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Research report

Trans-generation enrichment of clozapine-responsiveness trait in mice using a subchronic hypo-glutamatergic model of schizophrenia:A preliminary study

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HIGHLIGHTS

- There is still no validated animal model for clozapine-refractory schizophrenia.
- A heterogeneous response to clozapine in mice sub chronic-PCP model was shown.
- Response traits to clozapine were shown to be augmented in a forward-genetic design.
- Clozapine refractory mice were not able produce beyond the first generation.
- There was an trans-generation increase in brain GAD67 levels in males but not in females.

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ABSTRACT

Background: Schizophrenia patients who do not respond to clozapine treatment represent the most debilitating type of schizophrenia with unmet needs for novel interventions. To date there is no validated animal model for clozapine-refractory schizophrenia.
Methods: We used poor performance in the social preference (SP) test of C57/BL mice exposed to subchronic phencyclidine (PCP) as a correlate of negative signs of schizophrenia. Subsequently the mice were treated with clozapine and according to their SP they were defined as responding (i.e. clozapine/PCP ratio > 1.5 SD) or non-responsive to clozapine. In each generation the responding mice were mated to produce the next generation. Unfortunately, the clozapine- non-responsive mice failed to proliferate and were thus excluded from the analyses. This forward genetic paradigm was used to produce the next generation of clozapine-responding mice. We assessed brain glutamic acid decarboxylase-67 (GAD67) protein levels, as a GABA-ergic marker, in the F2 and F3 generations.
Results: Already in the F1 generation of male mice, but not females, it was possible to discriminate between clozapine-responders and non-responders. The rate of responders within each consecutive generation, increased. The increase was more pronounced in females. Up-regulation of GAD67 levels was detected between F2 and F3 only in male clozapine-responder mice, but not in females.
Conclusions: This preliminary proof-of-concept study succeeded in producing a trans-generation enrichment of clozapine-responsiveness trait in a hypo-glutamatergic animal model of negative signs of schizophrenia. This model may serve as a platform to better characterize the clozapine responsiveness trait and offer a model for clozapine-responsive schizophrenia.

1. Introduction

Schizophrenia is a chronic debilitating psychotic disorder, affecting almost 1% of the general population [1]. Current pharmacotherapy consists of antipsychotic compounds that affect mainly the positive symptoms and, to a considerably lesser extent, the negative symptoms and the cognitive deficits associated with schizophrenia. Large prospective studies from recent years have

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predicted that whereas 50–70% of patients have a sufficient responsiveness to standard antipsychotic compounds (both typicals and atypicals), approximately one third of the population suffering from schizophrenia has treatment-resistant schizophrenia (TRS) [2–4]. For this population, the recommended treatment is clozapine which has been shown to be superior in TRS patients [5–7]. Although this treatment is associated with numerous side effects, some of which could be fatal [8]. For this reason this compound is usually reserved as a third line of treatment, to be used only after failure of typical and atypical antipsychotic compounds [6,9].

Approximately 50%–70% of the TRS population does not respond to clozapine therapy [10]. These clozapine-resistant schizophrenia (CRS) patients are also referred to as ultra-resistant. For them there is an unmet need for an efficient and safe treatment. Current strategies include augmenting clozapine with other agents such as other antipsychotics or mood stabilizers. None of these strategies has proven to be satisfactory [6,8,10]. The key factor for improving treatment of CRS patients is in better understanding the genetic and biochemical nature of the differences between responsive and non-responsive populations. At present there are no established data on specific genetic polymorphisms that can predict response to clozapine, although several studies attempted to identify relevant candidate genes that modulate the response of schizophrenia patients to clozapine treatment [11,12].

We hypothesized that generating a reliable animal model of CRS may help to improve the understanding of the pathophysiology of these severely ill patients. Successful development of a CRS animal model may advance the development of new therapeutic interventions. Unfortunately, as mentioned above, there is no validated model of CRS to promote the investigation of drug resistance and the development of novel treatments.

Phencyclidine (PCP) and MK-801 (Dizoclepine) are non-competitive N-methyl-D-aspartate (NMDA) receptor antagonists which may induce a psychotic-like state that has some similarities to schizophrenia. PCP and ketamine when administered acutely to healthy people induce hyperactivity, paranoid delusions, hallucinations, formal thought disorder and cognitive impairments [13]. Olney et al. [14] propose that the presence of hypofunctional NMDA receptors modulate glutamate and GABA neurotransmission. Such modulation may lead to brain dysfunction accounting for both the acute symptoms and the chronic course of schizophrenia. PCP and MK-801 are increasingly being used as a research tool to model psychosis in pre-clinical studies.

Subchronic PCP administration in animals has been shown to produce persistent effects on stereotypy and an increase in enduring social and cognitive deficits of particular relevance to schizophrenia [15,16]. More specifically, subchronic PCP and other NMDA receptor antagonists impair performance on tests dependent on hippocampal function, such as acquisition of a spatial continuous recognition memory, as well as attention set-shifting that may be sub-served by frontal cortical functions [17,18]. The exploratory preference (i.e. the tendency to explore novel mice or objects) of the PCP-treated mice increased significantly after subchronic administration of clozapine ($P < 0.001$), but not haloperidol ($P = 1.00$) [19]. Since the social impairment in this animal model of schizophrenia has been shown to be sensitive to clozapine treatment we used this behavioral test in our attempt to develop an animal model of CRS.

This model may be used to identify the expression of brain proteins relevant to the neurobiology of schizophrenia, such as the GABAeric system, through glutamic acid decarboxylase-67 (GAD67) expression. It was suggested to be implicated in schizophrenia [20] as well.

We therefore aimed to establish an animal model for clozapine-responders and – non-responders and to enrich those traits through a forward genetic paradigm. Next, we intended to examine differences between clozapine-responders and – non-responsive regarding GAD67 expression in the brain.

Developing colonies of animals based on behavioral features is an important approach to study the biochemical and genetic basis of various traits. Several lines of animals were developed to study the genetic and biochemical origins underlying mood and anxiety disorders [21–24].

Increased frequency of a trait in a population subjected to selective breeding was used as a tool to enrich the genetic component of a specific trait [25,26]. For example, genetic involvement in catalepsy was revealed by increasing the rate of animals with catalepsy across generations of animals selected for this trait [27].

In this study, we aimed to develop a novel CRS mouse model based on selective breeding approach. We attempted to obtain trans-generational enrichment of clozapine treatment responsiveness or non-responsiveness traits in an animal model of schizophrenia-associated social deficit, in both male and female mice. To this aim we used the PCP-interference with the Social Preference Test (SPT) as the benchmark for responsiveness or resistance to clozapine. Our second objective was to identify a relevant biochemical correlate (brain GAD67) for responsiveness or non-responsiveness to clozapine treatment.

2. Methods

2.1. Animals

Male and female C57/BL (8 weeks old) weighing 25–30 g were purchased from Harlan, Israel (Jerusalem, Israel). Mice were housed in groups of 4–5 mice under a controlled 12/12-h light–dark cycle (light from 7:00 a.m. to 7:00 p.m.), with room temperature at $23 \pm 1\ ^{\circ}C$ and humidity at $55 \pm 5\%$. The mice had free access to water and food pellets. The experimental procedure was approved by the Animal Care and Use Committee of Tel-Aviv University.

2.2. Study protocol

2.2.1. Drug administration

C57/BL wild type mice (males: n = 9 and females: n = 9) were treated sub-chronically with PCP (10 mg/kg s.c, for 10 days) followed by a washout period of 10 days and then clozapine (4 mg/kg, p.o) for eight days.

2.2.2. Behavioral tests

2.2.2.1. Social preference test (SPT). Each animal underwent a SPT three times: at baseline, after PCP administration (on the last day of the washout period) and following 8-days of clozapine treatment.

The SPT apparatus is divided into three compartments. The two lateral ones contain two transparent plastic cups with air vents. Prior to the day of the test, mice are habituated to the apparatus for two consecutive days. On the test day, the mice are habituated to the arena for a period of 10 min, during which time two black partitions hide completely the other compartments containing the cups. Thereafter, the partitions are removed and an unfamiliar C57/BL mouse is placed in one of the cups (animate stimulus; social chamber). The other cup remains empty (inanimate stimulus: non-social chamber). The test mouse is free to explore the chambers for another 10 min. Videos were analyzed using the Ethovision 7 software (Noldus, Wageningen, The Netheralnds), analysis allowed tracking either the center of the animal or the nose. Parameters analyzed included total time spent in each chamber; total nose pokes to the cups and total time in which the test mouse was in proximity to the cup—as a representation of social exploration. The social behavior parameter is determined by the preference index, which