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Melatonin attenuates behavioural deficits and reduces brain oxidative stress in a rodent model of schizophrenia

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ABSTRACT

Melatonin is a neurohormone that is linked to the aetiopathogenesis of schizophrenia. The aim of this study was to assess the potentials of oral melatonin supplement in the management of induced schizophrenia-like behavioural and brain oxidative status changes, using an animal model. The relative degrees of modulation of ketamine-induced behaviours by haloperidol, olanzapine or melatonin were assessed in the open-field, Y-maze, elevated plus maze and the social interaction tests. 12-week old, male mice were assigned to six groups of ten each (n = 10). They were pretreated with daily intraperitoneal ketamine at 15 mg/kg (except vehicle) for 10 days, before commencement of 14 day treatment with standard drug (haloperidol or olanzapine) or melatonin. Ketamine injection also continued alongside melatonin or standard drugs administration for the duration of treatment. Melatonin, haloperidol and olanzapine were administered by gavage. Treatments were given daily, and behaviours assessed on days 11 and 24. On day 24, animals were sacrificed and whole brain homogenates used for the estimation of glutathione, nitric oxide and malondialdehyde levels. Ketamine injection increased open-field behaviours; while it decreased working-memory, social-interaction and glutathione activity. Nitric oxide and malondialdehyde levels also increased after ketamine injection. Administration of melatonin was associated with variable degrees of reversal of these effects. In conclusion, melatonin may have the potential of a possible therapeutic agent and/or adjunct in the management of schizophrenia.

1. Introduction

Schizophrenia is a complex neuropsychiatric disorder, whose aetiopathogenesis has been linked to both genetics and the environment [1,2]. It is known to exhibit wide variations in symptomatology, response to treatment, course and eventual outcome [3]. About four decades ago, Wyatt et al. [4] suggested that schizophrenia is not a single disease, but rather a syndrome of clinically and biologically-distinct entities; and presently, schizophrenia has been associated with dysfunctions in several neurotransmitter [5], (serotonergic [6,7], cholinergic [8] and glutamatergic [9,10]) systems, resulting in alterations in brain biochemistry and clinical phenotypes that are (in many cases) individualised [3].

To facilitate our understanding of schizophrenia and its management, experimental models have been developed [11] that mimic the behavioural and biochemical changes that follow alterations observed in the various neurotransmitter systems associated with schizophrenia. Currently, pharmacological models using *N*-methyl-D-aspartate glutamate receptor (NMDA-R) antagonist like ketamine, phencyclidine and MK801 have become very important. Their use is premised on observations that these drugs successfully induce both positive and negative symptoms of schizophrenia [3].

Ketamine (in subanaesthetic doses) induces schizophrenia-like behaviours in rodents [12,13], and humans [14] by blocking N-methyl d-aspartate receptors (NMDA-R). In rodents, repeated administration of subanaesthetic dose of ketamine (10–50 mg/kg) has been associated with behavioural changes like social withdrawal [3], hyperlocomotion [13], prepulse-inhibition deficits [15] and memory loss [16], following alterations in glutamate or dopamine levels. The ketamine model of schizophrenia has been reported to have superior face-validity compared to phencyclidine; because it is less toxic, and repeated administration induces behavioural changes [17] similar to those observed in schizophrenia [18].

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Ketamine administration has also been reported to be associated with changes in oxidant status; and studies have shown that depending on dose and/or duration of administration, ketamine can have either prooxidant or antioxidant effects. Reus et al. [19] reported antioxidant potential of ketamine, with the reversal of alterations in serum levels of tumour necrosis factor alpha (TNF-α) and interleukins (IL) 1 and 6 in rats following maternal deprivation. da Silva et al. [20,21] reported administration of ketamine was associated with increased glutathione/lipid peroxidation and myeloperoxidase activity in all regions of the brain; as well as increased levels of hippocampal IL-4 and IL-6.

In the management of schizophrenia, dopamine antagonists such as haloperidol are known to effectively reverse the positive symptoms; but they are plagued by issues such as inability to improve cognitive impairment, social withdrawal, and a decreased life expectancy that has been associated with metabolic derangements [22]. Atypical antipsychotics such as olanzapine, which are relatively newer, can treat both positive and negative symptoms of schizophrenia. However, studies have also shown that there is no clear evidence that they are more effective or better-tolerated than the older drugs [23,24]. The moral dilemma of balancing adequate control of psychotic symptoms against treatment-associated (possible contribution to) decreased longevity and long-term health problems is believed to be a major issue in the management of schizophrenia today. Hence, the search for newer drugs with better efficacy or tolerability continues.

An emerging area in schizophrenia research focuses on the impact of immunomodulatory drugs such as melatonin [21]; and recently, there have been renewed interests in the links between melatonin and schizophrenia. Several studies have reported abnormal functioning of melatonin in the pathophysiology of schizophrenia [25–29]. Wulff et al. [30] reported an association between schizophrenia, circadian rhythm dysfunction and melatonin levels in schizophrenic patients. There is also accumulating clinical evidence suggesting the involvement of melatonin in the pathogenesis of schizophrenia [27,28]. Melatonin has also been used in the control of schizophrenia-associated sleep disorders [31] and drug-related tardive dyskinesia [32].

Melatonin is a neurohormone that is primarily synthesized and secreted by the pineal gland [33,34], with a role in entraining circadian rhythms. It is also an important antioxidant, anti-inflammatory and analgesic [28], that helps in the regulation and synchronization of cell physiology and in combating stress [35]. Effects of exogenous melatonin has been studied extensively [33,34,36–38]. In humans, daytime administration of melatonin promoted sleep and resulted in sleep-like brain activity patterns in the precuneus and hippocampus [39]; while in rodents, several studies have reported anticonvulsant [40], anxiolytic [38], antidepressant [37,38], and memory-facilitating [33,34,41] effects of exogenous melatonin. Reports of studies from our laboratory assessing the behavioural effects of melatonin in mice, reported a central depressant effect; with a decrease in open field behaviours [33] and reversal of the behavioural and systemic effects of exposure of prepubertal mice to chronic unpredictable stress [34] However, there have also been studies that reported proconvulsant effects, [42] suppression of night-time memory formation [43] and impairment of acquisition but not the expression of contextual fear in rats [44].

Melatonin has been reported to be beneficial in the reversal of behavioural and biochemical alterations associated with ketamine-induced schizophrenia in mice [21]. While studies have evaluated melatonin's effectiveness as an adjunct in the management of schizophrenia in humans [45,46], there is still a dearth of preclinical information on its potential as a sole agent. In this study, we induced a mouse model of schizophrenia by administering ketamine through intraperitoneal injection (daily) for a period of ten days [16], and maintained a continuous daily injection till the end of the experiments. It is known that animal models of schizophrenia that are based on repeated administration of NMDA receptor antagonists show both constructive and phenomenological validity [12]; available antipsychotics have also been proven to be effective against the abnormalities observed in these models [47]. The rationale for this study was the need to determine the ability of initial or repeated administration of melatonin to reverse behavioural and brain oxidative changes induced by ketamine, and the extent to which its effects compare with standard antipsychotics. We therefore tested the hypothesis that exogenously administered melatonin can significantly counteract ketamine-induced behavioural and brain oxidative changes in mice.

2. Materials and methods

2.1. Drugs

Melatonin (5 mg tablets of melatonin as *N*-Acetyl-5-Methoxytryptamine, Pure Naturals LLC, Delaware, USA), Haloperidol (Haldol®, 10 mg, Janssen-Cilag, Germany), Olanzapine (Prexal®, 5 mg, Afrab-chem Ltd, Lagos, Nigeria), Ketamine (Ketamine hydrochloride USP, 50 mg ketamine base per ml, Ketalar®, Rotexmedica Gmbh, Trittau, Germany). Glutathione, nitric oxide and lipid peroxidation (MDA) assay kits (Biovision Inc., Milpitas, CA, USA).

2.2. Animal care

12-week old, male Swiss mice (Empire Breeders, Osogbo, Osun State, Nigeria) were used in this study. Mice were housed in groups of five, in plastic cages in temperature-controlled quarters (22–25 ° Celsius) with 12 h of light daily (lights on at 7.00 a.m). They were fed commercial standard chow (Calories: 29% protein, 13% fat, 58% carbohydrate) from weaning. Animals received food and water *ad-libitum* except during behavioural tests. All procedures were conducted in accordance with the approved institutional protocols and within the provisions for animal care and use prescribed in the scientific procedures on living animals, European Council Directive (EU2010/63).

2.3. Experimental method

Mice were randomly assigned into two main groups (1 and 2), based on behavioural tests to be conducted. In main group 1, mice were exposed to the social interaction test; while in 2, mice were exposed to the open-field, Y-maze and elevated plus maze (EPM) tests. 120 mice were randomly assigned to two groups of sixty animals (N = 60), for experiment one and two respectively. Mice in each main group were then randomly divided into six groups (1–6) of ten (n = 10) each. Animals in group 1 received intraperitoneal (i.p) injection of vehicle (distilled water) at 2 ml/kg, while groups 2–6 received i.p ketamine at 15 mg/kg daily, for 10 days. From day 11 to 24, mice in group 1 (vehicle) were given distilled water (i.p at 2 ml/kg and oral at 10 ml/kg); group 2 (ketamine control) received daily i.p ketamine and oral distilled water. Animals in groups 3–6 received daily i.p. ketamine and oral administration of haloperidol (4 mg/kg), olanzapine (2 mg/kg), or one of two doses of melatonin (MEL) (5 or 10 mg/kg), respectively. MEL, haloperidol or olanzapine were administered (daily for 14 days) by gavage to simulate use in humans. Doses of MEL [34], haloperidol [48] or olanzapine [49] administered were calculated by dissolving measured quantities in distilled water. Melatonin was dissolved (at room temperature) at 10 mg/10 ml of distilled water.