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REVIEW

Genetic toxicology of abused drugs: a brief review

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Although numerous studies have been conducted on abused drugs, most focus on the problems of addiction (dependence) and their neurotoxicities. Now accumulated data have demonstrated that the genotoxicity and/or carcinogenicity of abused drugs can also be detrimental to our health. In this review, commonly abused substances, including LSD, opiates (diacetylmorphine, morphine, opium and codeine), cocaine, cannabis, betel quid and khat, are discussed for their potential genotoxicity/carcinogenicity. The available literature in the field, although not as abundant as for neurotoxicity, clearly indicates the capability of abused drugs to induce genotoxicity.

Introduction

Drugs or substances with abuse potential usually possess the property of physical and/or psychological dependence (addiction). Individuals who abuse these drugs or substances, through positive (euphoric) and negative (withdrawal) reinforcement, often enter a miserable relapse cycle (Figure 1). Drug abuse not only undermines an individual's health, but also results in major medical expense and social problems. Because of their profound social impact, the mechanisms underlying drug abuse and dependence, with a major interest in the neurotoxic and behavioral effects, have been the research focus for decades (Brown *et al.*, 1985; Szara, 1986; Wetherington *et al.*, 1996). In recent years, the spread of infectious diseases, such as acquired immunodeficiency syndrome (AIDS) and hepatitis (B and C), through needle sharing has also been a major concern (Haverkos, 1988). In the USA, more than half of the 40 388 new human immunodeficiency virus (HIV) infections were drug related (Centers for Disease Control and Prevention, 1995).

The etiology of drug abuse/dependence is a complex interplay of psychological and biological factors. While neurotoxicity and psychiatric disorders will continue to be the main themes of drug abuse research, genetic vulnerability to drug abuse has gradually emerged as a new field of study. One aspect of this new field, conducted by family, twin and adoptee studies, is to demonstrate the likelihood of a genetic contribution underlying drug abuse risks. Further genetic loading studies have indicated that adults with drug abuse potential have a high frequency of other co-morbid psychiatric diagnoses, such as alcoholism, attention decifit/hyperactivity disorder, Tourette syndrome, antisocial personality disorder, depression, panic attacks and anxiety disorders (reviewed by Comings, 1996). The other aspect, emerging with the development of molecular medicine, is to explore the potential genotoxicity and/or carcinogenicity associated with abused drugs/substances. Many techniques, such as the *Salmonella typhimurium* (Ames) mutation assay, mammalian cell *hprt* mutation assay and sister chromatid exchange (SCE), micronuclei and DNA repair assays, have been used to detect the genotoxicity of abused drugs. Here we review the data on abused drug-induced genotoxicity and carcinogenicity. The term 'abused drug' used in this review refers to some well-known addictive drugs/substances, either licit or illicit, that have been commonly abused and associated with genotoxicity or carcinogenicity. However, the genotoxicity and carcinogenicity of tobacco and alcohol will not be included in the text because their genotoxicities/carcinogenicities are well established and have been evaluated in detail elsewhere (IARC, 1986, 1988).

Early genotoxicity studies: the LSD issue

D-Lysergic acid diethylamide (LSD or LSD-25) was first synthesized by Albert Hoffman at Sandoz Laboratories in 1938. He isolated lysergic acid from ergot (*Claviceps purpurea*) in trying to make a chemical agent that would act as a circulatory stimulant. LSD was later found to have little medical benefit and was banned because of its hallucinogenic effect (reviewed in Ulrich and Patten, 1991). Besides ergot, another source for the clandestine production of LSD is the seeds of morning glory plant (Hoffer, 1964; Smith, 1981).

Two early reports on LSD, showing its ability to cause chromosome aberrations *in vitro* (cultured human leukocytes; Cohen *et al.*, 1967) and *in vivo* (leukocytes from LSD users; Irwin and Egozcue, 1967), initiated many studies to evaluate the cytogenetic effects of the drug. However, the results of subsequent investigations were contradictory. The *in vitro* studies revealed that chromosome damage occurred with high concentrations and/or with prolonged exposures which could not be achieved in humans given reasonable doses. Most, if not all, *in vivo* indications that LSD ingestion was associated with a significant increase in the frequency of chromosomal breakage were based on studies of subjects who had ingested 'street' LSD under non-clinical conditions (reviewed in Dishotsky *et al.*, 1971). Studies on patients treated with LSD under clinical conditions did not indicate similar increases (Corey *et al.*, 1970). The contradiction can be attributed to several causes: (i) the difficulties of evaluation, which include discrepancies between the alleged and actual composition of illicit drugs (Krippner, 1970); (ii) unreliability of the estimated LSD

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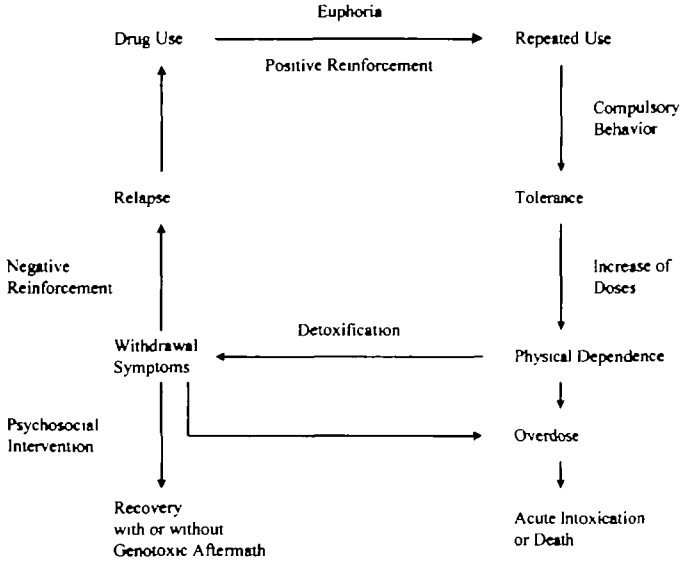


Figure 1. The cycle of drug use, dependence and relapse.

dosages obtained from interviewing subjects who used illicit LSD (Cheek *et al.*, 1970); (iii) viral infection (Nichols, 1966); (iv) multiple drug use that complicates interpretation of the data (Cohen *et al.*, 1967; Egozcue *et al.*, 1968). It was therefore concluded that LSD, at reasonable doses, had no definite effect on human chromosomes (Dishotsky *et al.*, 1971). Today the doses of LSD used are only a fraction of what was taken in the 1960s (Ulrich and Patten, 1991) and that may make LSD-induced chromosome aberrations even less likely to occur in real life.

However, it cannot be assumed that LSD is non-mutagenic because of its inability to induce structural chromosomal aberrations *in vivo*. In fact, positive results were achieved with dominant lethal tests after single and repeated administration of LSD to ICR mice (Sram *et al.*, 1974). Mutagenic activity of LSD was also observed in *Escherichia coli* (Vann *et al.*, 1970) and in barley (Singh *et al.*, 1970). Although the mutagenicity of LSD has been hypothesized as being due to its ability to intercalate in nucleic acids (Smythies and Antun, 1969; Wagner, 1969; Dishotsky *et al.*, 1971; Sram and Goetz, 1974), the underlying mechanisms, such as the LSD-induced mutational spectrum and the reversibility of intercalation, remain to be solved.

Opiates: The first abused drugs identified as carcinogens

Diacetylmorphine (heroin)

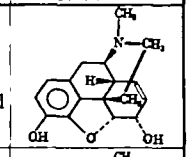
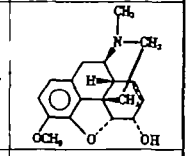
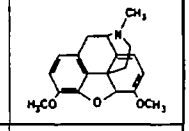
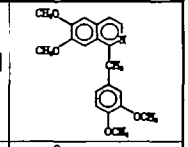
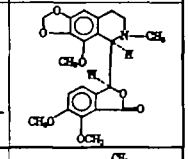
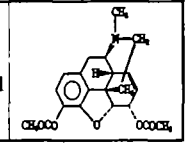
The early cytogenetic studies on the effects of LSD triggered subsequent investigations into opiates. Early cytogenetic experiments showed that diacetylmorphine abuse was associated with chromosome aberrations. One study, involving comparisons of 16 opiate addicts on a methadone program with a control group, revealed a significant elevation of chromosome aberrations following 72 h culture of leukocytes from the addictive group (Falek *et al.*, 1972). When the 'street' diacetylmorphine addicts entered a methadone maintenance treatment program (MMTP), the elevated levels of chromosome aberrations persisted for three months and then declined to control levels after 1 year in the MMTP (Falek and McFadden, 1973; Falek and Hollingsworth, 1980a,b). In Rhesus monkeys (*Macaca mulatta*), a similar elevated level of chromosome aberrations in their white blood cells was observed with chronic diacetylmorphine treatment (Fischman *et al.*, 1977). Another investigation was conducted on 72 h cultures of leukocytes taken from newborns 12 h to 31 days post-partum. After comparing 34 newborns of diacetylmorphine-addicted mothers (28 of whom were in MMTP) with 22 control newborns, it was found that chromosome aberrations were six to seven times higher in the drug-exposed newborns than those in the controls (Abrams, 1975). Similar cytogenetic results were obtained in studies on diacetylmorphine-treated pregnant Rhesus monkeys and their offsprings (Fischman *et al.*, 1983). In the same study, a doubling in sister chromatid exchange (SCE) level over controls was also observed. Thus, all of these studies support the conclusion that *in vivo* diacetylmorphine use resulted in an increase in chromosome aberrations and SCE levels. However, while diacetylmorphine can elevate the levels of SCE and chromosome aberrations *in vivo*, diacetylmorphine *per se* is not a DNA damaging agent. Diacetylmorphine was not found to bind covalently to DNA (Lee and Loh, 1975), does not induce DNA repair and does not increase the mutation frequency in many prokaryotic and eukaryotic tests (reviewed in Brusick, 1978). Intravenous diacetylmorphine abusers have been shown to be at greater risk for neoplastic diseases than the general population (Sapira *et al.*, 1970; Harris and Garret, 1972). Diacetylmorphine, as a non-mutagen which increases cancer rates, was thus proposed as a tumor promoting agent (Shafer *et al.*, 1983). However, the possibility that diacetylmorphine exerts its carcinogenic effect at the initiation stage via metabolites such as morphine could not be excluded. In addition, for diacetylmorphine-induced carcinogenicity, oncogenic viral infection in i.v. drug abusers and decreased immune responsiveness could also be contributing factors besides genetic damage resulting from diacetylmorphine abuse (Donahoe *et al.*, 1985, 1986; Falek *et al.*, 1991).

Opium

Opium is the dried exudate from unripe seed capsules of *Papaver somniferum*. The alkaloids of opium are conventionally divided into two distinct chemical classes, phenanthrene derivatives and benzylisoquinoline derivatives. Morphine, codeine and thebaine are the main phenanthrene derivatives in opium. The benzylisoquinoline derivatives, including papaverine and noscapine, are not addictive and therefore are not discussed in this review. The structures and chemical names of the main opium alkaloids and derivatives are shown in Table I.

In Iran, epidemiological studies indicated that opium smoking was associated with esophageal and urinary cancers in humans (Hewer *et al.*, 1978; Kmet, 1978; Sadeghi *et al.*, 1979). Dichloromethane extracts from pyrolyzed samples of sukhteh (the residue or dross from the opium pipe) gave dose-dependent increases in mutations of *S.typhimurium* TA100 and TA98 in the presence of rat liver microsomal activation (Hewer *et al.*, 1978; Bartsch *et al.*, 1980). On the other hand, crude opium samples showed little or no mutagenic activity and therefore the mutagens appear to be formed in the pipe as a result of pyrolysis during opium smoking. A variety of compounds were later isolated from the opium pyrolysates and identified as mutagens in *S.typhimurium* TA98 tests. These mutagens were implicated as the cause of opium smoking-induced cancers (Friesen *et al.*, 1985, 1987)

Table I. Structures and chemical names of main opium alkaloids and derivatives

Group	Trivial name	Systematic name	Structure
Phenanthrene	Morphine	(5α,6α)-7,8-Didehydro-4,5-epoxy -17-methylmorphinan-3,6-diol	
	Codeine	(5α,6α)-7,8-Didehydro-4,5-epoxy-3-methoxy-17-methylmorphinan-6-ol	
	Thebaine	(5α)-6,7,8,14-Tetradehydro-4,5-epoxy-3,6-dimethoxy-17-methylmorphinan	
Benzylisoquinoline	Papaverine	1-[(3,4-Dimethoxyphenyl)methyl] -6,-7-dimethoxyisoquinoline	
	Noscapine	[S-(R*,S*)]-6,7-Dimethoxy-3-(5,6,7,8-tetrahydro-4-methoxy-6-methyl-1,3-dioxolo[4,5-g]isoquinolin-5-yl)-1(3H)-isobenzofuranone	
Derivative of opium alkaloid	Heroin (diacetyl-morphine)	(5α,6α)-7,8-Didehydro-4,5-epoxy -17-methylmorphinan-3,6-diol diacetate	

Morphine

Morphine, a parent compound and metabolite of diacetylmorphine (heroin), is widely used in the management of moderate to severe pain. Although only limited information is available, there is evidence that *in vivo* administration of morphine to mice can increase the frequency of chromosome aberrations in bone marrow cells (Swain *et al.*, 1980) and induce micronuclei in bone marrow cells and lymphocytes (Das and Swain, 1982; Sawant and Couch, 1995). In contrast, *in vitro* morphine treatment has failed to induce chromosome aberrations in cultured human lymphocytes (Falek *et al.*, 1972) or micronuclei in mitogen-stimulated murine splenocytes (Sawant and Couch, 1995). It is therefore reasonable to assume that metabolic activation is involved in the induction of chromosome aberrations or micronuclei. Furthermore, morphine-induced micronuclei in mice can be reduced by naloxone, an opioid antagonist, indicating that this genetic damage is at least in part opioid receptor mediated. Although the principal metabolite of morphine *in vivo*, morphine-3-glucuronide (Glare and Walsh, 1991), does not participate in receptor-mediated responses, the possibility of the involvement of other metabolites cannot be ruled out (Sawant and Couch, 1995).

Mutagenic effects of morphine were not observed in *Drosophila*, *Salmonella* and yeast test systems (reviewed in Madden *et al.*, 1979). However, one recent study has indicated that the mutation frequency and the frequency of Comet tails of fragmented DNA were dose-dependently increased when human HUT-78 cells were treated with morphine alone for 4 days or morphine in combination with a brief ethylmethanesulfonate (EMS) exposure (Shafer *et al.*, 1994). Since DNA damage resulting from the brief EMS exposure was repairable, the results of morphine-induced *hprt* gene mutagenesis suggested that direct or indirect mutagenesis could be initiated if the exposure to morphine persisted through one or more cell cycles. Thus, morphine could act through long-term inhibition of replication or repair processes, converting transient alterations into permanent mutations. Morphine can also affect the repair of DNA damage caused by UV light (Madden and Falek, 1991). Morphine-induced DNA fragmentation has been associated with apoptosis in murine thymocytes *in vivo* (Fuchs and Pruett, 1993). Both opiate and glucocorticoid receptors are involved in morphine-induced apoptosis. Recently, it has been reported that administration of morphine to rats increased the ethylation of esophageal DNA by *N*-nitrosodiethylamine and may reduce the first pass clearance of *N*-nitrosodiethylamine by the liver, although only at high doses of morphine (Ribeiro and Swann, 1997). Morphine could therefore be classified as a co-mutagen.

Codeine

Codeine, a natural component found in opium, is used in a variety of pharmaceuticals, including analgesics, sedatives, hypnotics, antiperistaltics and antitussive agents. Codeine has been nominated for evaluation by the National Cancer Institute and the Food and Drug Administration in the US National Toxicology Program (NTP) because it is a widely used opiate drug (NTP, 1996). The NTP study showed that codeine

phosphate (CP), with and without S9 metabolic activation enzymes, induced dose-related increases in sister chromatid exchange in Chinese hamster ovary (CHO) cells. CP was not mutagenic in any of four strains of *S.typhimurium*, in the presence or absence of S9. There was no evidence of carcinogenic activity of codeine after 2 year feeding studies in F344/N rats and in B6C3F1 mice. However, further analysis with the computer automated structure evaluation (CASE) and multiple computer automated structure evaluation (MULTICASE) structure–activity relational expert systems for prediction of carcinogenic activity has suggested that codeine could be a rodent carcinogen (Zhang *et al.*, 1996).

Coca and cocaine

Cocaine, a major alkaloid isolated from coca leaf (Kleber, 1991), is one of the most widely abused drugs in Western societies. Prenatal cocaine exposure occurs in 6–15% of pregnancies in large North American cities (Chasnoff *et al.*, 1990; Gillogley *et al.*, 1990; Vega *et al.*, 1993; Holzman and Paneth, 1994). Cocaine is a well-known teratogen and it has been found that its abuse in humans significantly reduces the weight of the fetus, increases the stillbirth rate related to abruptio placentae and is associated with a higher malformation rate (Bingol *et al.*, 1987). Maternal use of cocaine during pregnancy has been shown to be associated with urogenital anomalies (Chavez *et al.*, 1989), cardiovascular anomalies (Lipshultz *et al.*, 1991) and microcephaly (Volpe, 1992). Cocaine has been found to inhibit DNA synthesis in developing rat brain regions (Anderson-Brown *et al.*, 1990). Further investigation has shown that cocaine exposure for 24 h can inhibit the incorporation of thymidine and, to a lesser extent, uridine and leucine, in cultured cortical glial and C6 glioma cells (Garg *et al.*, 1993). Such inhibition of macromolecular syntheses in glial cells may be the mechanism involved in cocaine-induced fetal brain growth retardation.

In male albino Swiss mice, a single dose (60 mg/kg) of cocaine could induce liver injury, which was characterized by specific changes in DNA poidy and apoptosis (Cascales *et al.*, 1994). Exposure of fetal mouse brain co-cultures to cocaine resulted selectively in the apoptosis of neurons. The major metabolites of cocaine had no detectable effects on neurons, indicating that apoptosis was due to cocaine itself (Nassogne *et al.*, 1997).

Although the carcinogenicity of cocaine remains to be determined, cocaine has been predicted as a carcinogen by the CASE system (Rosenkranz and Klopman, 1990).

Cannabis and cannabinoids

Cannabis derivatives (marijuana and hashish) are probably the most widely abused herbal drugs in the world. Although marijuana has long been considered as a relatively benign drug, new evidence suggests that its effects in the brain resemble those of 'hard' drugs such as heroin (Wickelgren, 1997). For example, the symptoms of emotional stress caused by marijuana withdrawal have been associated with activation of corticotropin-releasing factor, a peptide that has already been linked to anxiety and stress during opiate, alcohol and cocaine withdrawal (de Fonseca *et al.*, 1997). In addition, δ-9-tetrahydrocannabinol (THC), the major psychoactive compound in cannabis, results in a similar effect as heroin on mesolimbic dopamine transmission through a common μ_1 opioid receptor mechanism located in the ventral mesencephalic tegmentum (Tanda *et al.*, 1997).

Marijuana and its constitutive cannabinoids, including THC, cannabinol (CBN), and cannabidiol (CBD), have been shown to markedly induce cytogenetic changes in both *in vivo* and *in vitro* mammalian cells (reviewed in Zimmerman and Zimmerman, 1991). These aberrations include chromosomal breaks, deletions, translocations, errors in chromosomal segregation and hypoploidy. In alveolar macrophages recovered from the smokers of marijuana, either alone or in combination with tobacco smoking, DNA single-strand breaks have been detected with the alkaline unwinding assay (Sherman *et al.*, 1995). These results clearly indicate that marijuana is a DNA damaging agent. THC and other cannabinoids were also found to impair DNA and RNA synthesis in cell cultures (Leuchtenberger *et al.*, 1973). However, in rats administered 50 mg/kg THC from day 2 to day 22 of gestation, the DNA and RNA levels of the offspring appeared unaffected, while the brain protein levels were significantly lower than in the control group at days 7 and 14. The protein levels of the offspring in the treated group increased rapidly so that there were no differences between the control and treated groups at 21 days of age. Therefore, the effects of THC on both somatic and brain growth were concluded as being transient rather than permanent (Morgan *et al.*, 1988).

Chronic treatment of male CF-1 mice with THC did not result in any significant increase in the frequency of dominant lethal mutations (Berryman *et al.*, 1992). In contrast, marijuana smoke condensates were mutagenic in the Ames test (Busch *et al.*, 1979). Marijuana smoking has been suspected to be associated with cancers of the mouth, jaw, tongue and lung in 19–30 year olds (Caplan and Brigham, 1990; Nahas and Latour, 1992). In addition, a case–control study, conducted on 204 pairs of children, has shown that there was a 10-fold increased risk of leukemia in children who were exposed to marijuana *in utero* (Robison *et al.*, 1989).

Betel quid

The habit of betel quid (BQ) chewing, together with tobacco chewing or smoking, has been associated with an increased risk of oral cancers in South-East Asia and the South Pacific islands (IARC, 1985). Although there was insufficient evidence demonstrating that chewing BQ alone (i.e. without tobacco) was carcinogenic to humans, recent studies have indicated that the ingredients of BQ can also be genotoxic or carcinogenic. The composition of BQ, varying in different geographic locations, generally consists of betel nut (*Areca catechu*), *Piper betle* leaf and slaked lime, with or without tobacco (Jeng *et al.*, 1994). About 85–95% of the total alkaloid content (2–4%) of betel nut consists of arecoline (Wenke and Hoffmann, 1983). Other betel nut alkaloids are arecaidine, guvacoline and guvacine. The aqueous extract of betel nut and arecoline have been shown to induce dose-dependent elevations in the frequencies of SCE and chromosomal aberrations in cultured CHO cells (Dave *et al.*, 1992). Furthermore, when mice were injected with arecoline, significant numbers of chromosome aberrations were observed in bone marrow cells (Panigrahi and Rao, 1982). Intraperitoneal administration to mice of arecaidine has also been shown to increase SCE frequencies in bone marrow cells (Panigrahi and Rao, 1984).

The addition of lime to BQ constituents generates reactive oxygen species (ROS), which induce cytogenetic damage in

Table II. Epitome of the genetic toxicity of commonly abused drugs

Abused drug	Experimental system	Genetic toxicity	Reference
LSD	Cultured human leukocytes (*in vitro*)	Chromosome aberration (+)	Cohen *et al.* (1967)
	Leukocytes from LSD users (*in vivo*)	Chromosome aberration (+)	Irwin and Egozcue (1967)
	LSD users (*in vivo*)	Chromosome breakage (±)	Dishotsky *et al.* (1971)
	ICR mice (dominant lethal tests)	(+)	Sram *et al.* (1974)
	Escherichia coli	Mutagenic activity (+)	Vann *et al.* (1970)
	Barley	Mutagenic activity (+)	Singh *et al.* (1970)
Opiates			
Heroin	Cultured addicted human	Chromosome aberration (+)	Falek *et al.* (1972)
	Rhesus monkeys (WBC)	Chromosome aberration (+)	Fischman *et al.* (1977)
	Cultured leukocytes from newborns of heroin addicted mothers	Chromosome aberration (+)	Abrams (1975)
	Rhesus monkey	Chromosome aberration (+)	Fischman *et al.* (1983)
	Rhesus monkey	Sister chromatid exchanges(+)	Fischman *et al.* (1983)
Opium pyrolysates	*Salmonella typhimurium* TA98	Mutagenic activity (+)	Friesen *et al.* (1985, 1987)
Morphine	Mice bone marrow cells (*in vivo*)	Chromosome aberrations (+)	Swain *et al.* (1980)
	Bone marrow cells	Micronuclei (+)	Das and Swain (1982)
	Lymphocytes	Micronuclei (+)	Sawant and Couch (1995)
	Cultured human lymphocytes	Chromosome aberrations (–)	Falek *et al.* (1972)
	Murine splenocytes	Micronuclei (–)	Sawant and Couch (1995)
	Drosophila; *Salmonella*; yeast	Mutagenic effects (–)	Madden *et al.* (1979)
	Human HUT-78 cells	Mutagenesis (+)	Shafer *et al.* (1994)
	Murine thymocytes (*in vivo*)	DNA fragmentation (+)	Fuchs and Pruett (1993)
	Rats	Co-mutagen (+)	Ribeiro and Swann (1997)
Codeine	Chinese hamster ovary (CHO) cells	Sister chromatid exchanges (+)	Zhang *et al.* (1996)
	Salmonella typhimurium	Mutagenic activity (–)	
	F344/N rats; B6C3F1 mice	Carcinogenic activity (–)	
	Rodent (CASE; MULTICASE)	Carcinogenic activity (+)	
Cocaine	Developing rat brain	DNA synthesis inhibition	Anderson-Brown *et al.* (1990)
	Male albino Swiss mice	DNA poidy changes; apoptosis	Cascales *et al.* (1994)
	Mouse brain neurons	Apoptosis	Nassogne *et al.* (1997)
	CASE	Carcinogen	Rosenkranz and Klopman (1990)
Cannabis	Mammalian cells	Chromosomal breaks; deletions; translocations; errors in chromosomal segregation; hypoploidy	Zimmerman and Zimmerman (1991)
	Alveolar macrophages (alkaline unwinding assay)	DNA single-strand breaks	Sherman *et al.* (1995)
	Male CF-1 mice (dominant lethal mutations)	(–)	Berryman *et al.* (1992)
	Ames test	Mutagenic activity	Busch *et al.* (1979)
Betel quid	Cultured CHO cells	Sister-chromatid exchanges (+); chromosomal aberrations (+)	Dave *et al.* (1992)
	Mice bone marrow cells	Chromosomal aberrations (+)	Panigrahi and Rao (1982)
	Mice bone marrow cells	Sister chromatid exchanges (+)	Panigrahi and Rao (1984)
	Hamster cheek pouch	Cytogenetic damage	Nair *et al.* (1992)
	S.typhimurium TA100; TA1535; TA98; TA1538	Mutagenic activity (+)	Shirname *et al.* (1983)
	S.typhimurium TA102	Mutagenic activity (+)	Shirname *et al.* (1984)
	CHO cells	Micronuclei	Lee-Chen *et al.* (1996)
Khat	Mice (dominant lethal mutations)	(+)	Tarig *et al.* (1990)
	Rats	Embryotoxic effect (+)	Soufi *et al.* (1991)
	Rats	Teratogenic effect (+)	Islam *et al.* (1994)

+, positive effect; –, negative effect; CASE, Computer Automated Structure Evaluation; MULTICASE, Multiple Computer Automated Structure Evaluation.

hamster cheek pouch and may contribute to cytogenetic damage observed in the oral cavity of BQ chewers (Nair *et al.*, 1992). Of the various betel nut extracts, the catechin fraction, at alkaline pH achieved by the use of lime, was shown to be the most active producer of ROS. Treatment of DNA with betel nut extract *in vitro* at pH > 9.5 resulted in the formation of 8-hydroxy-2′-deoxyguanosine, which suggests that the hydroxy radical (HO•) is generated under the conditions prevailing during BQ chewing (Nair *et al.*, 1987, 1990).

Arecoline is mutagenic to *S.typhimurium* TA100, TA1535, TA98 and TA1538 (Shirname *et al.*, 1983, 1984). Hydroxychavicol, a major component of the *Piper betle* inflorescence, which is uniquely added to BQ in Taiwan, has been demonstrated to give positive results in *S.typhimurium* TA102 without metabolic activation and to induce micronuclei in CHO cells (Lee-Chen *et al.*, 1996). Several nitroso derivatives, such as *N*-nitrosoguvacoline (NG), *N*-nitrosoguvacine (NGC), 3-(methylnitrosoamino)propionitrile (MNPN) and 3-(methylnitrosoamino)propionaldehyde (MNPA), are formed by the *N*-nitrosation of arecoline and sodium nitrite *in vitro* (Wenke and Hoffmann, 1983) and have been found in the saliva of various betel quids (Wenke *et al.*, 1984a; Nair *et al.*, 1985; Stich *et al.*, 1986). Both MNPN and MNPA are carcinogenic to F344 rats (Wenke *et al.*, 1984b; Prokopczky *et al.*, 1987, 1991; Nishikawa *et al.*, 1992), indicating that nitrosation may also play an important role in BQ-induced cancers. A hospital-based case–

control study of matched pairs, conducted recently in Taiwan, indicates that while alcohol, tobacco and BQ pose synergistic effects in oral cancers, there was a statistically significant association between oral cancer and BQ chewing alone (Ko *et al.*, 1995).

Khat

Khat (*Catha edulis*), a plant containing cathinone, which is likened to a natural amphetamine, is widely abused by chewing in Southern Arabia and East Africa (Kalix, 1991, 1992; Balint *et al.*, 1991). Khat has been shown to induce dominant lethal mutations in mice, embryotoxic and teratogenic effects in rats, as well as oral cancers in humans (Tarig *et al.*, 1990; Soufi *et al.*, 1991; Islam *et al.*, 1994).

Future prospects

The progress of molecular biology has brought together the fields of genetics and toxicology. In the 1970s, *in vitro* genotoxicity screens, based on the assumption that induction of mutations was an initial step in the progression toward malignancy, have been applied for carcinogenicity prediction and risk assessment (Ames *et al.*, 1973; McCann *et al.*, 1975). The application of these screening tests to determine genotoxic potential have clearly protected us from being exposed to highly toxic chemicals. However, with increased understanding of the molecular mechanisms of carcinogenesis, we now know that not all carcinogens are genotoxic. The non-genotoxic carcinogens may directly or indirectly exert their effects through modulation of cell cycle and gene expression (Baylin, 1997). Therefore, additional efforts should be made on the non-genotoxic carcinogens that cause damage through mechanisms other than direct damage to DNA (Schwetz and Casciano, 1998). Recent advances, such as the application of molecular epidemiology approaches as well as transgenic cell and/or animal systems that express activated oncogenes, will be useful for the evaluation of carcinogenicty. Furthermore, new molecular and biochemical techniques will enable us to detect altered nucleotide sequence rather than a mutant phenotype. Thus, the rapid development of genetic toxicology will also lead to a better understanding of the molecular mechanisms of abused drug-induced toxicities.

The alteration of an oncogene *per se* or its expression could be cancerous. It has been shown that some addictive substances, such as opioids, cocaine and amphetamine, can induce expression of the c-*jun* and c-*fos* oncogenes in brain tissues of rats and mice (Graybiel *et al.*, 1990; Mackler and Eberwine, 1991; Nguyen *et al.*, 1992; Gogas *et al.*, 1993; Persico *et al.*, 1993; Johansson,B. *et al.*, 1994). Because drug abuse tends to be a long-term and repetitive behavior, such drug-induced oncogene expression could persist for long periods and increase the likelihood of cancer development. In contrast, genetic control of apoptosis (programed death) involves activation of the c-*myc* gene, which triggers passage of the differentiated resting cells from the G_0 to the G_1 phase of the cell cycle (Martin *et al.*, 1994). The progression to S phase is blocked by activation of a tumor suppressor oncogene, *p53*. Since apoptosis is interpreted as an active deletion of damaged cells to pave the way for regeneration, it would be of great interest to further investigate the role of cocaine in inducing apoptosis, which has been observed in the liver of male albino Swiss mice and in the neurons of fetal mouse brain (Cascales *et al.*, 1994; Nassogne *et al.*, 1997).

Exposure of germ cells to carcinogens and mutagens could lead to the occurrence of heritable tumors in the offspring. Therefore, it would be plausible to assume that exposure of parents to mutagenic or carcinogenic drugs of abuse could cause cancers in children. Indeed, there is a strong association between childhood rhabdomyosarcoma and the paternal use of 'recreational' drugs such as murijuana and cocaine (Grufferman *et al.*, 1993). Recent studies indicate that cancers resulting from germ cells mutations due to parental drug abuse could pose a potential threat to our next generation (reviewed in Little and Vainio, 1994).

In addition to the genotoxic effects exerted by the abused drugs *per se*, recent attention has also been directed towards understanding the implications of genetic differences associated with pharmacodynamic parameters mediating the actions of drugs (Shuster, 1984; Marley *et al.*, 1992, 1993). Current evidence from family, adoption and twin studies provides strong support for genetic components to interindividual differences in vulnerability to drug abuse (Uhl and Persico, 1994). One of the candidates for studying drug abuse pharmacogenetics is the human P450 system, the enzymes by which a wide variety of drugs are metabolized (DeVane, 1994; Inaba *et al.*, 1995). For example, the Far Eastern and Caucasian populations are strikingly different in metabolizing codeine due to a debrisoquine/sparteine hydroxylation polymorphism resulting from *CYP2D6* gene mutations (Johansson,I. *et al.*, 1991). In addition to codeine, many other addictive drugs, such as dihydrocodeine (Mikus *et al.*, 1994), hydrocodone (Otton *et al.*, 1993), methamphetamine (Lin *et al.*, 1995), methylene dioxymethamphetamine (Brady *et al.*, 1986) and phencyclidine (Owens *et al.*, 1993), have also been shown to be metabolized by CYP2D6. Thus, susceptible individuals may be more liable to the genotoxic effects of abused drugs if these drugs are poorly metabolized due to polymorphism of the target genes. Furthermore, the effects of multiple chemical interactions, especially alcohol and/or tobacco with abused drugs, could be complicated and should be taken into account, since polydrug use is common among drug abusers (Henningfield *et al.*, 1990; Sastry, 1991; Berryman *et al.*, 1992).

Finally, modification of lifestyle, which means all aspects of the way people behave, has been indicated as able to reduce the age-specific risk of cancer (Doll, 1990). For example, alcohol and tobacco are important modifiers of lifestyle. These two 'drugs of solace' have been shown to elicit a major impact on the incidence of human cancer. Besides, a study conducted on four religious groups has shown that modification of lifestyle by discouraging tobacco or alcohol use had merit in terms of reducing the overall risk of cancer (Troyer, 1988). Although drug abusers may enjoy rather different lifestyles from other members of the population, they may indeed expose themselves to a lifestyle that is prone to developing more cancers. Therefore, epidemiological studies on the relationship among cancers and various factors relating to the lifestyles of drug abusers will also be of great interest and are worthy of further investigation.

Conclusions

The mechanism of drug dependence has been the focus of drug abuse research. It is therefore not surprising that studies on drug-induced neurotoxicity have formed the mainstream and the carcinogenicity and/or genotoxicity of abused drugs have been overlooked for some time However, accumulated

data on commonly abused substances have indicated that these substances can also possess genotoxicity or carcinogenicity in addition to their addictive properties (summarized in Table II). Although these *in vitro* genotoxicity studies may still pose problems for risk estimation, they have already resulted in some plausible molecular mechanisms of carcinogenicity. Several *in vitro* studies have mimicked *in vivo* conditions by adopting an experimental design of physiological concentrations of drugs and long-term exposure. For example, morphine treatment with a low dose (5×10^{-8} M) and long-term exposure (4 days) was shown to be mutagenic in human HUT-78 cells (Shafer *et al.*, 1994). The *in vitro* experiments conducted at physiological concentrations with long-term exposure will be complementary to the *in vivo* studies, which are inevitably compromised by high concentrations and maximum tolerable doses (MTD).

Drug abuse tends to be a long-term and repetitive behavior and susceptible individuals who can metabolize drugs more actively to addictive or reactive metabolites owing to their variant genetic components resulting from mutations would be especially vulnerable. Thus drug-induced genotoxic and/or carcinogenic effects on the abusers can be diverse and profound. In an era of extreme drug abuse, further investigation in these new directions will help determine the seriousness of drug abuse, which is determined not only by the addiction liability but also by the genotoxicity and carcinogenicity.

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