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Alpha-lipoic acid alone and combined with clozapine reverses schizophrenia-like symptoms induced by ketamine in mice: Participation of antioxidant, nitrergic and neurotrophic mechanisms

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ABSTRACT

Oxidative stress has important implications in schizophrenia. Alpha-lipoic acid (ALA) is a natural antioxidant synthesized in human tissues with clinical uses. We studied the effect of ALA or clozapine (CLZ) alone or in combination in the reversal of schizophrenia-like alterations induced by ketamine (KET). Adult male mice received saline or KET for 14 days. From 8th to 14th days mice were additionally administered saline, ALA (100 mg/kg), CLZ 2.5 or 5 mg/kg or the combinations ALA + CLZ2.5 or ALA + CLZ5. Schizophrenia-like symptoms were evaluated by prepulse inhibition of the startle (PPI) and locomotor activity (positive-like), social preference (negative-like) and Y maze (cognitive-like). Oxidative alterations (reduced glutathione — GSH and lipid peroxidation — LP) and nitrite in the prefrontal cortex (PFC), hippocampus (HC) and striatum (ST) and BDNF in the PFC were also determined. KET caused deficits in PPI, working memory, social interaction and hyperlocomotion. Decreased levels of GSH, nitrite (HC) and BDNF and increased LP were also observed in KET-treated mice. ALA and CLZ alone reversed KET-induced behavioral alterations. These drugs also reversed the decreases in GSH (HC) and BDNF and increase in LP (PFC, HC and ST). The combination ALA + CLZ2.5 reversed behavioral and some neurochemical parameters. However, ALA + CLZ5 caused motor impairment. Therefore, ALA presented an antipsychotic-like profile reversing KET-induced positive- and negative-like symptoms. The mechanism partially involves antioxidant, neurotrophic and nitrergic pathways. The combination of ALA + CLZ2.5 improved most of the parameters evaluated in this study without causing motor impairment demonstrating, thus, that possibly when combined with ALA a lower dose of CLZ is required.

1. Introduction

Schizophrenia is a highly disabling and multifaceted illness with positive, negative and cognitive symptom domains (Carpenter, 2007). The pathophysiology of this mental disorder is related, but not limited to: i) alterations in neurotransmitter systems, such as dopaminergic and glutamatergic (Coyle et al., 2010; Miyake et al., 2011); ii) alterations in neurotrophins (Favalli et al., 2012); iii) oxidative imbalance (Zhang et al., 2010) and iv) alterations in nitric oxide (NO) signaling (Bernstein et al., 2005).

Ketamine (KET) repeated administration to rodents mimics positive, negative and cognitive symptoms of schizophrenia (Meltzer et al., 2013; Monte et al., 2013). These behavioral alterations are accompanied by oxidative imbalance and nitrite alterations (Monte et al., 2013).

The predictive validity of the KET-induced model of schizophrenia is achieved only by atypical antipsychotics (Becker and Grecksch, 2004). Clozapine (CLZ) was the first atypical antipsychotic drug developed and presents a unique mechanism of action when compared to other drugs of this class (Leo and Regno, 2000). This drug transiently occupies D2 receptors (D2Rs) besides interacting with other neurotransmitter systems. Due to its unique mechanism of action, CLZ is the most effective drug for treatment-resistant schizophrenia (Leucht et al., 2013). Clozapine seems to have anti-inflammatory properties in putative brain areas related to schizophrenia, but in some studies it was not efficient in the reversal of oxidative alterations in animal models of schizophrenia (Ribeiro et al., 2013). On the other hand, CLZ is implicated in

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pro-inflammatory alterations in insulin responsive cells and in obesity-associated cell types, possible mechanisms related to the development of metabolic syndrome by this drug (Contreras-Shannon et al., 2013). Indeed, CLZ is associated with important side effects depending on the dose and plasma concentration (Yusufi et al., 2007).

Alpha-lipoic acid (ALA) or thioct acid is a natural acid synthesized in human tissues. This drug is used for symptomatic diabetic neuropathy (Moini et al., 2002) and cardiac autonomic neuropathy (Ziegler and Gries, 1997). Mechanistic evidences suggest that ALA (Maczurek et al., 2008; Deslauriers et al., 2014): i) inhibits the formation of hydroxyl radicals and also scavenges reactive oxygen species, thereby increasing the levels of reduced glutathione (GSH); ii) scavenges lipid peroxidation (LP) products; iii) down-regulates the expression of redox-sensitive pro-inflammatory proteins including TNF and inducible NO synthase; and iv) decreases D2Rs. The first suggestion for ALA in schizophrenia emerged in the 50s (Giamattei, 1957). In the last decade a clinical study suggested that ALA can ameliorate the adverse metabolic effects induced by antipsychotic drugs (Kim et al., 2008). A recent study reported that the combination of ALA and omega-3 polyunsaturated fatty acids was not effective in the relapse prevention after antipsychotic discontinuation in first-episode schizophrenia (Emsley et al., 2014). On the other hand, preclinical evidences suggest that ALA administration during preadolescence/adolescence period could prevent schizophrenia-like behavioral alterations in mice (Deslauriers et al., 2014).

Thus, based on the importance of oxidative stress to the pathophysiology of schizophrenia (Zhang et al., 2010) and on the evidences that ALA would present antipsychotic effects, we hypothesized that: i) the administration of ALA could reverse the behavioral and neurochemical (oxidative, neurotrophic and nitrergic) alterations induced by KET and ii) the combination of CLZ and ALA could reverse the behavioral and neurochemical (oxidative, neurotrophic and nitrergic) alterations induced by KET with better results than each drug alone.

2. Material and methods

2.1. Ethics

The study followed legal and ethical requirements provided by the NIH Guide for the Care and Use of Laboratory Animals (NIH 1996) and the Brazilian College of Animal Experimentation (COBEA) Law no. 11794/2008. The local ethical committee of Federal University of Ceara approved the protocol.

2.2. Animals

Adult male Swiss mice (25–30 g) maintained at a controlled temperature (23 ± 1 °C) with a 12 h dark/light cycle (lights on at 7:00 AM) and free access to water and food were used.

2.3. Drugs and treatment

Alpha-lipoic acid was orally administered (Sigma-Aldrich, St. Louis, USA, ALA 100 mg/kg). Ketamine hydrochloride (König, Brazil, KET 20 mg/kg) or clozapine (Leponex® Novartis, Brazil, CLZ 2.5 or 5 mg/kg) was intraperitoneally administered. The doses of ALA (Macêdo et al., 2012), CLZ (Moreira and Guimarães, 2005) and KET (Monte et al., 2013) were based on previous studies.

2.4. Study design

After randomization each animal received one daily injection of KET or saline for 14 days to simulate an acute treatment of psychotic episodes (Byrne, 2007; Monte et al., 2013). Additionally from 8th to 14th days the animals were subdivided in groups to which each of the following drugs was added 30 min after saline or KET: saline, ALA, CLZ 2.5 or 5 mg/kg. The combination groups received ALA + CLZ2.5 or ALA + CLZ5 30 min after saline or KET. Therefore, a total of twelve groups were used in the present study. Clozapine was used as standard antipsychotic.

Thirty minutes after the last drug administration prepulse inhibition of the startle reflex (PPI), locomotor activity (open field test), working memory (Y-maze task) and social interaction were registered. Afterwards mice were sacrificed by decapitation and the prefrontal cortex (PFC), hippocampus (HC) and striatum (ST) dissected, rapidly frozen and stored at −70 °C until assayed.

2.5. Behavioral tests

2.5.1. Prepulse inhibition of the startle reflex (PPI)

After acclimatization to the background noise, mice were presented with a series of 10 stimuli (pulse alone — 120 dB, 50 ms duration — habituation phase). Thereafter, the PPI modulation of the acoustic startle was tested using 74 trials pseudo-randomly divided into seven different categories (20 s interval): 20 presentations of pulse alone (120 dB, 50 ms duration), 8 presentations of each prepulse (PP) intensity alone (70, 75 or 80 dB, 3000 Hz frequency, 20 ms duration) and 10 presentations of each prepulse intensity + pulse (with 50 ms interval). Mean amplitude of startle response to pulse-alone (P) and prepulse-pulse (PP + P) trials was calculated for each subject. The level of PPI in each mice was determined by expressing the PP + pulse startle amplitude as a percentage decrease from pulse-alone startle amplitude, according to the following formula: %PPI = 100 − [100 × (PP/P)] (Levin et al., 2011). Startle amplitude was used to assess motor alterations.

2.5.2. Open-field test

An arena with nine squares (Archer, 1973). The observed parameters were the number of squares crossed (horizontal activity) and number of rearings (vertical activity).

2.5.3. Y-maze test

Each mouse was allowed to freely move through the maze during 8 min. The series of arm entries was recorded visually. An alternation was defined as entries in all three arms on consecutive occasions. The percentage of alternation was calculated as total of alternations/(total arm entries − 2) (Yamada et al., 1996; Dall'Igna et al., 2007).

2.5.4. Social interaction test (SIT)

The apparatus consisted of a box divided into three chambers with a small opening in the dividers. In each of the two side chambers an iron cage was placed with a probe mice or empty. Mice were allowed 5 min of exploration. Afterwards an unfamiliar, same-sex probe mouse from the same experimental group was placed in one of two restraining cage (Radyushkin et al., 2009). Social preference was defined as: (% time spent in the social chamber) − (% time spent in the opposite chamber).

2.6. Determination of oxidative stress parameters

2.6.1. Reduced glutathione (GSH)

The method was based on Ellman's reagent (DTNB) reaction with free thiol groups as described elsewhere (Sedlák and L' Hanus, 1982). GSH level was expressed as μg of GSH/g wet tissue.

2.6.2. Lipid peroxidation (LP)

Lipid peroxide formation was analyzed by measuring the thiobarbituric acid reacting substances (TBARS) in the homogenates as described elsewhere (Ohkawa et al., 1979). It was expressed as μg of malondialdehyde (MDA)/g wet tissue.