggregate concentration value for propoxyphene (Chaturvedi et al., 1983a). In multi-drug (or chemical) deaths, the levels of those drugs should be carefully interpreted, as there may be interaction among the drugs, leading to the rapid onset of toxicity of a greater intensity (Chaturvedi et al., 1983a; 1983b).

Reactive metabolites irreversibly bind to macromolecules, causing permanent cell toxicity. In the acetaminophen toxicity, the reactive intermediate NAPQI is primarily responsible for the liver necrosis. This intermediate undergoes electrophilic reaction with GSH, a detoxification route. Induction or inhibition of the cytochrome P-450 system should influence the acetaminophen toxicity, but this is not always true. Phenobarbital, an inducer of the P-450 system, increases the toxicity of acetaminophen in mice, but decreases its toxicity in hamsters. In hamsters, phenobarbital induces glucuronyltransferase, as well. Inhibition of the P-450 system decreases the toxicity in both species. On the basis of the metabolic principles, two general approaches can be used to decrease the toxic reactions. These approaches are: (i) molecular modification to block toxic metabolite formation and (ii) coadministration of other agents, either to promote the formation of nontoxic metabolites, or to decrease reactive metabolite toxicity. N-Methylation of acetaminophen lowers the formation of the reactive metabolite *p*-quinone imine, but decreases the analgesic activity and increases convulsant activity (Nelson, 1982); introduction of the N-acetyl moiety in procainamide lowers its toxicity. The effective use of SH-containing compounds to treat acetaminophen overdose has been documented. Administration of cysteine to maintain adequate levels of GSH and of sodium sulfate to maintain the sulfation reaction has been effective against the acetaminophen toxicity.

Overall, the extensive knowledge of a drug's metabolism facilitates predicting its effects on biological systems and correlating those effects with the amounts of the drug and/or its metabolites present in the target tissue(s).

SUMMARY

Foreign compounds are primarily biotransformed in the body by the microsomal drug metabolizing enzyme systems. Metabolites could be active, inactive, and/or reactive. Most of the time, metabolites are more polar than the parent compounds and are thus more easily eliminated. Identification and quantification of the metabolites are integral parts of forensic toxicology. In some cases, the concentrations of metabolites can be correlated with the intoxication of a drug. Several selective and sensitive techniques are available for the analysis of metabolites. Lack of metabolite reference standards, interference with endogenous substances, and low levels of metabolites in biological tissues/fluids may pose difficulty in metabolite analyses. However, proper modifications of the analytical procedures, utilization of sophisticated instrumental techniques, and application of biochemical metabolic principles

have been successful in the identification and quantitation of metabolites. Knowledge of drug metabolism can assist in developing safe drugs and in recommending proper treatments in the event of possible intoxication. Additionally, demonstrating the presence of metabolites, including parent drugs, provides compelling evidence for exposure to the parent drug and facilitates interpretation of toxicological findings and accident investigation.

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