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Research article

Neuroprotective effect of combined therapy with hyperbaric oxygen and madopar on 6-hydroxydopamine-induced Parkinson's disease in rats

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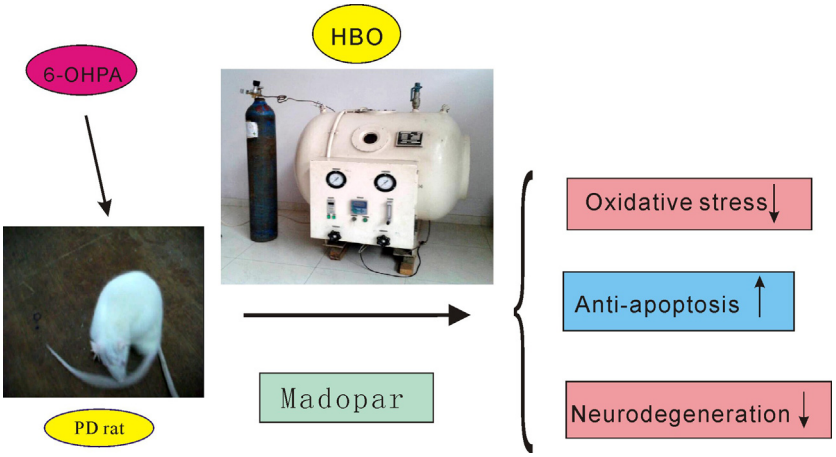
HIGHLIGHTS

- The combination therapy attenuated apomorphine-induced turns in PD rats.
- The combination therapy reduced oxidative stress and modulated the level of anti-apoptosis.
- The combination inhibited neurodegeneration of PD rats.

GRAPHICAL ABSTRACT



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ABSTRACT

Parkinson's disease (PD) is a common movement disorder in the elderly. In the present study, we examined whether the combination of hyperbaric oxygen (HBO) and madopar therapy provided a neuroprotective effect on dopaminergic neurons in the substantia nigra using a rat model of PD. Rotational assessments revealed that both HBO and combination therapy significantly attenuated apomorphine-induced turning in PD rats. Our results indicated that the combination therapy increased glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities and reduced the malondialdehyde (MDA) content in the SN. Furthermore, the combination therapy resulted in significant protection against the loss of neurons, and specifically tyrosine hydroxylase (TH)-positive neurons, in the SN and also alleviated the production of glial fibrillary acidic protein (GFAP). The levels of Bcl-2 were increased and Bax were decreased following the HBO or combination therapy. In brief, the neuroprotective effect of combined therapy with HBO and madopar against 6-OHDA-induced PD rats may rely on its ability to reduce oxidative stress and protect against Bax/Bcl-2-mediated apoptosis.

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder that is characterized by resting tremor, rigidity and

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bradykinesia and is a common movement disorder in the elderly [13,20]. The conventional therapy using levodopa and dopamine agonists for PD focuses primarily on relieving the motor symptoms, but this therapy does not prevent dopaminergic neuron degeneration [8,25]. Thus, there is a great demand for novel therapies that prevent neuronal death. Increasing evidence suggests that one crucial factor in the pathogenesis of PD is oxidative stress, which represents the physiological response to reactive oxygen species (ROS) production [18] in PD patients. Increased ROS production and an imbalance of the antioxidant defense and repair mechanisms lead to the loss or apoptosis of dopaminergic neurons in the substantia nigra (SN) [4]. Thus, the reversion of cellular oxidative damage represents an effective strategy for PD treatment [11].

Hyperbaric oxygen (HBO) therapy is a unique method used for the treatment of various illnesses and clinical conditions, such as carbon monoxide poisoning, cerebral ischemia, and even PD [3,35]. Increasing evidence suggests that superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase activity are increased after repeated HBO treatments [9,10], although some reports show that HBO treatment may cause excessive generation of ROS, which induces neuronal cell damage [2]. On the other hand, HBO therapy attenuates hypoxia in affected tissues, which is helpful for the protection of neuronal function. There is a great deal of recent evidence indicating that HBO therapy prevents neuronal damage and improves neurological outcome after brain ischemia [26,33]. Therefore, in our current study, 6-hydroxydopamine hydrochloride (6-OHDA)-lesioned rats were used as a model of PD to explore the protective effects of combined therapy with HBO and madopar on 6-OHDA-induced behavioral, biochemical, and pathological changes and to determine the underlying mechanisms by which the combination therapy modulates the hemi-parkinsonian factors involved in neuronal degeneration and oxidative stress.

2. Materials and methods

2.1. Animals

A total of 85 male Wistar rats weighing 250–290 g were obtained from the Experimental Animal Center of Guangxi Medical University [SYXK 2009-0002] and were allowed to acclimate in quarantine for one week prior to experimentation. The experimental animals were treated according to the Guidance Suggestions for the Care and Use of Laboratory Animals issued by the Ministry of Science and Technology of the People's Republic of China.

2.2. Unilateral 6-OHDA lesion

Surgery was performed as previously described [24], with minor modifications. Briefly, after assessing their preoperative rotation behavior to confirm that there were no abnormal rats, the rats were anesthetized using sodium pentobarbital (30 mg/kg, i.p.). Then, the rats were fixed in a stereotaxic frame. According to the atlas from [28], the coordinates of the right caudate putamen (CPu) are located 0.2 mm anterior to Bregma, 3.0 mm lateral to the sagittal suture, and 5.5 mm below the surface of the skull. Next, each animal, except for those in the sham group (injected with 10 μL of 0.2% ascorbate–saline solution), was injected with 10 μL of fresh 6-OHDA solution (diluted in 0.2% ascorbate–saline solution; final concentration of 2.0 g/L) into the CPu using a microinjector (Shanghai, China). One week later, the rats were injected with the same dose of 6-OHDA into the same location again. Two weeks later, the rats were subcutaneously injected with apomorphine (0.1 mg/kg) to induce rotation to the left side [7]. The rats exhibiting contralateral rotations faster than 7 rotations per min were considered as valid models of PD.

2.3. Study design

The qualified PD model rats (65.75%) were randomly separated into 4 groups (Groups B–E) containing 12 rats per group. Group B served as the model group, and was intragastrically administered normal saline once daily for 14 consecutive days. Group C was intragastrically given madopar (L-DOPA + benserazide, 25 + 6.25 mg/kg) for 14 days [15]. Group D received HBO treatment. Besides HBO, Group E was given madopar (the same dose of group C) treatment. Group A served as the sham group, and was intragastrically administered the same volume of normal saline as group B. The rotational behavior of the animals was tested again with apomorphine (0.1 mg/kg, s.c.) after 14 days of treatment (Fig. 1). The data are presented as the total number of rotations over 30 min.

2.4. Hyperbaric therapies

The animals were placed in hyperbaric chambers as previously described [10]. Then, the pressure was increased to 0.25 MPa at a rate of 100 kPa per min, and each treatment lasted for 60 min. Decompression was performed at a uniform rate over 10 min. HBO treatment was performed once daily for consecutive 14 days.

2.5. Measurement of SOD, GSH-Px and MDA levels in SN tissue homogenates

At the end of the experiment, six rats from each group were anesthetized using sodium pentobarbital (30 mg/kg, i.p.), and their brains were then removed. According to the rat brain stereotaxic coordinates [28], the right substantia nigra (SN) was isolated and homogenized in ice-cold physiological saline (10% w/v) to produce a 5% homogenate. The contents of SOD, GSH-Px and MDA were determined using commercially available kits (Nanjing Jiancheng Bioengineering Institute, China).

2.6. Histological and immunohistochemical examination of SN tissue

After 14 days of treatment, the remaining six rats from each group were anesthetized using sodium pentobarbital (30 mg/kg, i.p.) and subjected to a thoracotomy. The animals were rapidly perfused using 250 mL of normal saline followed by 250 mL of 4% paraformaldehyde at 4 °C to remove any remaining blood in the brain. The right SN samples were isolated, processed by routine histology procedures, embedded in paraffin, cut into 4 μm-thick section and mounted on the slide. The samples were stained with hematoxylin and eosin (HE) for histopathological examination or incubated with different antibodies for immunohistochemical examination.

According to the method of previous study [21,22,34], the waxed specimens were incubated with primary antibody (tyrosine hydroxylase (TH) antibody, or GFAP antibody, or Bax antibody, or Bcl-2 antibody, 1:100 dilution, Zhongshan Goldenbridge Biological Technology, Beijing, China) overnight at 4 °C, washed three times with 0.1 mol/L PBS for 3 min each, and incubated with biotinylated goat antirabbit immunoglobulin G (Zhongshan Goldenbridge Biological Technology, Beijing, China) at 30 °C for 25 min. After washing three times with 0.1 mol/L PBS for 3 min each, the specimens were incubated with a streptavidin–biotin complex at 30 °C for 20 min. Then, they were rinsed five times in 0.1 mol/L PBS for 3 min, incubated with diaminobenzidine for 15 min at room temperature, counterstained with hematoxylin, cleared, mounted and examined.

Cell counts and the density of GFAP-immunopositive fibers were analyzed in five independent sections each from all of the experimental groups and quantified using a microscope at a magnification