Laboratory studies

Effects of vinpocetine and ozagrel on behavioral recovery of rats after global brain ischemia

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ABSTRACT

Brain ischemia leads to severe disruption of the nervous system and recovery is often prolonged. Rehabilitative post-ischemia pharmacological treatment may therefore be important for behavioral recovery, especially for cognition and motor behavior. The present study investigated the effects of combined vinpocetine and ozagrel administration on the behavioral recovery of rats from global brain ischemia. The results suggest that the combined treatment leads to significantly better improvement compared to single drug administration. We conclude that the combined use of vinpocetine and ozagrel may provide beneficial effects to patients suffering from brain ischemia.

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1. Introduction

Brain ischemia leads to severe cognitive and behavioral deficits. Acute pharmacological interventions, within several hours of brain ischemia, may be useful for neuroprotection; while in later periods damage may be irreversible and accompanied by long-term neurodegeneration. Pharmacological treatments in the later period aim at neurorehabilitation and represent the situation most commonly seen in clinical practice.

Vinpocetine is extracted from the periwinkle plant, and is used to dilate the cerebrovascular system, improve microcirculation and decrease platelet aggregation [1–5]. Vinpocetine has been used in a number of clinical conditions to improve cerebrovascular circulation and to prevent blood coagulation. In addition, vinpocetine has anti-neuroinflammatory properties, and therefore has been used to treat many age-associated neurodegenerative diseases, including Alzheimer's disease and Parkinson's disease [6–8].

Ozagrel is an antiplatelet agent, which inhibits thromboxane A2 synthesis. It was approved in 1988 by the World Health Organization to be used to improve postoperative cerebral vasoconstriction and the associated cerebral ischemia [9–11]. It was found to restore motor system deficits [12], but some studies demonstrated kidney dysfunction with high doses. The present study investigated the effects of vinpocetine-ozagrel combined administration on the behavioral recovery of rats after global brain ischemia. We examined both neuron density in the CA1 region and hippocampus dependent short-term memory in rats in a four-vessel occlusion model of global brain ischemia. We aimed to provide a rationale for the use of these agents in the clinical care of patients after brain ischemia.

2. Materials and methods

2.1. Animal model of four-vessel occlusion

Forty male Sprague-Dawley (Harlan Laboratories, Indianapolis, IN, USA) rats (300–400 g) from the Animal Experimental Center of Xinxiang Medical University were used. Four animals were used in a preliminary experiment. The animals were randomly assigned into four groups: sham operated group; non-treated control group (C); 7 days vinpocetine-ozagrel treatment group (VO); and 7 days vinpocetine treatment group (V) (n = 9 each). All procedures were in accordance with the ethical guidelines for animal research provided by our local Ethics Committee.

For the global ischemia model, the rats were anesthetized with isoflurane (initiated and maintained with 5% and 1.5% isoflurane, respectively). Then the animals' heads were fixed to coagulate the vertebral arteries at the first cervical vertebra. Both common carotid arteries (CCA) were isolated but not wounded. The rats were left to recover in their home cage for another 24 hours before CCA occlusion, using aneurysm clips, for 10 minutes. The clips were then removed and the animals were left to recover in a warm environment before being placed back in their home cage. For the sham operated group, no CCA occlusion was performed.
2.2. Pharmacological treatments

Sodium ozagrel (20 mg/kg) and vinpocetine (100 mg/kg) were given orally from day 5 after the surgery, and continuing for another 7 days before behavior assessment (12 days after surgery). The dosage of both drugs demonstrated no adverse effects and no animal deaths were observed in the preliminary trial period.

2.3. Y maze test

Because the Morris water maze requires long-term training, 12 days after the surgery we performed the Y maze test to evaluate immediate spatial learning and short-term spatial memory formation, both of which are dependent on hippocampal function. Briefly, three 40 cm long arms with 120 degree intervals were used; the arms were 20 cm high and 15 cm wide. The rats were allowed free exploration of the Y maze for 8 minutes, and the number of spontaneous alternations (three consecutive entries into three different arms) were recorded. The locomotor activity was evaluated as the total number of entries into all arms.

2.4. Histology

Fourteen days after the surgery, and after all behavior tests, the animals were sacrificed for histological analyses. The animals were perfused with 4% paraformaldehyde as a fixative and the brains were removed. Coronal hippocampal slices were cut on a freezing microtome and 40 μm sections (10 slices from the left side of the brain of each animal) were stained using cresyl violet for Nissl staining to count the number of surviving neurons in the CA1 region of the hippocampus.

2.5. Statistics

The data were represented as mean ± standard error of the mean. Statistical tests were carried out with the Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) software. p < 0.05 was considered as significant in paired t-tests.

3. Results

3.1. Changes in neuron density of CA1 after global brain ischemia

In the sham operated group the neuron density was 515 ± 24 cells/mm². However, in C group at 14 days after global brain ischemia, neuron density was 186 ± 80 cells/mm², suggesting a loss of neurons following global ischemia.

The neuron density of CA1 in the VO group was 342 ± 67 cells/mm², which was significantly different from the untreated C group (p < 0.01). Additionally, in the V group the neuron density of CA1 was 277 ± 49 cells/mm², suggesting a partial beneficial effect of vinpocetine (Fig. 1).

3.2. Y maze behavior

We then investigated whether the changes in neuron density of CA1 correlated with the behavior changes seen in the Y maze, as this is a hippocampus-dependent task. In line with data we have previously described, we found a reduction in alternation behaviors after brain ischemia. However when compared to the control group, there were significant improvements in the VO and V groups for the number of arm entries, suggesting motor behavior restoration with 7 days of treatment with vinpocetine and/or ozagrel (p < 0.05). Interestingly, the alternation behavior, which reflects cognitive function, was different between the treatment groups. The VO group showed significant improvement while the V group did not, suggesting that vinpocetine treatment selectively restored motor behavior when used alone (Table 1).

4. Discussion

Brain ischemia leads to severe cognitive and behavioral deficits. Post-ischemia recovery and rehabilitation has received increasing attention in recent years. Additionally, the critical clinical care of brain ischemia patients often requires major procedures, including secondary surgeries and general anesthesia, which can also lead to a severe reduction in cognitive ability. Therefore post-ischemia care with pharmacological treatments is an area of interest in neurorehabilitation and behavior recovery.

Vinpocetine is used to dilate the cerebrovascular system, improve microcirculation and decrease platelet aggregation. The clinical applications of vinpocetine range from age-associated neurodegenerative diseases, including Alzheimer's disease and Parkinson's disease, to ischemia, as well as other cerebrovascular system diseases [1–3,13–17]. Ozagrel is an antiplatelet agent, and is used to target motor deficits after brain ischemia [9,12,18–20]. It is conceivable that the combination of the two drugs would target both the cognitive and motor systems, encouraging functional recovery. Indeed, we found that the use of vinpocetine alone is neuroprotective and can improve cognition as tested by the Y maze; however vinpocetine alone did not restore motor function as well as the combined vinpocetine-ozagrel treatment.

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Spontaneous alternation (%)</th>
<th>Number of arm entries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operation</td>
<td>74.2 ± 6.5</td>
<td>29.2 ± 4.7</td>
</tr>
<tr>
<td>Ischemia Control</td>
<td>32.7 ± 9.7 ***</td>
<td>12.1 ± 5.1</td>
</tr>
<tr>
<td>VO</td>
<td>58.1 ± 3.9 * #</td>
<td>19.9 ± 4.2 *</td>
</tr>
<tr>
<td>V</td>
<td>36.1 ± 4.3 *** #</td>
<td>18.2 ± 2.9 *</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard error of the mean.
* p < 0.05 versus sham group.
** p < 0.01 versus sham group.
# p < 0.05 versus control group.
In conclusion, the combined use of vinpocetine and ozagrel is a promising treatment for neurorehabilitation after brain ischemia. The present study commenced treatment 5 days after the ischemic event, representing a clinically relevant time for treating patients. In future studies we expect to examine this treatment commencing at different time points with a varied length of treatment in order to find the optimal treatment window in post-ischemia brain rehabilitation.

Conflicts of Interest/Disclosures

The authors declare that they have no financial or other conflicts of interest in relation to this research and its publication.

References