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COMMENTARY

COCAINE-MEDIATED HEPATOTOXICITY

A CRITICAL REVIEW

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In recent years, cocaine use in this country has reached epidemic proportions and, as a society, we are beginning to realize the social, economic, and medical impact resulting from the abuse of this drug [1]. Its popularity in humans stems from its rapid CNS effects which include a sense of well-being and euphoria. Thus, cocaine has been extensively studied psychopharmacologically.

A report by Marks and Chapple [2] suggests that cocaine may cause hepatotoxicity in humans; they reported that chronic abusers of both heroin and cocaine were generally found to have high levels of serum transaminases, and some exhibited jaundice. This new and potentially serious health finding has served to catalyze much interest in the area of cocaine-mediated hepatotoxicity, not only in the elucidation of the role that cocaine plays in mediating this liver damage in the heroin–cocaine users, but also in the determination of whether cocaine alone actually induces hepatotoxicity.

In this article, we will review the studies related to cocaine-mediated hepatotoxicity with special emphasis on mechanistic investigations, and we will use these reports to suggest new and possible fertile areas of research.

History and use of cocaine

Although coca leaves were not introduced into Europe until the 1500's, it is known that the Peruvian Indians have chewed coca leaves as a CNS stimulant since pre-Columbian times [3]. In 1855, Gardeke became the first to extract the active ingredient of coca leaves and, five years later, Niemann isolated the alkaloid, characterized it chemically, and named it "cocaine" [3]. Its structure was first elucidated by Willstatter *et al.* [4] who, in addition to those initial investigations, synthesized cocaine free from other stereoisomers. The configuration of cocaine has been determined more recently to be (−)-2β-carbomethoxy-3β-benzoyloxytropane [5, 6]. Von Anrep first studied the pharmacological effects of cocaine in 1880. Aschenbrandt and Freud later described the effect of cocaine on the CNS and cited its ability to produce euphoria and to decrease fatigue. In 1884, Koller discovered its unique local anesthetic and vasoconstrictive properties, and cocaine soon after became widely accepted as a local and regional anesthetic. In addition, cocaine became the first effective drug for the treatment of nasal congestion due to seasonal allergies. By the end of the nineteenth and twentieth centuries, cocaine had attained great popularity, was contained in many proprietary medications, and was advocated as a cure for opium, morphine, and alcohol addiction [3].

As cocaine use increased, society became alarmed over its addictive effects, toxic potential, and variable effects on human behavior. Because of this concern, governments began to restrict the use of cocaine as early as 1897; by the early 1900's, it was subject to strict regulation [7], making all but restricted medical use of cocaine illegal.

The combination of renewed affluence and interest in drug use in the late 1960's and early 1970's, however, drastically altered the image of cocaine; cocaine soon became known as a "status" drug. This reputation of cocaine as a "drug of the elite" has served to increase its popularity with respect to illicit drug use in all circles of society since the mid-1970's. In marked contrast to many other drugs of abuse, the use of cocaine has been increasing steadily from that time to the present [1, 7].

Pharmacology of cocaine

Cocaine is a drug with actions in both the peripheral and central nervous systems. It is both a local anesthetic and a sympathomimetic agent. Cocaine, as a local anesthetic, produces conduction block without depolarizing the nerve membrane at low concentrations [8]. It diffuses through the membrane in its less prevalent non-ionized form; once inside the cell, it is protonated to an ionized species. It is this ionized species that binds to a "receptor" site on the inner surface of the membrane. It is generally accepted that cocaine competes with calcium at the site that controls membrane permeability [9, 10].

The most widely accepted theory of the sympathomimetic action of cocaine is that it blocks re-uptake of norepinephrine into the sympathomimetic nerve terminal and that, in doing so, cocaine allows higher concentrations of norepinephrine to interact with the physiological receptor site. Studies have demonstrated that cocaine inhibits the uptake of norepinephrine and epinephrine in several tissues and causes the elevation of blood levels of norepinephrine [3].

In the periphery, the effect of cocaine is generally

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a combination of its local anesthetic action and its sympathomimetic action. Cocaine application topically to the eye causes mydriasis [11] and constriction of blood vessels [12]; in the heart, cocaine elicits both positive and negative inotropy and chronotropy [3].

The mechanism of cocaine's action in the CNS remains unclear. Cocaine has been shown to inhibit the re-uptake of dopamine, serotonin, and tyramine in the CNS [3]. In small doses, cocaine is a CNS stimulant, causing increased activity and respiratory rate. However, larger doses lead to medullary depression and death from respiratory depression or cardiac arrest.

Metabolism of cocaine

Cocaine is metabolized in humans by two distinct pathways. The first, which accounts for better than 90% of cocaine's biotransformation, involves various hydrolytic reactions. The second route is an oxidative process centered around the tropane nitrogen (Fig. 1).

Chronologically, the hydrolytic pathway was discovered first. Surprisingly, very little was known about the metabolism of cocaine until 1969 when Fish and Wilson [13] isolated benzoylecgonine from human urine after intramuscular administration of cocaine. Stewart *et al.* [14] later discovered that benzoylecgonine can be produced merely by chemical hydrolysis as opposed to enzymatic processes. Nevertheless, hydrolytic enzymatic reactions involving cocaine do take place. Serum pseudocholinesterase rapidly hydrolyzes cocaine to give ecgonine methyl ester and benzoic acid [15, 16]. More recent studies have confirmed this enzymatic reaction and have found that esterases located in the liver also catalyze this biotransformation [14].

Although the various hydrolytic pathways inactivate cocaine pharmacologically, the minor oxidative route appears to be responsible for the hepatotoxicity of this drug. Leighty and Fentiman [17], Nayak *et al.* [18], and Misra *et al.* [19] have documented extensively the oxidative pathway for cocaine biotransformation. From these investigations, it is now established that cocaine can be demethylated, or N-oxidized followed by demethylation, to give norcocaine. Once norcocaine is generated, it is then rapidly oxidized to *N*-hydroxynorcocaine [18, 19]. The fate of this metabolite was unknown until recently. Investigations have revealed that *N*-hydroxynorcocaine can be further metabolized producing norcocaine nitroxide [20, 21]. Most investigators now believe that this free radical is ultimately responsible for the hepatotoxicity elicited by cocaine.

Hepatotoxicity of cocaine

Clinically, the first observation that cocaine might mediate hepatic damage was noted by Marks and Chapple [2]. These investigators demonstrated that a group of human cocaine and heroin users exhibited elevated serum transaminase levels and, in some cases, exhibited jaundice. While these data did not definitely determine hepatotoxicity resulting from cocaine usage alone, this work nevertheless served to stimulate animal research in the area of cocaine-mediated hepatotoxicity.

The observations of Shuster *et al.* [22] and Evans *et al.* [23] clearly implicated cocaine as a potent hepatotoxin via the intraperitoneal route when administered both acutely and chronically. These investigators have reported that cocaine administration causes severe liver damage in the form of fatty infiltration, midzonal and periportal necrosis, and marked elevation of serum glutamic-oxaloacetic transaminase (SGOT) levels in mice—quite similar hepatotoxic manifestations to those reportedly produced by carbon tetrachloride [24]. Until recently, published investigations had failed to demonstrate acute cocaine-mediated hepatic injury in mice without prior induction of the cytochrome P-450 system. Recently, however, this phenomenon has also been characterized in non-induced mice [25] in the form of dose- and time-dependent decreases in several important hepatic enzymes.

The work of Evans and Harbison [26] and Evans *et al.* [23] first suggested that a metabolite of cocaine, rather than cocaine itself, was responsible for the hepatotoxicity, from data using cytochrome P-450 inducing agents. These investigators suggested that the active metabolite of cocaine might be an N-oxide intermediate that was formed during the initial dealkylation of cocaine to norcocaine. Subsequent data later indicated, however, that the actual hepatotoxin was a further metabolite of norcocaine oxidation [27].

More recent work by Thompson *et al.* [28] sug-

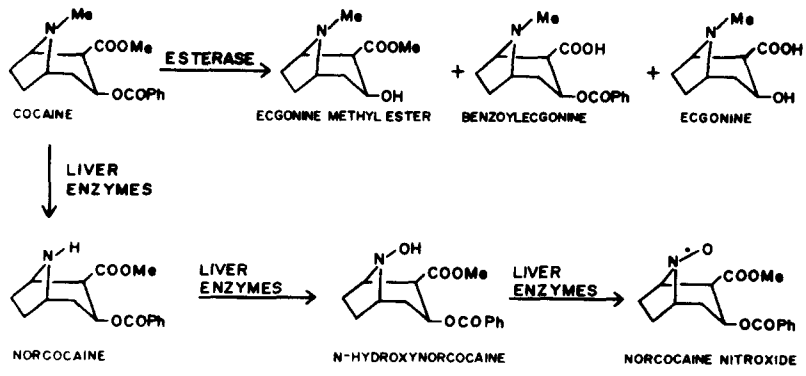

Fig. 1. Proposed scheme of cocaine hydrolytic and oxidative metabolism.

gested that the bioactivation of cocaine to a more hepatotoxic metabolite is carried out by the cytochrome P-450 monooxygenase system and confirmed data implicating a norcocaine metabolite as the reactive species. When tested for their abilities to elevate SGOT levels, hydrolytic metabolites of cocaine were found not to be hepatotoxic; both norcocaine and *N*-hydroxynorcocaine, however, were shown to produce marked hepatotoxicity. Furthermore, cytochrome P-450 inhibitors such as SKF-525A blocked this liver damage, while inducing agents greatly enhanced the hepatotoxicity from cocaine, norcocaine, and *N*-hydroxynorcocaine administration. Thus, it was concluded that the cytochrome P-450 system was responsible for all metabolic conversions of each of the three compounds. Unfortunately, this study chose to measure drug metabolism only in terms of hepatotoxic manifestations, as opposed to the actual isolation of metabolites. It may therefore be incorrect to conclude that only one oxidative enzyme system catalyzes all the reactions based merely on data derived from the end result of complete metabolism. A more realistic inference from the data is that a further metabolite of *N*-hydroxynorcocaine is responsible for the observed hepatotoxicity and that the cytochrome P-450 system participates in at least one metabolic conversion of the multi-step bioactivation of cocaine.

Indeed, recent studies on the elucidation of enzymes involved in cocaine bioactivation have demonstrated that both cytochrome P-450 and FAD-containing monooxygenase catalyze steps in the oxidation of cocaine [29–31]. The initial dealkylation of cocaine to norcocaine was shown to be accomplished by two alternate pathways: one involving direct cytochrome P-450 oxidation and the other being catalyzed sequentially by both FAD-containing monooxygenase and cytochrome P-450 via a cocaine N-oxide intermediate. The N-hydroxylation of norcocaine to produce *N*-hydroxynorcocaine was found to be catalyzed by FAD-containing monooxygenase, independent of cytochrome P-450. The final step in cocaine oxidation, the one-electron oxidation of *N*-hydroxynorcocaine to produce norcocaine nitroxide, was demonstrated to be a cytochrome P-450-mediated reaction. In these same studies, it was determined that the back (reductive) reaction producing the hydroxylamine from the nitroxide occurs *in vitro* and is mediated by flavin-containing enzymes and reduced pyridine nucleotides. Superoxide was shown to be generated during the reductive process (Fig. 2).

Several investigators have reported that cocaine administration to mice results in the depletion of hepatic reduced glutathione [25, 28]. In the same studies, the enhancement of intracellular glutathione levels by pretreatment with cysteine was shown to protect the liver against cocaine-induced hepatotoxicity in phenobarbital-induced and non-induced mice. Similarly, depletion of intracellular glutathione content by pretreatment with diethyl maleate was also shown to potentiate the hepatotoxicity.

Current thought concerning the actual identity of the hepatotoxic cocaine metabolite generally centers around the formation of a reactive nitroxide. It is known that the oxidation of norcocaine *in vitro* at ambient temperatures with hydrogen peroxide produces a stable nitroxide radical [32]. Using a microsomal preparation, it has also been shown that an EPR-detectable norcocaine nitroxide signal is produced from *N*-hydroxynorcocaine oxidation [20, 31]. Evans [20] has attempted to correlate the formation of this nitroxide with the covalent binding of radiolabeled cocaine to cellular proteins. Unfortunately, the position of the radiolabel was on the methyl group of the cocaine molecule and was subject to extensive *in vivo* hydrolysis under normal conditions. Furthermore, nitroxide radicals are known to be relatively unreactive chemically. More recently, it has been demonstrated that the free radical of norcocaine nitroxide is unreactive with either microsomal or cytosolic proteins [31]. This observation eliminates the possibility that covalent binding of cocaine to microsomal proteins occurs by simple reaction of the nitroxide moiety with proteins.

In the case of cocaine-mediated hepatotoxicity, it is tempting to ascribe the observed depletion of hepatic glutathione to the direct reaction of reduced glutathione (GSH) with norcocaine nitroxide. However, purified norcocaine nitroxide has been shown not to be reactive with either reduced glutathione or cysteine [31]. Thus, the *in vivo* depletion of hepatic reduced glutathione after cocaine administration is not due to a direct reaction of the nitroxide with glutathione.

As stated earlier, it has been demonstrated that superoxide radical is generated during the reduction of norcocaine nitroxide to *N*-hydroxynorcocaine. The generation of this radical species suggests that

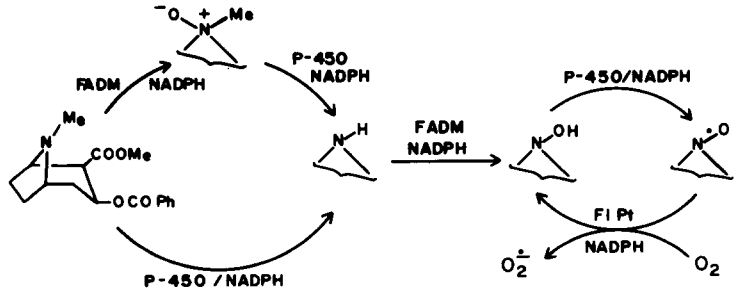

Fig. 2. Metabolic scheme showing enzymes postulated to be involved in the oxidation of cocaine to norcocaine nitroxide.

lipid peroxidation may be stimulated *in vitro* and *in vivo* during the metabolism of cocaine. Recent studies have determined that cocaine and its three oxidative metabolites stimulate microsomal lipid peroxidation *in vitro*, as analyzed by the formation of thiobarbituric acid-reactive products [33]. There was found to be a direct relationship between the ability to stimulate *in vitro* peroxidation and the extent of cocaine metabolism; norcocaine nitroxide stimulated the greatest peroxidation, followed by the hydroxylamine, norcocaine, and, finally, cocaine. This same study demonstrated that an acute injection of cocaine to non-induced mice resulted in enhanced *in vivo* microsomal lipid peroxidation several hours after cocaine challenge. Similarly, it has been shown, using EPR spin-trapping techniques, that lipid peroxyl radicals are formed upon the incubation of *N*-hydroxynorcocaine and reducing equivalents with hepatic microsomal suspensions [34]. The formation of these lipid peroxyl radicals was retarded when dialyzed cytosol was added to the above incubation mixture; the formation was further retarded when both reduced glutathione and dialyzed cytosol were added.

The results of the studies to date allow us to speculate on the mechanism of cocaine-mediated hepatic damage. We propose that the cocaine-induced loss of hepatic reduced glutathione is primarily a result of NADPH depletion caused by utilization of NADPH and by the production of hydrogen peroxide during cocaine oxidative metabolism. It is suggested (Fig. 3) that a futile redox cycle is set up between the oxidation of *N*-hydroxynorcocaine to norcocaine nitroxide and the reduction of norcocaine nitroxide to *N*-hydroxynorcocaine, both at the expense of NADPH. This cycle results in the loss of cellular NADPH and the production of hydrogen peroxide (produced from the cytochrome P-450-mediated oxidation of *N*-hydroxynorcocaine and from the dismutation of superoxide generated as a by-product of norcocaine nitroxide reduction). Hydrogen peroxide is reduced by glutathione peroxidase which concomitantly converts reduced glutathione to oxidized glutathione. The back reduction of oxidized glutathione by glutathione reductase, it is postulated, is partially blocked because levels of NADPH, a necessary cofactor for the enzyme, are reduced by the futile redox cycle. As a result, there is a build-up of oxidized glutathione (GSSG) which is then actively excreted by the cell in order to maintain its GSH:GSSG ratio in a "normal" range [35, 36]. The compromised glutathione reductase function, along with the active excretion of oxidized glutathione, accounts for the observed cocaine-induced glutathione depletion. This decrease in reduced glutathione then leads to decreased glutathione peroxidase activity, since GSH is the sole reductant for the enzyme. As a result, both hydrogen peroxide and superoxide may serve to stimulate lipid peroxidation. With lipid peroxidation damaging cellular membranes, many hepatic enzyme activities, especially those dependent on membrane integrity for proper functioning, are decreased and cellular death is inevitable.

Future directions

If one accepts the current mechanism suggested for cocaine-mediated hepatotoxicity (i.e. critical loss of hepatic reducing equivalents), it becomes obvious that certain sectors of the human population may be particularly susceptible to the hepatotoxic effects of cocaine due to their genetic makeup. Of special interest is the genetically-determined group of individuals who are heterozygous for the gene controlling the enzyme glucose-6-phosphate dehydrogenase. These heterozygous individuals possess decreased activities of glucose-6-phosphate dehydrogenase and may, therefore, generate fewer reducing equivalents (NADPH) as compared to normal individuals. Clinically, the red blood cells of these people are seen to exhibit enhanced frequency of hemolytic anemia in the presence of peroxidizing agents [37]. It is possible that this reduced level of NADPH may also render the livers of these individuals particularly sensitive to hepatic peroxidative damage from cocaine.

Since the current data implicate the oxidative route of cocaine metabolism in the production of cocaine-induced hepatotoxicity, it may also be that those individuals with decreased plasma pseudocholinesterase activity [38] are especially susceptible to the hepatotoxic effects of cocaine. Decreased hydrolysis or detoxification of cocaine might then result in a shunting of more cocaine through the oxidative bioactivation pathway. Presumably, this enhanced oxidation of cocaine would result in the generation of greater amounts of hepatotoxic metabolite, and greater resulting hepatic damage from cocaine. Experimentally, a similar situation was produced in mice by the administration of the esterase inhibitor diazinon which enhanced cocaine hepatotoxicity [28]. Future work in these areas of

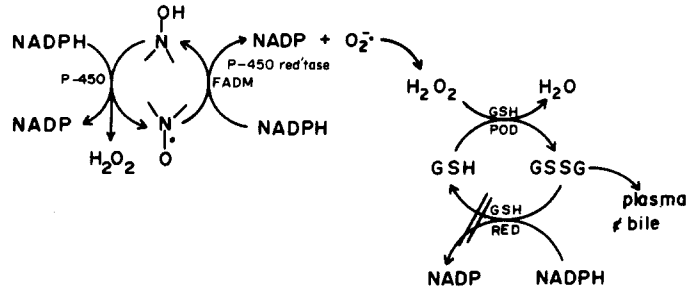

Fig. 3. Proposed mechanism for cocaine-mediated hepatotoxicity.

genetically-determined enzyme deficiencies would serve to further define the mechanism of cocaine-mediated hepatotoxicity as well as to determine "high-risk" groups within the human population.

It is known that the psychopharmacological actions of cocaine can lead to its physical dependence; however, until the studies by Misra *et al.* [39], the metabolic profile of cocaine in the central nervous system was unknown. It was found that, in the rat, cocaine is not only hydrolyzed to benzoylecgonine and ecgonine, but is also biotransformed to norcocaine and benzoylnorecgonine. Given that this latter pathway has been demonstrated to be the metabolic route responsible for cocaine-mediated hepatotoxicity and that FAD-containing monooxygenase is known to be present in brain tissue (D. M. Ziegler, personal communication, 1983), it becomes important to determine if the oxidative biotransformation of cocaine contributes to the known CNS toxicity of cocaine.

Nasal administration of cocaine appears to be the most popular route of administration, yet little is known about the biotransformation of cocaine in the nasal mucosa. Cytochrome P-450 and FAD-containing monooxygenase are known to be located in the nasal mucosa and have been shown to generate formaldehyde during the oxidation of at least one drug, dimethylaniline [40]. Nasal mucosal damage from cocaine may, in part, be due to the production of formaldehyde, as the result of cocaine N-demethylation by cytochrome P-450. Verification of such a hypothesis remains to be demonstrated.

The mechanism proposed for cocaine-mediated hepatotoxicity is an elaborate scheme involving the production of superoxide, hydrogen peroxide, and lipid peroxidation. However, this theory has yet to be independently verified; additional research into this and other potential mechanisms must be conducted for the complete study of cocaine-mediated hepatotoxicity.

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