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Research report

Synergistic effects of ceftriaxone and erythropoietin on neuronal and behavioral deficits in an MPTP-induced animal model of Parkinson's disease dementia

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HIGHLIGHTS

- MPTP caused impairments in working memory and recognition as well as neurodegeneration.
- Ceftriaxone reduced MPTP-induced behavioral and neuronal deficits.
- Erythropoietin reduced the MPTP-induced neurodegeneration.
- Combination with the above two drugs showed greater behavioral and neuronal effects.

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ABSTRACT

Both ceftriaxone (CEF) and erythropoietin (EPO) show neuroprotection and cognitive improvement in neurodegenerative disease. The present study was aimed at clarifying whether combined treatment with CEF and EPO (CEF + EPO) had superior neuroprotective and behavioral effects than treatment with CEF or EPO alone in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced Parkinson's disease (PD) rat model. The rats were injected with CEF (5 mg/kg/day), EPO (100 IU/kg/day), or CEF + EPO after MPTP lesioning and underwent the bar-test, T-maze test, and object recognition test, then the brains were taken for histological evaluation. MPTP lesioning resulted in deficits in working memory and in object recognition, but the cognitive deficits were markedly reduced or eliminated in rats treated with CEF or CEF + EPO, with the combination having a greater effect. Lesioning also caused neurodegeneration in the nigrostriatal dopaminergic system and the hippocampal CA1 area and these changes were reduced or eliminated by treatment with CEF, EPO, or CEF + EPO, with the combination having a greater effect than single treatment in the densities of DAergic terminals in the striatum and neurons in the hippocampal CA1 area. Thus, compared to treatment with CEF or EPO alone, combined treatment with CEF + EPO had a greater inhibitory effect on the lesion-induced behavioral and neuronal deficits. To our knowledge,

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this is the first study showing a synergistic effect of CEF and EPO on neuroprotection and improvement in cognition in a PD rat model. Combined CEF and EPO treatment may have clinical potential for the treatment of the dementia associated with PD.

1. Introduction

Glutamatergic hyperactivity and oxidative stress contribute to neurodegeneration and cognitive deficits in Parkinson's disease (PD). Ceftriaxone (CEF), a beta-lactam antibiotic, increases glutamate transporter 1 (GLT-1) expression and removal of released glutamate and ameliorates glutamate excitotoxicity [1]. In models of ischemia and stroke in Wistar rats, 5 days of pretreatment with CEF (200 mg/kg/day) induced neurohistological changes [2,3], while 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 improvement in spatial memory [4]. Therapeutic effects of CEF have also been observed in animal models of neurodegeneration [1,5–8]. 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 a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD rat model [9,10].

Erythropoietin (EPO), a hormone secreted from the kidneys, increases hemoglobin production and is used to treat anemia [11]. However, in an MPTP-induced PD rat model, EPO administration was found to increase nitric oxide levels in the substantia nigra and to be neuroprotective [12]. A study using a PD model in mice showed that EPO increases the production and activity of glutathione peroxidase in astrocytes in the substantia nigra, thus increasing the antioxidative function of astrocytes [13]. A study in rats showed that EPO treatment has a neuroprotective effect in hypoxia-induced brain damage and this was suggested to be due to an anti-excitotoxicity effect [14]. Further more, treatment with EPO increased the expression of glutamate transporter and activity of glutamine synthetase, and thus enhanced extracellular glutamate clearance and metabolism and prevented glutamate-induced cell injury [15,16]. These results indicate that CEF and EPO have a common effect of reducing glutamate-related excitotoxicity and thus may suggest a synergistic neuroprotection of combined treatment with CEF and EPO.

MPTP lesioning in the substantia nigra pars compacta (SNc) results in a pathophysiology similar to that seen in PD [17,18] and causes motor dysfunction [19,20] and hyperactivation in the glutamatergic system [21]. In addition, cognitive impairment has been observed in MPTP-lesioned rats, which shows disturbances of learning in the two-way active avoidance test [22] and the Morris water maze test [20,22,23]. Our previous studies demonstrated that MPTP-lesioned rats show memory and recognition deficits, thus serving as a model of PD dementia (PDD) [24–27]. The purpose of this study was to elucidate the effects of combined treatment with CEF and EPO on cognitive and neuronal functions in this MPTP-induced PD rat model.

2. Materials and methods

2.1. Animals

Male Wistar rats (12-weeks-old, weighing 460.4 ± 5.9 g; BioLASCO 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 [28]. 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. 1358). 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 rats (n = 48) were divided into a "sham-operated" group (n = 10) and a larger "MPTP-lesioned" (n = 38) group and underwent stereotaxic surgery on day 0. Brain surgery was performed as described in our previous reports [9,10,24,26,27,29]. Briefly, the rats were anesthetized by intraperitoneal injection (i.p.) of Zoletil (20 mg/kg; Virbac, Carros, France), then the "MPTP-lesioned" rats 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 (AP: −5.0 mm, ML: ±2.0 mm, DV: −7.7 mm from the bregma, midline, and skull surface, respectively) [30], while the sham-operated group was bilaterally infused with 2 μl of saline. Starting on the day of surgery (day 0), the sham-operated group received daily i.p. injections of saline (1 ml/kg/day; Sham + saline group; n = 10), while the MPTP-lesioned rats were divided into 4 groups that were injected daily with saline (1 ml/kg/day, MPTP + saline group, n = 10), CEF (5 mg/kg/day, MPTP + CEF group, n = 8), EPO (100 IU/kg/day, MPTP + EPO group, n = 10), or CEF (5 mg/kg/day) plus EPO (100 IU/kg/day) (MPTP + CEF + EPO group, n = 10). Rats from the same home cage underwent the same treatment. 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 for recovery before being returned to their 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 [20,31].

The rats were then subjected to a battery of behavioral tests performed as in our previous studies [9,10,24,26,27,29], namely a bar test on days 1–7, a T-maze test on days 8–10, and an object recognition test on days 11–13. All behavioral tests were started at least 2 h after the beginning of the light phase (7:00 h) and were performed in a dim observation room (21 l× red light) with sound isolation reinforced by a masking white noise of 70 db, as in our previous studies [25,27,29]. The rat's performance in the T-maze and object recognition tests was monitored by a video camera positioned above the apparatus and scored using a home-made video image analysis system (VIAS) [32]. The spatial resolution of the VIAS was 0.7 cm and the rate of image processing higher than 14 pictures/s. The test equipment and objects used in this study were cleaned using 20% ethanol and thoroughly dried before each trial. On day 14 after surgery, the rats were euthanized by exposure to CO_2, transcardially perfused with phosphate-buffered saline (PBS) fol-