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Research report

Neuroprotective effects of ceftriaxone treatment on cognitive and neuronal deficits in a rat model of accelerated senescence

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ABSTRACT

Rats of OXYS strain are characterized by genetically defined accelerated senescence. Ceftriaxone (CEF) exerts neuroprotective effects by decreasing the excitotoxicity and activation of antioxidant system. Here, we studied the effects of CEF (50 or 100 mg/kg/day, i.p., 36 days) on cognitive and neuronal deficits in 5-month-old OXYS rats. Chronic CEF administration in a dose of 100 mg/kg partially inhibited impairments of movement and restored the deficit in the novel object recognition in OXYS rats. Neuromorphologically, control OXYS rats exhibited a lowered neuronal density in the hippocampal CA1 area and there was a tendency to decrease in the substantia nigra pars compacta compared to Wistar controls. Both doses of CEF increased the density of pyramidal neurons in the CA1 area in OXYS rats. Control OXYS rats demonstrated a tendency to lower tyrosine hydroxylase (TH) immunoreactivity in the striatum compared with Wistar rats, while CEF treatment at a dose of 50 mg/kg significantly augmented this parameter. In control OXYS rats, the levels of neurogenesis in the subgranular zone of the dentate gyrus of the hippocampus were significantly higher than in Wistar rats indicating compensatory processes that probably prevented the further induction of neurogenesis by CEF. Restoration of the recognition function and neuronal density in the CA1 area in OXYS rats after CEF treatment might be related to activation of the mechanisms that provide survival of newborn and mature neurons. The data suggested CEF as a promising pharmacological tool for the prevention of cognitive decline at accelerated aging.

1. Introduction

Due to global population aging, dementia caused by neurodegeneration has received increasing attention. It is estimated that the United States alone has over five million patients with dementia.

Over the last few years, a large amount of experimental data has demonstrated that accelerated senescent OXYS rats are a suitable model of aging. OXYS rats were produced in the Institute of Cytology and Genetics of Siberian Branch of the Russian Academy of Sciences (Novosibirsk, Russia) by selective breeding of Wistar rats that were highly sensitive to the cataractogenic effect of D-galactose [1]. OXYS rats have a shortened lifespan and show early development of age-related pathological phenotypes similar to geriatric disorders observed in humans, including senile osteoporosis [2–5], cataract [6], retinopathy [7,8], decreased sexual motivation and reproductive dysfunction [9–11]. They also demonstrate cognitive deficit and neurodegeneration

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as early as 3 months of age [12,13] and have high levels of free radicals [14] and oxidative damage to DNA and proteins in liver mitochondria and cytosol [15–17]. These features make it possible to use OXYS rats to evaluate the efficacy of treatments for functional impairments in aging.

Glutamate, an excitatory neurotransmitter, plays a role in excitotoxicity and aging-related neurodegeneration [18]. Excessive release of glutamate can overstimulate *N*-methyl-D-aspartate (NMDA) receptors, causing calcium overload in neurons and triggering apoptotic cell death [19–21]. Thus, glutamatergic hyperactivity and oxidative stress contribute to neurodegeneration and cognitive deficits in aging. A recent study showed dysregulation of glutamatergic neurotransmission in several brain regions in patients with neurodegenerative disorders, for example, in Parkinson's disease (PD) [22]. Moreover, blockade of glutamatergic activity may attenuate neuronal and cognitive deficits at aging [23]. The blockade of NMDA receptors by using GLYX-13 has been found to be effective in the treatment of aging [24]. Reduction of glutamatergic hyperactivity has therefore been suggested as an effective therapeutic intervention for neurodegeneration and cognitive deficits in aging. Glutamate released at the synaptic cleft is taken up by glial cells via glutamate transporter-1 (GLT-1), thus terminating glutamate function at the synapse [25]. Increased clearance of glutamate from the synapse helps to prevent glutamate excitotoxicity [26–28] and could be an alternative strategy for protecting neurons from excitotoxic cell death.

Ceftriaxone (CEF), a beta-lactam antibiotic, increases GLT-1 expression and removal of released glutamate and ameliorates glutamate excitotoxicity [29]. Several studies have demonstrated the antiexcitotoxic potential of this compound [30,31]. In animal models, pretreatment with CEF (200 mg/kg/day) prevented ischemia and stroke-induced neurohistological and molecular changes in Wistar rats [32,33]. Treatment of rats with the same dose of CEF for 7 or 14 days during hypoxic exposure was found to increase GLT-1 expression, resulting in sequestration of excess glutamate in glial cells, protection of hippocampal neurons from excitotoxicity, and improved spatial memory retrieval [34]. Therapeutic effects of CEF have also been observed in animal models of amyotrophic lateral sclerosis [29], Huntington's disease [29,35,36], and spinal muscular atrophy [37,38]. Previous studies demonstrated that CEF rescues the impairment of hippocampal synaptic plasticity and memory formation in AQP4 knockout mice [39] and reduces DAergic degeneration and motor dysfunction in a 6-hydroxydopamine (6-OHDA)-induced PD model [40]. Our recent studies demonstrated that treatment with CEF at dosages of 100 and 200 mg/kg/day inhibits neurodegeneration in the hippocampus and nigrostriatal dopaminergic (DAergic) system and improves cognitive function in an 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD rat model [41–43].

Movement and cognitive impairments are associated with aging, and these deficits correlate with DAergic and hippocampal neurodegeneration. The present study was aimed to examine the potential neuroprotective effects of CEF in the treatment of cognitive and neuronal deficits in rats of OXYS strain with hereditary defined accelerated senescence.

2. Materials and methods

2.1. Experimental animals

14-week-old male Wistar rats weighing 440.1 ± 17.0 g and OXYS male rats of the same age weighing 304.9 ± 6.0 g from The Federal Research Center "Institute of Cytology and Genetics", Siberian Branch of the Russian Academy of Sciences (Novosibirsk, Russia) were used. Rats were housed in groups of five in acrylic cages (40 × 60 × 20 cm) in an animal room under standard conditions (a natural light-dark cycle (16 h light and 8 h dark), temperature: 18–22 °C, relative humidity: 50–60%, standard food and water *ad libitum*). Each animal was handled for 5 min/day on three consecutive days, before taking into experiment. Rats were divided into four experimental groups: Control (Saline-treated) Wistar males ("Wistar + saline" group, $n = 10$), Control (Saline-treated) OXYS males ("OXYS + saline" group, $n = 10$), Ceftriaxone-treated at a dose of 50 mg/kg OXYS males ("OXYS + Cef50" group, $n = 10$), and Ceftriaxone-treated at a dose of 100 mg/kg OXYS males ("OXYS + Cef100" group, n = 10).

All experimental procedures were performed according to the NIH Guide for the Care and Use of Laboratory Animals and approved by the Animal Care Committee of Chung Shan Medical University (IACUC approval No.: 1018). All efforts were made to minimize the number of animals used and their suffering.

2.2. General procedures

Starting from the age of 14-week old (day 1), Wistar rats received 36 daily intraperitoneal (i.p.) injections of saline while the respective groups of OXYS rats received 36 daily i.p. injections of saline or CEF (50 or 100 mg/kg/day). Rats were weighed weekly during the experiment to correct drug dosages.

In the last week of treatment, i.e. at the age of 19-week old, all animals were subjected to tests for behavioral phenotyping: the test for locomotor and exploratory activity on the day 33 of the treatment and the novel object recognition test on the days 34–36 as in our previous studies [44–48]. All behavioral observations were performed during the light phase between 12:00 and 18:00 h. For behavioral testing, the animals were placed individually in a clean cage (25 × 41 × 19 cm), and transported to a dim observation room (28 lx of the red light) with sound isolation reinforced by a masking white noise of 70 dB. Performance in the behavioral tests was monitored using a video camera (Sony, China) positioned above the apparatus and processed with original EthoVision XT software (Noldus, Netherlands). The test equipment and objects used in this study were cleaned using 20% ethanol and thoroughly dried before each test trial. On the day 37 the rats were sacrificed by exposure to CO_2, transcardially perfused with phosphate-buffered saline (PBS) and followed by 4% paraformaldehyde in PBS, then their brains were taken for further neuromorphological study.

2.3. Drug administration

Ceftriaxone was purchased from Roche (Switzerland). The rats were injected daily with either saline (1 ml/kg; Wistar + saline and OXYS + saline groups) or CEF at the dose of 50 (OXYS + Cef50 group) or 100 mg/kg/day (OXYS + Cef100 group) for 36 days. The rationale behind the CEF dosages (50, 100 mg/kg/day) adopted in the current study was based on our recent study showing neuroprotective effects of CEF in the subthalamic nucleus and hippocampal CA1 at the dosage of 200 and 100 mg/kg/day [5]. In this study, we also examined the effectiveness of CEF at the lower dose (50 mg/kg/day).

2.4. Behavioral tests

2.4.1. Open field test

Locomotion of the rats was measured in an acrylic open box (60 × 60 × 60 cm) as described earlier [11]. Distance travelled (in cm) by each rat was registered for 10 min.

2.4.2. Novel object recognition test

Recognition ability was measured using the novel object recognition test. The apparatus, an open box (60 × 60 × 60 cm), and the test procedure were identical to those in our previous reports [42,44–48]. Each rat was subjected to three exposure sessions at 24 h intervals (during days 34–36), then, 5 min after the exposure session on day 36, a test session was performed (Fig. 2A). Four different objects novel to the rats before the experiment were used for each rat to explore. Three of the objects ("A", "B", and "C") were fixed on the floor at a distance of 27 cm away from three corners of the arena. The experimental