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Neuropsychopharmacotherapeutic efficacy of curcumin in experimental paradigm of autism spectrum disorders

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ABSTRACT

Aim: Neuroinflammatory response triggered by the stimulation of matrix metalloproteinases plays a pivotal role in the development of autistic phenotype. MMPs stimulate inflammatory cytokines release along with mitochondrial deficits that ultimately lead to neuronal dysfunction and precipitate autistic symptoms. The aim of the present study was to explore the neuropsychopharmacotherapeutic efficacy of curcumin in the experimental paradigm of autism spectrum disorders.
Materials and methods: 1 M propanoic acid (4 μl) was infused over 10 min into the anterior portion of the caudoputamen to induce autistic behavior in rats. Curcumin (50, 100 and 200 mg/kg) was administered per orally starting from 2nd day of surgery and continued up to 28th day. Rats were tested for various neurobehavioural paradigms like social interaction, stereotypy, locomotor activity, anxiety, novelty, depression, spatial learning and memory as well as for repetitive and pervasive behavior. In addition, biochemical tests for oxidative stress, mitochondrial complexes, TNF-α and MMP-9 were also carried out.
Key findings: Intracerebroventricular injection of propanoic acid produced neurological, sensory, behavioral, biochemical and molecular deficits which were assessed as endophenotype of autism spectrum disorders. Regular treatment with curcumin for four weeks significantly and dose dependently restored neurological, behavioral, biochemical and molecular changes associated with autistic phenotype in rats.
Significance: The major finding of the study is that curcumin restored the core and associated symptoms of autistic phenotype by suppressing oxidative-nitrosative stress, mitochondrial dysfunction, TNF-α and MMP-9 in PPA-induced autism in rats. Therefore, curcumin can be developed as a potential neuropsychopharmacotherapeutic adjunct for autism spectrum disorders (ASD).

1. Introduction

Autism spectrum disorders (ASD) is a complex syndrome which is characterized by a heterogeneous group of neuropsychiatric disorders that affects the brain in the developmental stage [70]. US Center for Disease Control & Prevention (CDC) has reported the prevalence of ASD to be 1 in 68 children in 2014 as compared to 1 in 88 in 2010. Autism Society of India has reported the prevalence rate of autism to be 1 in 250. Currently 10 million people are suffering from ASD in India. ASD shows significant skewness with respect to occurrence in boys having a sex ratio of 4:1 [19,27,62]. ASD is characterized by core symptoms like loss of social interaction, non-social approach, impairment of communication as well as by associated symptoms like irritable nature, anxiety, aggression, epilepsy and sensory processing disorder [5,25,70].

Neuroinflammatory response triggered by the stimulation of matrix metalloproteinases plays a pivotal role in the development of autistic phenotype. MMPs stimulate inflammatory cytokines release along with mitochondrial deficits that ultimately lead to neuronal dysfunction and precipitate autistic symptoms [51,58]. For the past two decades the scientific community is focusing on the exploration of neuropsychopharmacotherapeutic potential of phytochemicals in the plethora of human ailments. Curcumin (diferuloyl methane) is the primary curcuminoid which is present in the Indian spice turmeric (*Curcuma longa*) and is regarded as "Indian Solid Gold". Its several pharmacological activities like being anti-inflammatory, antioxidant, anti-carcinogenic and neuroprotective have been highlighted in various studies [2,15,30,34,46,56,61,64,71,74,81]. With this background, the current study was designed to explore neuropsychopharmacotherapeutic potential of curcumin against PPA-induced autistic behavior in Sprague–Dawley rats.

2. Material and methods

2.1. Animals

Male Sprague–Dawley rats (250–280 g), 3–4 months old, bred in the Central Animal House Facility of the Panjab University, Chandigarh (India) were used. The animals were housed under standard laboratory conditions, maintained on a 12 h light and dark cycle and had free access

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to food (Ashirwad Industries, Chandigarh, India) and water. The experimental protocols were approved by the Institutional Animal Ethics Committee of the Panjab University, Chandigarh, and conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals.

2.2. Drugs

Propanoic acid and curcumin were purchased from Sigma (St. Louis, MO, USA). TNF-α ELISA and MMP-9 assay kits were purchased from R & D Systems (USA). All other chemicals used for biochemical estimations were of analytical grade.

2.3. Induction of ASD-phenotype

Simons Foundation Autism Research Initiative (SFARI) is a discrete scientific research program within the Simons Foundation's overall suite of programs. SFARI has created a modular database for Autism Research (http://gene.sfari.org). Their database describes various modules like Human gene module, Animal model module, Protein Interaction Module and gene-scoring module. SFARI describes various genetic models, in bred strains, induced models as well as rescue models for autism. This model has been selected from their database with reference to MacFabe et al. [44]. There are other research reports, e.g., by Shultz et al. [66] which also describe this model. Intracerebroventricular injection of 1 M propanoic acid (1 M PPA) was performed according to the procedure of MacFabe et al. [44]. Rats were anesthetized with thiopentone sodium (Neon Laboratories, India, 45 mg/kg, i.p.). Their scalps were shaved, cleaned and cut to expose the skulls. Their heads were positioned in a stereotaxic frame and a midline sagittal incision was made in the scalp of each rat. Burr hole was drilled in the skull and a calibrated Hamilton syringe was inserted into the anterior portion of the caudoputamen (1.3 mm anterior to bregma, 1.8 mm right of midline, and 0.5 mm below dura) [52]. A solution containing 1 M PPA (Sigma Chemical Co), diluted in PBS to an infusion volume of 4 μl, was infused over 10 min. After withdrawal of the syringe, the burr hole was filled with dental cement and fixative was added on it. The skin was sutured followed by daily application of antiseptic Neosporin® powder. Postoperatively, the rats were fed with oral glucose and normal pellet diet for 4 days, followed by normal pellet diet alone. Sham animals received ICV injection of the same volume of sodium acetate, PBS, propanol control.

2.4. Treatment schedule

Rats were randomly selected and divided in eight groups of five animals each. First group consisted of naïve control animals, second group consisted of sodium acetate control (4.0 μl of 1 M solution in PBS), third group consisted of PBS control (4.0 μl PBS), fourth group consisted of propanol control (4.0 μl of 1 M Propanol in PBS), fifth group was the induction group consisting of 1 M PPA (4.0 μl in PBS), sixth group consisted of autism induced animals treated with curcumin (50 mg/kg/day; peroral), seventh group consisted of autism induced animals (1 M PPA, 4.0 μl in PBS) which were treated with curcumin (100 mg/kg/day; peroral), eighth group consisted of autism induced animals (as above) treated with curcumin (200 mg/kg/day; peroral). The effective dose of PPA was selected on the basis of preliminary studies conducted by us using 3 doses of PPA, i.e., 1 M PPA, 0.52 M PPA and 0.26 M PPA. Out of which 1 M was found to be the best. Compounds were dissolved in PBS vehicle and buffered to pH 7.5 using HCl or NaOH. Curcumin was started from the third day after surgery till 28th day of protocol after which the animals were sacrificed under deep anesthesia. Starting from the third day of the experiment till 28th day, control groups received only the vehicle of curcumin and other groups received the suspension of curcumin (50, 100 and 200 mg/kg; peroral). Preliminary dose range (10–200 mg/kg; peroral) studies of curcumin were carried out in our laboratory. The doses of curcumin were selected on the basis of our preliminary studies and published data [3,39,64]. Curcumin was suspended in 0.5% w/v sodium carboxymethylcellulose immediately before the administration in a constant volume of 5 ml/kg body weight. The neuro-behavioral tests were performed on 7th, 14th and 21st day in our studies. The tests were done in the following order: reciprocal social interaction, repetitive self grooming, three-chamber test, actophotometer, partition test, marble-burying, open field, rotarod, elevated plus maze and novel object recognition. These behavioral tests were performed in two consecutive days. Appropriate lag time was given for each test and the tests were kept in such an order that tasks which induce stress like the resident-intruder test and the three-chamber task were interspaced by such tasks which caused less stress and where single rat was evaluated for the task such as repetitive self-grooming. This way we could avoid confounding effect from one task to another. As the time-period for some tasks was very long, they were spread up to three days (rather than single day) to avoid confounding effect.

The induction of autistic response started from the 7th day as we could observe from some of the neurobehavioural tests. However, we found statistically significant changes in all the neurobehavioral tests on the 21st day. Therefore, we have displayed the data of the 21st day only, except for forced swim test, where we have shown the data for the 14th day, as on the 21st day there was no significant change in the results of forced swim test. Rats were tested for various behavioral paradigms. On the 14th day forced swim test was done and on the 21st day rats were tested for learning and memory as well as for repetitive behavior, which is characteristic of autistic disorder and it continued for 8 days. Morris water test continued for 8 days, i.e., from 21st–28th day with some modifications [9,75]. The animals were sacrificed under deep anesthesia, blood was collected from the tail-vein and serum separated. Whole brains were rapidly removed and the samples were stored at −80 °C until processed for biochemical and mitochondrial complex estimations [38]. The sodium acetate control group was taken as the acidic analog of PPA to see the effect of administration of acidic compound while PBS group served as sham control and propanol represented the alcoholic analog of PPA. The neurological, sensory and behavioral scoring was done by a blinded evaluator.

2.5. Neurobehavioural tests

2.5.1. Reciprocal social interaction

Reciprocal social interaction was observed between two rats; one an unfamiliar male rat from other home cage and the other one from each group. Total time spent in social and non-social interaction respectively was individually measured in the 10 min session, as described by Silverman et al. [67].

2.5.2. Three-chamber test for social preference and social novelty preference

Time spent in each chamber as well as the time in physical contact with each of the wire cages was recorded for 10 min for each session, as described by Karvat and Kimchi, [33].

2.5.3. Repetitive self-grooming

The time spent on grooming the head or the rest of the body was recorded for 10 min, as described by Moretti et al. [47].

2.5.4. Partition test

Time spent at the partition represents the amount of interest in the social partner. Different social partners can be sequentially placed in one compartment to evaluate social preference and social memory in the subject rat, as described by Silverman et al. [67].