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Opinion

Genotoxic Aspects of Psychoactive Substances

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Abstract: Exposure to psychoactive substances is undoubtedly a serious public health issue that should be carefully analyzed from different perspectives. Regarding the types of toxic effects inflicted by these xenobiotics, it is already known that many of the common abused psychoactives have shown positive genotoxicity findings in complementary genetic toxicology assays. Nevertheless, while there are several experimental articles and reviews on this topic, it is also clear that additional information, particularly mechanistic studies, is still needed. This article addresses these issues, pointing out some aspects of the potential genotoxicity of psychoactive substances that should be further explored, and suggests some possible approaches that could be valuable in future toxicological studies.

Keywords: psychoactive substances; genotoxicity; mutations; genetic damage; abused drugs

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The diversity of psychoactive substances that humans have been in contact with from ancient times to the present is quite large [1]. The health-associated issues are, thus, complex and continue to represent important challenges in modern societies. Psychoactive drugs should be analyzed in relation to multiple health aspects, some of which are still less studied. In this context, the genotoxic potential of psychoactive substances is a topic of great interest. Indeed, for these xenobiotics, other types of toxicity have been thoroughly studied throughout recent decades, particularly to better characterize the neurotoxic effects as well as some critical aspects concerning the toxicity on target organs such as liver, kidneys, and heart. A great deal of effort has also been undertaken to understand the addictive behavior and the determinant factors associated with the physical and psychological dependence. While these and other deleterious effects, namely, driving under the influence of psychoactive substances [2], are of utmost importance in clinical and forensic settings, it is also pertinent to examine these substances from different toxicological perspectives, namely, characterizing the potential genotoxic burden inflicted upon exposure. In addition, some psychoactive substances (e.g., marijuana) have been assessed in several cohort studies, focusing on the incidence of specific organ damage and in the formation of neoplasms in the exposed populations. Nevertheless, additional epidemiological studies are still required, as reviewed in [3]. It should be emphasized that in many users and addicts to psychoactives, the exposure is often carried out during long periods of time, starting at a young adult age and often presenting a repetitive pattern towards chronicity. Moreover, some individuals, due to their specific genotypes, for instance, in relation to biotransformation genes, could be at increased risk. This vulnerability can be ascribed to the pivotal role of some active metabolites in genetic toxicology.

The assessment of genotoxicity can be carried out either in vitro or in vivo, resorting to several short-term assays that have been developed, and thoroughly used, to identify agents that can induce genetic damage by different mechanisms. Genotoxicity tests have been used for decades in different contexts, including for regulatory purposes, and are essentially employed for the prediction of carcinogens, being also valuable for the interpretation of the results obtained in carcinogenicity studies.

Genotoxicity testing encompasses genetic alterations that occur either at the DNA sequence or at the chromosome level. The endpoints traditionally evaluated include gene mutations in prokaryotes (e.g., bacterial reverse mutation test—the classical *Salmonella* Ames test), gene mutations in mammalians (e.g., hypoxanthine-guanine phosphoribosyltransferase (HPRT) and thymidine kinase (TK) forward mutation assays), and cytogenetic alterations in mammalian cells (e.g., chromosome aberrations, micronuclei, sister-chromatid exchanges) [4]. There are several other tests available, some of them currently performed, namely, methodologies devoted to evaluating different types of DNA lesions (e.g., DNA breaks, DNA-adducts, oxidized bases, single-cell gel electrophoresis assay/comet assay, γ-H2AX assay) as well as tests performed with mammalian germ cells and with nonmammalian systems, such as yeast, plants, drosophila, or mussels. In silico (computational) genetic toxicology approaches have also been developed for the prediction of genotoxicity resorting to structural alerts and chemical properties [4].

While the study of the genetic toxicology of psychoactive substances has been less studied than other forms of toxicity, it should be mentioned that several authors have addressed this problem and reported positive genotoxicity findings for these substances. Genetic toxicity data have been obtained in a variety of endpoints and experimental conditions, using, in many of these reports, the same assays recommended for the preclinical testing of new drugs.

Early experimental reports on the genotoxicity of psychoactive substances were published from the late 1960s to the 1980s. Zimmerman and Zimmerman [5] reviewed the genetic damage induced by marijuana and its constitutive cannabinoids and concluded that these drugs were deleterious, presenting clastogenic and aneugenic effects in mammalian cells. In 1998, Li and Lin [6] reviewed the genetic toxicity data available for common drugs of abuse, particularly lysergic acid diethylamide (LSD), opiates, coca/cocaine, cannabis/cannabinoids, betel quid/arecoline, and khat and concluded that these drugs clearly displayed the capability of inducing genetic damage. More recent reviews on specific psychoactive drugs have been published. For instance, we comprehensively reviewed the genotoxicity of cocaine in its different forms [7]. Several experimental studies carried out either in vitro, in vivo (rodents), or with human subjects exposed to this drug revealed the genotoxic potential of cocaine. Importantly, this review also provided some clues and possible explanations regarding the associated mechanisms of genotoxicity. Indeed, mechanistic information is considered one of the cornerstones of modern toxicology and is also a major challenge in the scope of the genetic toxicology of psychoactive substances. More recently, an updated review on the relationship between carcinogenesis, genotoxicity, oxidative stress, and inflammation induced by crack cocaine was also published [8].

Regarding the genotoxicity of marijuana and its constitutive cannabinoids, several reports have been published, demonstrating positive results in terms of mutagenic activity of marijuana smoke condensates (*Salmonella* mutation assay) as well as in other genotoxicity endpoints, including in assays performed with lymphocytes from exposed individuals (HPRT, DNA breaks, chromosomal aberrations) (reviewed in [5,6]). Maertens et al. [9] reported that in the presence of S9-mix, all of the marijuana smoke condensates analyzed were more mutagenic than the matched tobacco smoke condensates. However, in terms of the formation of micronuclei (MN), opposite results were found, with a concentration-dependent increase in this cytogenetic biomarker only for tobacco smoke condensates. Recently, the genotoxicity observed in the peripheral lymphocytes from marijuana mono-users was compared with those presented in marijuana and tobacco users. While the increase in the formation of γ-H2AX (early biomarker that reflects the formation of double-

strand breaks) was present in both groups, the formation of MN was only present in the individuals concomitantly exposed to both agents [10]. Marijuana smoke condensates displayed positive results in the cytokinesis-block micronucleus assay and comet assay in human lung cancer cells with differential p53 expression [11]. In another report, cannabidiol and cannabidivarin at low concentrations were shown to induce the formation of MN and to give positive results in the standard comet assay as well as in the modified assay performed with lesion-specific enzymes, detecting oxidative DNA damage in human-derived cells [12]. Regarding another class of widely consumed psychoactive substances, the genotoxic potential of arecoline and related areca nut compounds present in betel quid was also thoroughly addressed in a recent review [13].

Altogether, the data from the literature pointed out a genotoxic potential for most of the common psychoactive substances, especially for those with high risk of causing dependence. This fact should not be disregarded and should be integrated into the scope of cancer risk assessment. Nevertheless, negative or conflicting results have also been reported for these same drugs. It is also relevant to evaluate the possibility of confounding factors (e.g., tobacco smoke), which increase the complexity of this subject and the need for complementary approaches to consolidate our knowledge. In addition, other psychoactive natural agents, for instance, kava and its constituents, generally displayed negative results in the classical genotoxicity endpoints evaluated (reviewed in [14]).

The emergence of new psychoactive substances (NPSs), namely, synthetic cannabinoids (SCs), cathinones, piperazines, and tryptamines, as well as synthetic hallucinogens, synthetic opioids, and benzodiazepines [15,16], render this topic even more pertinent, since for many of them there is scarce or even no information available in terms of genetic damage. It should be reinforced that these NPSs represent an alarming problem. In fact, the increase in the number of intoxications and deaths associated with SCs is clearly a public health issue of great importance and major concern for policy makers [17]. Nevertheless, the number of reports addressing the genotoxicity of NPSs is still limited. In this context, according to [18], four psychedelic phenethylamines, never tested before, significantly increased the MN frequency in human lymphoblastoid TK6 cells at sub-cytotoxic concentrations, while 3,4-methylenedioxymethylamphetamine (MDMA), also included in the study, did not. In contrast, a study carried out with the synthetic cannabinoid JWH-018 in SH-SY5Y cells did not reveal genotoxicity using the comet assay [19], although this NPS was demonstrated to be genotoxic in another study using TK6 cells [20]. As mentioned above, the formation of active metabolite(s) should be considered in genetic toxicology. Indeed, some authors focusing on synthetic cathinones found an important role of metabolism in the genotoxic responses observed [21]. Another point worth mention is the fact that, within a given class of NPSs, it is pertinent to evaluate not only a representative compound but also other derivatives, since, as recently reported [22], small chemical differences may render distinct genotoxicity patterns.

Finally, it is important to highlight possible directions that could be explored in this specific topic of genetic toxicology. In fact, it is pertinent to employ methodologies and study complementary approaches that have shown to be useful in other areas of toxicology. Thus, different aspects considered relevant in this field and some examples of possible strategies for future studies are summarized in Table 1. Several of these further perspectives have been suggested in our previous reviews on the genotoxicity of specific drugs of abuse [7,13]. While some of these points have already been considered by different researchers in their studies, in our opinion, these topics could be subjected to a more detailed and integrated investigation.

Table 1. Some aspects and suggestions of experimental strategies that could be useful in future studies to increase our knowledge of the genotoxic potential of psychoactive substances.

Aspects to be Further Considered	Possible Experimental Approaches
Level of exposure	Evaluation of realistic or pharmacological concentrations and doses of the drugs used in the in vitro and in vivo experimental studies, respectively.
Duration of exposure	Assessment of genotoxicity endpoints in long-term exposure experiments with the purpose of mimicking the repeated and chronic behavior patterns found in many drug users and addicts.
Concomitant exposure	Evaluation of the genotoxicity displayed by mixtures of psychoactive substances, either illicit or prescribed, to assess possible toxicological interactions that may occur (synergistic, potentiation, additive, and antagonism).
Advanced cell models	Advanced cell models, alternative to the use of experimental animals and more representative of the physiological conditions, can adequately be adopted also in this field, namely, resorting to three-dimensional (3D) cultures or human stem cell-derived models with metabolic competence, e.g., [23–26].
Additional genotoxicity endpoints	Experiments using novel or emerging genotoxicity endpoints or integrating mechanistically complementary assays in the same study should be determinant to gather more information and obtain conclusive findings.
Mechanistic studies	Experiments exploring pathways associated with DNA damage and repair, biotransformation, redox signaling, and apoptosis could be important to comprehend in detail the genotoxic mechanisms involved (mode of action), which can be relevant for cancer risk assessment purposes.
In silico approaches	Computational tools could be important also in this topic of genetic toxicology, particularly to predict the genotoxicity of NPS.
Metabolome studies	Analysis of the endo- (i.e., intracellular metabolites) and exometabolome (i.e., extracellular metabolites secreted by the cells) of human cells exposed to genotoxic concentrations of the drugs may provide valuable mechanistic information. Toxicometabolomics is undoubtedly an emerging field with multiple applications in toxicological research, including for psychoactive substances (reviewed in [27]).
Adductomic studies	Analysis of the adductome of cells exposed to psychoactive substances using different techniques could be very useful to understand the mode of action of genotoxic psychoactive agents. Some comprehensive articles focusing on adductomics in different contexts have been recently published [28,29].
Human data and biomarkers	Conducting additional genotoxicity studies with human populations exposed at different drug levels could be very informative. Biomarkers of exposure, effect, and susceptibility should be studied in this context. The validated cytogenetic endpoints for cancer, i.e., chromosome aberrations and micronuclei in human lymphocytes, can be used as biomarkers of early biological effects. The association of these biomarkers with biomarkers of susceptibility (e.g., genetic polymorphisms in DNA repair and biotransformation genes) could lead to the identification of genotypes at differential risk.

In conclusion, the evaluation and mechanistic characterization of the genotoxicity induced by different psychoactive substances is a challenging and exciting research topic that deserves further investigation. Additional research approaches should be performed to gain further insights and gather more genetic toxicology data.

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