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RESEARCH ARTICLE

Genotoxicity and mutagenicity induced by acute crack cocaine exposure in mice

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Abstract

Context: Crack cocaine is an illicit drug derived from cocaine, in which use and abuse have increased around the world, especially in developing countries. *Objectives:* The aim of this study was to evaluate genomic damage in multiple organs of mice following acute exposure to crack cocaine. For this purpose, single cell gel (comet) assay in peripheral blood, liver, kidney, and brain cells was performed and micronucleus test for bone marrow and liver cells was also made in this setting. *Material and methods:* A total of 20 C57BL/10 male mice were distributed into four groups, as follows: 0, 4.5, 9, and 18 mg/kg b.w. of crack cocaine dissolved to 1% dimethyl sulfoxide by intraperitoneal (i.p.) route. All animals were sacrificed 24 h after i.p. injection. *Results:* The results showed that crack cocaine induced DNA damage in peripheral blood, and brain cells for higher doses used as depicted by single cell gel (comet) assay data. Analysis of kidney cells showed no genetic damage for all groups tested. The number of micronucleated cells did not increase after crack cocaine exposure in bone marrow or liver cells. *Conclusion:* In summary, crack cocaine is a genotoxic agent in peripheral blood, liver, and brain cells but not mutagenic in multiple organs of mice.

Keywords

50-36-2, crack cocaine, DNA damage, mice

History

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Introduction

Illicit drugs use and abuse corresponds a health problem around the world. Among them, cocaine is the second most commonly used illicit substance in Europe, with 4.6% of the total adult population having used this drug at least once in their lifetime (Mendoza et al., 2014). In Brazil, public health studies have revealed that 3 million regular users of cocaine, accounting for 20% of its consumption worldwide.

Crack cocaine is a very toxic by-product of cocaine, resulting from the addition of sodium bicarbonate to cocaine base paste (Falck et al., 2008), being presented as ‘‘rocks’’. Chemically, crack cocaine is characterized by a substance insoluble in water, thermostable, that vaporizes at temperatures above 93 °C, being rapidly absorbed by the upper airways and lung (Chang et al., 2005). Today, Brazil is the world’s largest market for crack cocaine (Laranjeira et al., 2012). However, few studies have assessed the potential effects of crack cocaine at the cellular and molecular level, in particular focusing genotoxicity in different organs

and tissues. For example, some studies have demonstrated significant effect on DNA content, increasing the degree of aneuploidy, with the action of crack being much more potent and rapid than that of cocaine hydrochloride (Narvaez et al., 2013). Crack cocaine was able to induce genetic damage in peripheral blood and buccal mucosa cells of users (de Freitas et al., 2014; das Graças Alonso de Oliveira et al., 2014). However, to the best of our knowledge, no studies have addressed if crack cocaine induces genetic damage in multiple organs so far.

The single cell gel (comet) assay is very sensitive method able to detect genetic damage in any eukaryotic cell (Tice et al., 2000). This is sensitive, fast, low-cost technique widely used in the literature able to detect several types of DNA lesions including single and double strand breaks, adducts and incomplete repair sites (Tice et al., 2000). Micronucleus (MN) arises from acentric fragments or whole chromosomes which are not included in the main nuclei of the daughter cells. The formation of MN can be induced by substances that cause chromosome breakage (clastogens) as well as by agents that affect the spindle apparatus (aneugens) (Beliën et al., 1995). The aim of this study was to evaluate genomic damage in multiple organs of mice following acute exposure to crack cocaine.

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Materials and methods

Animals and experimental design

All experimental protocols involving animals are conformed to procedures described in the Principles for the Use of Laboratory Animals Guidelines. The study was approved by the Animal Ethics Committee of Federal University of Sao Paulo, UNIFESP, SP, Brazil (Protocol no. 3251250314). A total of 20 C57BL/10 male mice weighing 20 g on the average, and 8 weeks age were distributed into four groups ($n=5$), as follows: 0, 4.5, 9, and 18 mg/kg b.w. of crack cocaine (CAS number: 50-36-2, the estimate of percent purity of crack cocaine was $\sim 75\%$) by intraperitoneal (i.p.) route. The dose levels used correspond, respectively, to 6.25, 12.5, 25% of the cocaine LD50 (lethal dose to 50%) by i.p. injection as demonstrated elsewhere (Salvadori et al., 1998). Crack cocaine was diluted in 1% dimethyl sulfoxide at final volume of 1 mL. The control group received the solvent only. All animals were sacrificed 24 h after i.p. injection. The drug was gently donated by Criminal Department of Limeira city, Sao Paulo State, Brazil for research purposes.

Single cell gel (comet) assay

The protocol used for peripheral blood, kidney, brain, and liver cells followed the guidelines outlined by Tice et al. (2000). Peripheral blood was collected by cardiac puncture and liver, brain, and kidney cells were obtained by liver tissue maceration with phosphate buffer solution (PBS) (Alvarenga et al., 2010). Cells were transferred to individual plastic tubes, containing 1 mL of cold phosphate buffer solution (PBS, Ca^{2+} , Mg^{2+} free, pH 7.3), and centrifuged for 5 min, 1000 rpm, at room temperature. The supernatant was removed and the cell suspensions ($\sim 10 \mu\text{L}$) were used for single cell gel (comet) assay. A volume of $10 \mu\text{L}$ of cellular suspension was added to $120 \mu\text{L}$ of 0.5% low-melting point agarose at 37°C , layered onto a pre-coated slide with 1.5% regular agarose, and covered with a coverslip. After brief agarose solidification in refrigerator, the coverslip was removed and the slides immersed in lysis solution (2.5 M NaCl, 100 mM EDTA – Merck™, Darmstadt, Germany; 10 mM Tris–HCl buffer, pH 10 – Sigma-Aldrich™, St Louis, MO, EUA; 1% sodium sarcosinate – Sigma-Aldrich™, St Louis, MO, EUA; with 1% Triton X-100 – Sigma-Aldrich™, St Louis, MO, EUA; 10% dimethyl sulfoxide – Merck™, Darmstadt, Germany) for about 1 h. Afterwards, the slides were washed in ice-cold PBS (Ca^{2+} , Mg^{2+} free, pH 7.3) for 5 min, left in electrophoresis buffer (0.3 mM NaOH and 1 mM EDTA – Merck™, Darmstadt, Germany, pH > 13) for DNA unwinding during 20 min, and electrophoresed in the same buffer for 20 min at 25 V (0.86 V/cm) and 300 mA. Following electrophoresis, slides were neutralized in 0.4 M Tris-HCl (pH 7.5, Sigma-Aldrich™, St Louis, MO, EUA), fixed in absolute ethanol and stored at room temperature until analysis in a fluorescence microscope at $400\times$ magnification. All steps were performed under reduced light.

A total of 50 randomly captured comets per animal (25 cells from each slide) were examined blindly by one expert observer at $400\times$ magnification using a fluorescent microscope (Olympus™, Orangeburg, NY). The microscope was

connected through a black and white camera to an image analysis system (Comet Assay II, Perceptive Instruments™, Suffolk, Haverhill, UK) calibrated previously according to the manufacturer's instructions. To measure DNA damage, we used the tail moment defined as the product of the tail length and the fraction of DNA in the comet tail (Tice et al., 2000).

Micronucleus test

After completing the experimental period, the MN test was performed in bone marrow and liver tissues. The bone marrow MN test was performed according to de Moura et al. (2014). For this purpose, femoral bones were collected and stored in sodium chloride 0.9%. The proximal epiphyses of the bones were removed and 1 mL of fetal bovine serum (FBS; Cultilab™, Campinas, São Paulo, Brazil) was injected into the medullar canal. A smear on glass slides was performed with the suspension formed by the bone marrow and fetal bone serum. After drying the slides, they were stained with Giemsa (Merck™, Darmstadt, Germany). For liver MN test, paraffin sections (3 μm) were stained by Feulgen and counterstained with Fast Green (Sigma-Aldrich™) (da Silva et al., 2014). A total of one thousand polychromatic erythrocytes or hepatocytes were analyzed per animal (de Moura et al., 2014). Slides were scored blindly using a light microscope with a $100\times$ immersion objective.

Statistical analysis

All the data are expressed as mean \pm standard deviation (SD). One-way analysis of variance was performed followed by Tukey's multiple comparisons test. Statistical analysis was performed using GraphPad Prism™ 6.0 program. $p < 0.05$ was considered to be significant.

Results

Comet assay

Peripheral blood, liver, brain, and kidney cells were evaluated for DNA damaging. Blood cells exposed to crack cocaine at dose of 18 mg/kg increased genetic damage when compared to control group. The doses of 4.5 mg/kg or 9 mg/kg were not able to induce genetic damage after acute exposure of crack cocaine. Brain cells were more sensitive to genotoxic insult because the doses of 9 mg/kg and 18 mg/kg were able to induce genetic damage. The dose of 4.5 mg/kg did not cause genetic damage in brain cells. Such findings are summarized in Figure 1. When liver cells were analyzed, interesting results were found. Hepatocytes exposed to crack cocaine demonstrated genetic damage only at the highest dose used (18 mg/kg). Liver cells exposed after 24 h to crack cocaine at 9 mg/kg and 4.5 mg/kg did not show remarkable differences ($p > 0.05$) when compared to control group (Figure 1). Finally, kidney cells were also evaluated in this setting. Crack cocaine did not induce genetic damage in kidney cells, for all doses adopted in this study. Such findings are summarized in Figure 1.

Micronucleus test

MN test in bone marrow showed that crack cocaine was not able to stimulate micronuclei formation in bone marrow cells

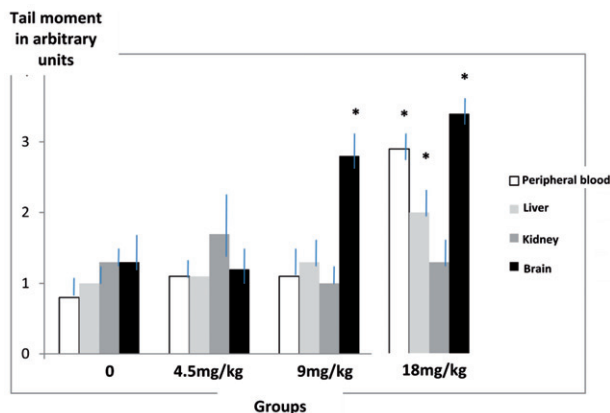


Figure 1. DNA damage (tail moment) in animals exposed to crack cocaine after 24 h. * $p < 0.05$ when compared to control group.

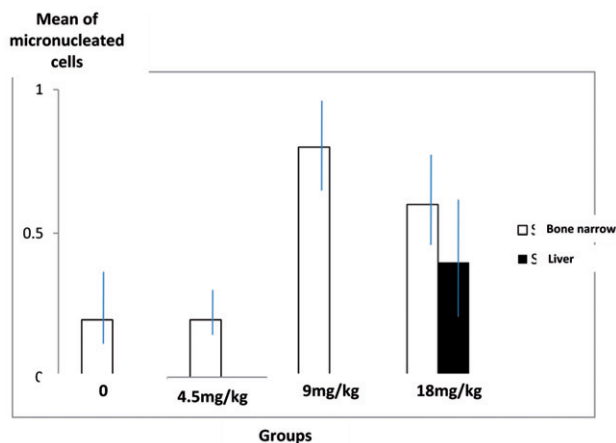


Figure 2. Mean \pm SD of micronucleated cells after acute crack cocaine exposure. $p < 0.05$ when compared to control group. $p > 0.05$.

for all doses tested, i.e. no mutagenic effect was detected in blood cells. In a similar manner, the number of micronucleated cells did not increase after crack cocaine exposure in liver cells. Such data are summarized in Figure 2.

Discussion

The aim of this study was to evaluate genetic damage in multiple organs of mice exposed to crack cocaine using convenient and rapid *in vivo* assay. The single cell gel (comet) and MN assays were used for evaluation of crack cocaine genotoxicity and mutagenicity, respectively as a consistent method for detecting genomic damage simpler than other conventional techniques for genetic analysis.

Our results demonstrated that crack cocaine was able to induce genetic damage in peripheral blood cells as depicted by tail moment results, in the highest doses used in this setting. Our results are fully in line with previous studies that demonstrated that crack cocaine is able to induce genetic damage in human blood cells (de Freitas et al., 2014). Taken together, our results demonstrated that crack cocaine is genotoxic in peripheral blood of mice after acute exposure.

According to the single cell gel (comet) assay guidelines, it is recommended to evaluate liver since the main metabolic organ (Tice et al., 2000). Our results revealed that crack

cocaine was able to induce genetic damage in liver cells after acute exposure at 18 mg/kg. Kidney cells did not increase DNA-strand breaks when compared to negative control for all doses used. These findings are new, and therefore difficult to discuss. During the process of metabolism of xenobiotics that occurs in liver, crack cocaine, and its sub-products are excreted in the kidney. Therefore, it seems that crack cocaine did promote genetic damage in liver cells when exposed at higher doses only. During excretion process, kidney cells are not sensitive to genotoxic insult promoted by crack cocaine. This requires further study.

Brain cells are an important target organ following crack cocaine exposure since the drug has stimulatory effects on central nervous system. Our results demonstrated that crack cocaine induced genetic damage in brain cells after acute exposure. By comparison, some studies have postulated that some biological mediators such as brain-derived neurotrophic factor (BDNF) and thiobarbituric acid reactive substances (TBARS) are being related to cerebral plasticity and impairment caused by crack cocaine abuse (Sordi et al., 2014; von Diemen et al., 2014).

Micronucleated cell indexes may reflect genomic damage (de Castro Marcondes et al., 2014). The detection of an elevated frequency of micronuclei in some tissues and/or organs indicates increased risk of cancer. Our results demonstrated that the MN frequencies were not significantly different between control and crack cocaine exposure in bone marrow cells. Liver cells did not show mutagenesis after acute crack cocaine exposure as well. By comparison, previous studies conducted by our research group have demonstrated that crack cocaine is able to induce MN in buccal mucosa cells (das Graças Alonso de Oliveira et al., 2014). This was also confirmed by others (Almeida et al., 2012). However, other studies have demonstrated no mutagenic effect induced by cocaine in bone marrow cells of mice (Salvadori et al., 1998). These discrepancies may be due to differences in the experimental design.

In summary, our results reveal that crack cocaine is able to induce genotoxicity but not mutagenicity in multiple organs of mice. Since these pathological events are important for developing chronic degenerative diseases such as cancer, these data are relevant to better understand the real health risks induced by crack exposure after first exposure. This is particularly important for public health because crack cocaine is endemic in developing countries such as Brazil.

Declaration of interest

This study was supported by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior). DAR is a recipient of CNPq fellowship.

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