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Research report

Ceftriaxone prevents the neurodegeneration and decreased neurogenesis seen in a Parkinson's disease rat model: An immunohistochemical and MRI study

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HIGHLIGHTS

- MEMRI and IHC staining were used to measure neuronal activity and density in rats.
- MPTP induced neurodegeneration in the hippocampus and DAergic system.
- MPTP decreased neurogenesis in the dentate gyrus of hippocampus.
- Ceftriaxone prevents MPTP-induced neuronal deficits.

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ABSTRACT

Manganese-enhanced magnetic resonance imaging (MEMRI) is a widely used technique for detecting neuronal activity in the brain of a living animal. Ceftriaxone (CEF) has been shown to have neuroprotective effects in neurodegenerative diseases. The present study was aimed at clarifying whether, in an 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced Parkinson's disease (PD) rat model, the known CEF-induced neuronal protection was accompanied by neurogenesis and decreased loss of neuronal activity. After MPTP lesioning (day 0), the rats were treated with CEF (100 mg/kg/day, i.p.) or saline for 15 days. They were then injected with $MnCl_2$ (40 mg/kg, i.p.) on day 13 and underwent a brain MRI scan on day 14, then the brain was taken for histological evaluation on day 15. The results showed that MPTP lesioning resulted in decreased neuronal activity and density in the nigrostriatal dopaminergic (DAergic) system and the hippocampal CA1, CA3, and dentate gyrus (DG) areas and reduced neurogenesis in the DG, but in hyperactivity in the subthalamic nucleus (STN). These neuronal changes were prevented by CEF treatment. Positive correlations between MEMRI R1 values and neuronal density in the hippocampus were evidenced. Neuronal densities in the hippocampus and SNc were positively correlated. In addition, the R1 value of the STN showed a positive correlation with its neuronal activity but showed a negative correlation with the density of DAergic neurons in the SNc. Therefore, MEMRI R1 value may serve as a good indicator for PD severity and the effect of treatment. To our knowledge, this is the first study showing that CEF prevents loss of neuronal activity and neurogenesis in the brain of PD rats. CEF may therefore have clinical potential in the treatment of PD.

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1. Introduction

Glutamatergic hyperactivity contributes to the neurodegeneration and cognitive deficits seen in Parkinson's disease (PD). Lesioning in the substantia nigra pars compacta (SNc) with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) results in a pathophysiology similar to that seen in PD [1,2] and causes motor dysfunction and hyperactivation in the glutamatergic system [3]. Ceftriaxone (CEF), a beta-lactam antibiotic, increases glutamate transporter 1 (GLT-1) expression and the removal of released glutamate and ameliorates glutamate excitotoxicity [4]. In models of ischemia and stroke in Wistar rats, 5 days of pretreatment with CEF (200 mg/kg/day) induced neurohistological changes [5,6]. Treatment 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 [7]. Therapeutic effects of CEF have also been observed in animal models of neurodegeneration [4,8–11]. Our recent studies in an MPTP-induced PD rat model demonstrated that treatment with CEF at dosages of 100 and 200 mg/kg/day prevented neurodegeneration in the hippocampus and nigrostriatal dopaminergic (DAergic) system and improved cognitive function [12,13]. However, it is not known whether CEF also affects the MPTP-induced decrease in neurogenesis in the hippocampus.

Manganese-enhanced magnetic resonance imaging (MEMRI) is a valuable imaging tool for direct measuring activity-dependent neuronal events in a living animal since its pioneer use in late nineties by Lin and Koretsky [14]. Manganese ion (Mn^{2+}) may enter neurons through voltage-gated Ca^{2+} channels [15] and be retained there with a biological half-life of 51–74 days as suggested from a study using the adult rat's brain [16]. Before MR imaging, the animal was injected intraperitoneally (i.p.) with $MnCl_2$ and neurons took up Mn^{2+} when activated. Since Ca^{2+} elevation is related to the firing activity in excitatory neurons, it has been quantitatively analyzed that Mn^{2+} accumulates in neurons depending on the neurons' activity [17]. In addition, the paramagnetic feature enable Mn^{2+} to shorten the longitudinal relaxation time (T1) of water protons and thus enhance the T1-weighted MR signal specific to the tissues where Mn^{2+} has accumulated [18], making it an excellent MRI-detectable contrast agent [19]. Therefore, tissue contrast in Mn^{2+}-induced T1 signal intensity in MEMRI may be contingent upon the differential accumulation of Mn^{2+} in active and silent brain regions which topography can be absolutely quantified by measuring the absolute T1 (or $R1 = T1^{-1}$) value [17,20].

A major drawback of the use of Mn^{2+} is the toxic side effects observed at high concentration (~mM). Mn^{2+} itself may alter neuronal activity by a variety of mechanisms, such as substituting for Ca^{2+} in the exocytotic process [21], enhancing the release of Ca^{2+} from intracellular stores [22], or activation of glutamate-gated cation channels, e.g. N-methyl-D-aspartate (NMDA) receptor [23] (for review, please see Ref. [15]). This is of concern as high-Mn^{2+} tissue levels often required to enhance contrast between structures. Therefore, there needs to be a compromise between avoiding toxicity and delivering adequate doses of manganese. For this regard, a wide range of Mn^{2+} solution volume and concentrations should be tested for the optimization before carrying out experiments [24] while recent studies in rat [25] and mice [17] have suggested alternations might be induced by concentrations of $Mn^{2+} > 200\ \mu M$ based on literature [26–28] and their own findings.

A previous study showed that systemic injection of $MnCl_2$ (40 mg/kg) causes enhancement of hippocampal MR signals (20.7%) without disrupting sensorimotor function or hippocampal-dependent memory in a rapid place learning task [29]. In combination with pharmacological manipulation, MEMRI can be used to detect changes in neuronal activity that are produced by the treatment. We have shown that CEF treatment prevents the decrease in the density of neuronal terminals in the striatum and cell bodies in the SNc and hippocampus seen in MPTP-treated rats [12,13]. However, whether these changes are accompanied by prevention of the loss of neuronal activity is not yet clear. The purpose of this immunohistochemical and MEMRI study was to elucidate the effects of treatment with CEF (100 mg/kg/day) on neuronal density, activity, and neurogenesis in the brain of rats with MPTP-induced PD.

2. Materials and methods

2.1. Animals

Male Wistar rats (12-week-old weighing 460.4 ± 5.9 g; Bio-LASCO Taiwan Co., Ltd., ROC) were housed in groups of four in acrylic cages (35 cm × 56 cm × 19 cm) in an animal room (21–25 °C) on a 12 h light-dark cycle (lights on at 07:00 h) with food and water available *ad libitum*. Before being used in the study, each animal was handled for 5 min/day on 3 consecutive days, starting one day after arrival, to reduce defensive behavior and the stress response to the experimenter [30]. All experimental procedures were performed according to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care Committee of Chung Shan Medical University (IACUC approval No. 1455). All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to *in vivo* techniques, if available.

2.2. General procedures

The animals were divided into three groups of 5 rats, a "sham-operated" group and two "MPTP-lesioned" groups, and underwent stereotaxic brain surgery and MPTP lesioning or sham operation on day 0 as described in our previous reports [12,13,31–35]. Briefly, the rats were anesthetized by i.p. injection of Zoletil (20 mg/kg; Virbac, Carros, France), then the 2 MPTP-lesioned groups were bilaterally infused with MPTP-HCl (1 μmol in 2 μl of saline; Sigma, MO, USA) into the SNc using the following coordinates adapted from the rat brain atlas (see Ref. [36] AP: (−5.0 mm, ML: ±2.0 mm, DV: −7.7 mm from the bregma, midline, and skull surface, respectively), while the sham-operated group was bilaterally infused with 2 μl of saline. Immediately after surgery, the rats were injected intramuscularly with penicillin-G procaine (0.2 ml, 20,000 IU) to prevent infection, then were housed individually in acrylic cages for a week before being returned to their initial home cages. During the first 5 post-operative days, 10% sucrose solution was provided *ad libitum* to prevent weight loss after surgery and reduce mortality [37,38]. Starting on day 0, the sham-operated group was injected i.p. daily with saline (1 ml/kg/day; Sham + saline group), while the MPTP-lesioned groups were injected i.p. daily with either saline (1 ml/kg/day, MPTP + saline group) or CEF (100 mg/kg/day, MPTP + CEF100 group) for 15 days. Rats from the same home cage underwent the same treatment.

Hydrated manganese chloride ($MnCl_2 \cdot 4H_2O$, Sigma–Ulrich, UK) was dissolved in saline at a concentration of 100 μmol/ml (20 mg/ml). On day 13, all rats received two i.p. injections of 1 ml/kg of $MnCl_2$ solution separated by 1 h (total dose 40 mg/kg) [29]. Twenty-four hours later (day 14), a time when Mn^{2+}-induced signal enhancement has been reported to reach a stable asymptotic level [29], the rats were transported to the MR center for MR imaging, anesthetized during imaging, and transported back to the animal room.

To measure neuronal density and neurogenesis, rats were injected with 5′-bromo-2′-deoxyuridine (BrdU), a marker of