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Crack cocaine inhalation induces cardiac atrophy and facilitates limbic-motor seizures in mice submitted to subconvulsive dose of pilocarpine

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Abstract

Epidemiological studies have reported significant increase in seizures incidence in crack cocaine users. Furthermore, *crack* has been correlated with cardiovascular physiopathology, which can increase the chance of sudden unexpected death (SUDEP). Here we analyzed the susceptibility to epileptic seizures and the structural myocardial response to crack cocaine exposure following by subconvulsive dose of pilocarpine. Mice exposure to crack cocaine/subconvulsive dose of pilocarpine presented self-sustaining seizures and cardiac atrophy accompanied by increased migration of fibroblasts. Our results suggested that crack cocaine reduced the threshold for seizures and facilitated heart inflammatory process that could lead to predisposition to the occurrence of SUDEP.

Keywords: *crack cocaine; epilepsy; cardiac atrophy; seizures; addiction; neurodegeneration.*

1. Introduction

Crack cocaine has been a public health problem due to low cost and rapid action in the central nervous system (CNS) which leads to addiction [10]. Crack cocaine use may generate seizures due to its actions in glutamatergic pathways, as well as dopaminergic pathways, acting in reward system [13].

Epilepsy is characterized as a neurological syndrome responsible for the excitation and excessive synchronization of neural networks and highlighted by *spontaneous recurrent seizures* (SRS) [3]. Temporal lobe epilepsy is the most common type and can be initiated by status epilepticus (SE) [11]. Furthermore, epilepsy can be associated to crack-cocaine use [19,24].

Despite the continued use of crack cocaine can be related to the development of epileptic condition, few studies have related the epileptic phenomenon with crack-cocaine use [9]. It is consistent relation between epilepsy and the high mortality caused by sudden unexpected death in epilepsy (SUDEP) [7]. In this direction the use of cocaine have been related with cardiovascular disease, including hypertension, arrhythmia, coronary vasoconstriction, and myocardial ischemia [17]. These cardiovascular complications associated with predisposition to epileptic individuals to present SUDEP can make crack cocaine user even more vulnerable to SUDEP. However, the triple correlation between cocaine-epilepsy-SUDEP is still unclear.

Therefore, in the present study, we tested the hypothesis that the use of crack-cocaine lead to decrease of the threshold to limbic-motor seizure evoked by subconvulsive dose of pilocarpine in Swiss mice. We also examined the cardiovascular parameters associated with SUDEP seem in ELT patients to link the expose of crack-cocaine to epilepsy and SUDEP.

2. Methods

2.1 Animals

Male Swiss mice (*Mus musculus*) (n=32) weighing 25-36 g were housed in a temperature-controlled (22±2ºC) room with a 12-h light cycle. All animals have remained at least 1 hour in establishing with white light before experiments. All experiments were developed according to with the American Association for Accreditation of Laboratory Animal Care standards, and were approved by the Ethical Committee for Animal Experimentation of the UFAL (Protocol 048/2013). Animals were divided into 4 groups: (1) control (n= 8), (2) pilocarpine 75 mg/kg (n= 8), (3) crack cocaine exposure (n= 8) and (4) crack cocaine exposure with pilocarpine 75 mg/kg (n= 8).

2.2 Drug acquisition and chromatographic analysis

The use of crack cocaine for research purposes was authorized by National Health Surveillance Agency (ANVISA) and the drug was donated by Alagoas State police through a Federal Prosecutor of Federal University of Alagoas. The crack cocaine used was from unique and unknown lot.

To confirm existence of active principle, we performed crack cocaine chromatographic. For this analysis, 800 mg of the sample was processed in a closed tube coupled to a tight-gas syringe, where it was subjected to pyrolysis (200 to 280°C) by means of a hot place. After, 100 µL of smoke was aspirated and injected into the gas chromatography mass spectrometry (GC/MS QP-2010 SE model SHIMADZU®l). The GC/MS was coupled with a computer equipped with data acquisition and processing software GC/MS solution and Nisti 2008 library.

2.3 Crack cocaine exposure and systemic pilocarpine administration

We used a modified model of Petros's system [25], which was inserted into an exhaust hood. A dose-response curve with several doses crack cocaine was established (data not shown) and the dose of 400 mg was chosen. Mice were exposed during 5 days (once a day) to crack cocaine inhalation according to the following sequence: animal adapting in the acrylic chamber (1 minute), pipe heating (2 minutes), drug burning (1 minute), smoke exposure remain (1 minute) and oxygen (3 minutes). After 72 h of the last exposure animals were intraperitoneally administered methyl-scopolamine (1 mg/kg) to block the peripheral effects 30 minutes previously the administration of subconvulsive dose of pilocarpine (75 mg/kg, ip.). It is expected that this subconvulsive dose did not induce seizures. To verify if crack cocaine would interfere with threshold of seizures, arbitrary scale of Racine was utilized during 90 min after pilocarpine to characterize limbic myoclonic seizures [16]. After 24 hours, animals were transcardially perfused.

2.4 Morphometric and stereological analysis

Heart was excised and wet weights were obtained for all experimental groups. The left tibia was removed, and the length was measured with calipers to assess the cardiac index using the heart weight/tibia length ratio. The heart fraction was measured from the histological thin sections using the stereological grid-point counting method and test area overlapping images were obtained and analyzed as Blindly through a video-microscopy system (Microscópio Leica DMRBE, Wetzlar Germany; video camera Kappa Gleichen, Germany; Monitor Sony Trinitron, Pencoed, England). The stereological method used two-dimensional quantitative data and mathematical equation to determine the three-dimensional composition of the heart through variables related to the integrity and volume of cardiomyocytes as well as infiltrating inflammatory cells [12].

3. Results

Crack cocaine pyrolysis showed the volatile compounds: benzoic acid (peaks 6, 7, 8) and ecgonine methyl ester (peak 11), which corresponded to 55.68% and 11.18%, respectively, of the sample composition (Fig.1). The heating of samples rapidly volatilizes cocaine and one of the products is methylecgonine, also known as anhydroecgonine methyl ester (AME).

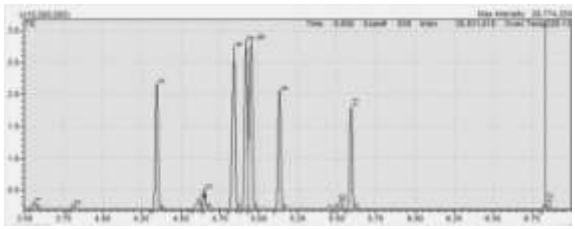

Figure 1. Chromatographic peaks of volatile compounds obtained of crack cocaine pyrolysis. 1. Carbon monoxide; 2. Nickel tetracarbonyl; 3. Benzoic acid methyl ester; 4. Magnesium; 5. Vanadium; 6, 7, 8. 2-methyl benzoic acid; 9. p-fenetidine; 10. Nickel.; 11. Anhydroecgonine methyl ester; 12. Nickel.

Only groups that received pilocarpine combined with crack cocaine exhibited self-sustained myoclonic seizures. Crack cocaine with pilocarpine presented self-sustained seizures incidence of class 2 (myoclonus of head and neck) in Racine's scale during 90 min, when seizures were aborted with diazepam (5mg/kg). Animals that received only pilocarpine presented isolated seizures and animals that received vehicle or crack cocaine alone do not presented any seizures behavior manifestation (Table 1).

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Table 1. Hyperlocomotor activity, isolated or self-sustaining seizures after crack exposure and pilocarpine administration

Group	N	Class	Hyperlocomotor activity	Isolated seizures	Self-sustaining seizures
No exposure	8	-	-	-	-
Pilocarpine	8	-	-	X	-
Crack cocaine	8	-	X	-	-

Crack cocaine administration alone not presented alteration in morphometric parameters of the heart. However, when it was performed in association between crack cocaine exposure and pilocarpine administration, a cardiac atrophy (0.05±0.001) was observed when compared to control (0.08±0.007) (p=0.002) (Fig.2).

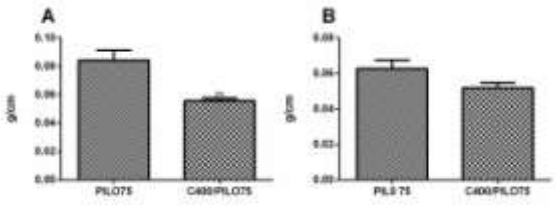

Figure 2. Morphometric parameters of the heart. A) Cardiac atrophy; B) Ventricular weight. **p <0.001 vs Pilocarpine. Results presented in mean ± standard deviation of the mean. T test unpaired.

For stereological analysis, we analyzed 95 photomicrographs (5 fields per animal) at 40x magnification. As analysis of the cardiomyocytes volume, cardiomyocytes number maintained unshaken then exposure to crack cocaine and pilocarpine administration (Fig. 3A-D). Nonetheless, pilocarpine subconvulsive dose associated to crack cocaine exposure promoted an increased migration of fibroblasts to myocardium, revealing its inflammatory effect (Fig. 3E-F).

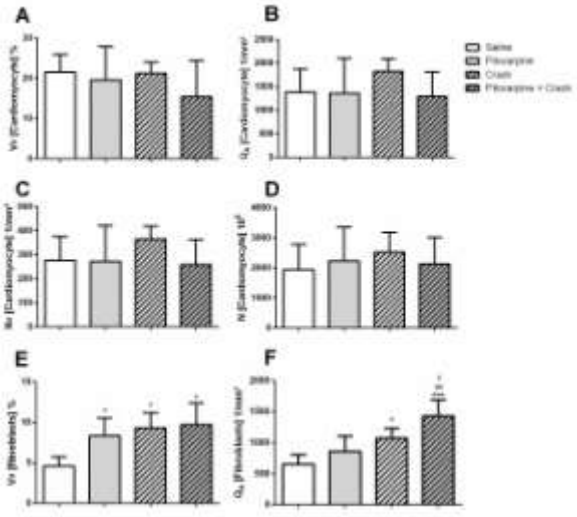

Figure 3. Stereology of cardiomyocytes of mice, Cell number, fibroblasts. A) Cardiomyocyte volume density (Vv); B) Cardiomyocyte profile density by area (Qa); C) Cardiomyocyte number density (Nv); D) Total number of cardiomyocytes (N); E) Fibroblast volume density (Vv) and F) Density of fibroblast profiles by area (Qa). *p <0.05, ***p <0.001 vs Saline, #p <0.01 vs Pilocarpine, $p <0.05 vs Crack. Results presented in mean ± standard deviation of the mean. One-Way ANOVA followed by the Student-Newman-Keuls post-test.

4. Discussion

Cocaine use and epilepsy have been related to cardiovascular changes, as dysrhythmias, depressed myocardial contractility and prolonged QRS complex and QTc duration [24]. In addition, crack cocaine ingestion was able to trigger status epilepticus, complex brady arrhythmias and ventricular arrhythmias [19]. Our study showed that after exposure to crack cocaine coupled to subsequent administration of a subconvulsive dose of pilocarpine animals developed self-sustained seizures, class 2 according to Racine's scale, and cardiac atrophy.

The amount of cocaine converted into AME may vary according to the purity of the sample, firing temperature and firing device; (50 to 80%) when heated to between 255 and 420°C and above 89% when heated to 650°C [14]. AME is generated exclusively from cocaine pyrolysis, thus being an analytical marker of crack cocaine [20]. Our results showed the presence of these compounds, especially methylecgonine or AME, which has been shown to be responsible for the neurotoxic effects of crack cocaine [6].

There has been growing awareness of SUDEP, which is now acknowledged as a serious problem for epileptic patients [2]. The increase of sympathetic activity has profound influence over the electrical and contractile functions of the heart [23]. The abnormal sympathetic signaling can produce severe toxic cardiac effects, such as increase in Ca^{2+} intracellular concentration [23] and possible myocardial apoptosis [18]. Furthermore, cocaine generates an excess of neurotransmitter in postsynaptic terminals after blocking presynaptic reuptake of catecholamines. Thus, excess catecholamines may have caused a vasoconstriction resulting from stimulation of α-adrenergic receptors in the vessel wall, compromising the proper blood supply to heart, consequently generating ischemia, which triggers cardiac atrophy due a compensatory mechanism to reduce the cell energy demand.

Moreover, it has already showed evidence of increased thrombogenicity due to elevated platelet agreeability produced by cocaine, a fact which confirms the hypothesis that ischemia may have occasioned a possible reduction of organ size [21]. In addition, cocaine also has a local anesthetic activity which compromises cardiac conduction, decreasing cardiac workload which may also cause atrophy [4].

Searching to understand cardiac atrophy, we analyze stereological parameters of the heart. We observed that the cardiomyocytes number remained unchanged. Cocaine is able to affect the immune system [15]. Furthermore, its adulterated form with other drugs or chemicals (crack cocaine) may have immunogenic substances [1]. Several authors have shown that intravenous cocaine administration increases the activity of natural killer cells in peripheral blood of humans and such cells may have a cytotoxic effect on cardiac myocytes, causing myocardial injury and inflammatory response [22]. Additionally, the increase of neurotransmitters (adrenaline and noradrenaline) may cause myocyte necrosis with inflammatory infiltrate [8], generating a heart inflammation that lead to the hypothesis of a myocarditis installed in groups treated with pilocarpine exposed to crack cocaine.

The lack of autonomic control, occurring in epilepsy [5], may be associated with suppression of insulin-like growth factor 1 (IGF1). Furthermore, muscle atrophy, neurohormonal activation, cytokine production, and left ventricular systolic dysfunction are associated with decreased levels of IGF1. However, further studies are needed to confirm the involvement of this pathway in this study.

Any harmful stimuli in the brain can affect your effector organs. Our study was able to show that animals with pre-disposition to seizures and exposed to crack cocaine have important histological markers that may be involved to SUDEP and to inflammatory phenomena caused by the activation of the immune system by the immunogens present in the drug, suggesting a myocarditis.

5. Conclusions

Our data suggest that the animals exposed to crack cocaine shown higher frequency and duration of epileptic seizures, presented important cardiovascular alterations that certainly compromise the life of the patient, besides the strong evidence of being connected to SUDEP. Further studies are required to identify which mechanisms lead to these alterations.

Acknowledgments

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