Nimodipine Improves Regional Cerebral Blood Flow and Suppresses Inflammatory Factors in the Hippocampus of Rats with Vascular Dementia

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OBJECTIVE: To study the effect of nimodipine on hippocampal regional cerebral blood flow (rCBF) and proinflammatory cytokines in rats with experimental vascular dementia.

METHODS: Male Sprague Dawley rats were randomly divided into four groups (n = 15/group): sham operated controls (group A); focal cerebral ischaemia (group B); vascular dementia (group C); and vascular dementia treated with 20 mg/kg nimodipine daily (group D). The Morris water maze test evaluated learning and memory, and magnetic resonance perfusion-weighted imaging was used to measure rCBF. Hippocampal levels of nuclear factor-κB (NF-κB), tumour necrosis factor-α (TNF-α) and interleukin 1β (IL-1β) were measured.

RESULTS: Compared with group C, rats in group D demonstrated significantly improved learning ability and significantly increased hippocampal rCBF. The levels of NF-κB, TNF-α and IL-1β were significantly lower in group D than in group C. Hippocampal nerve cell morphology was abnormal in group C but near normal in group D.

CONCLUSIONS: Nimodipine improved the symptoms of cognitive impairment, increased rCBF, reduced hippocampal cytokine levels and alleviated neuronal injury in the hippocampus of rats with experimental vascular dementia.

KEY WORDS: CALCIUM CHANNEL BLOCKER; CEREBRAL BLOOD FLOW; COGNITIVE FUNCTION; CYTOKINES; HIPPOCAMPUS; MORRIS WATER MAZE TEST; NIMODIPINE; VASCULAR DEMENTIA

Introduction

Vascular dementia is the most frequent psychiatric complication of stroke and is often difficult to treat.¹ Neurobiochemistry studies²,³ and cellular and molecular mechanism studies have all reported the frequent involvement of hippocampal damage in the physiopathology of vascular dementia in stroke patients.⁴,⁵ The neuroanatomy hypothesis postulates that infarcts located in strategic areas play a key role in the mechanism of cognitive impairment and provide a means of quantification.⁶ Studies have shown that abnormal cytokine networks are important in the development of nerve cell damage that
leads to cognitive impairment after stroke.7–9 Tumour necrosis factor-α (TNF-α) impairs the recovery of synaptic transmission following hypoxia in rat hippocampal slices.10 It has been shown that cytokine levels increase significantly in patients with vascular dementia and that there is a strong correlation between cytokine levels and vascular dementia severity.11

Vascular dementia is triggered by various cerebrovascular diseases, with 80% being ischaemic cerebrovascular disease.12 Vascular dementia is induced by vascular factors, chronic hypoxia, reduction in regional cerebral blood flow (rCBF) and molecular events that precede major ischaemia.13 It has been suggested that targeting vascular factors and improving cerebrovascular lesions could mitigate the symptoms of vascular dementia.14 Studies have focused on the involvement of nonvascular factors in vascular dementia, including oxidative stress, glutamate and nitric oxide.15,16 Future vascular dementia research should focus on improving permanent cerebral ischaemia.

Therapies such as fastigial nucleus electrical stimulation have been introduced for the treatment of vascular dementia.1,17 Few medical treatments for dementia have shown any clear therapeutic effects, however.18 The calcium channel blocker nimodipine dilates cerebral blood vessels and can pass through the blood–brain barrier,19 providing neuroprotective effects by selectively improving rCBF and inhibiting neuronal necrosis and apoptosis.20–22 Nimodipine has been shown to have a good therapeutic effect with few side-effects23 and it is recommended in some countries as a treatment for vascular dementia.24,25

The neuroprotective effect of nimodipine has been studied in detail,26–28 but evidence regarding the mechanism of action of nimodipine in vascular dementia is scarce. The present study investigated the effects of nimodipine on rCBF, histopathological changes and the levels of cytokines in the hippocampus of rats with experimental vascular dementia.

Materials and methods

ANIMALS

Male Sprague Dawley rats (16–18 months old, 300–450 g body weight) were provided by the Animal Experimental Centre of Liaoning Medical College, Jinzhou, China. Animals were housed with free access to food and water at a mean ± SD constant temperature of 22 ± 2°C, humidity of 55 ± 5%, and a 12-h light/12-h dark cycle. The study protocol was reviewed and approved by the Animal Ethics Committee of Liaoning Medical College. Animals were handled according to the guidelines for the capture, handling and care of mammals, approved by the American Society of Mammalogists.29

ANIMAL MODEL AND EXPERIMENTAL GROUPS

Rats (n = 70) were randomized into four groups before surgery according to a computer-generated randomization schedule: group A, sham operated control animals; group B, focal cerebral ischaemia model; group C, vascular dementia model; and group D, vascular dementia model treated with nimodipine.

Rats were anaesthetized with 10% chloral hydrate (0.3 ml/100 g; Sigma-Aldrich, St Louis, MO, USA) intraperitoneal injection, and a midline incision was made to expose the bilateral common carotid arteries. The vascular dementia model was induced in groups C and D using the bilateral middle cerebral artery occlusion–reperfusion method as described previously.30 Briefly, ischaemia was induced by intraluminal filament (mean ± SD 18.5 ± 0.5 mm) occlusion of the middle
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cerebral artery for 1 h. Mean body temperature (taken rectally) was maintained during surgery at 37.0 ± 0.5°C using a heating pad. Postoperative neurological function was scored on a five-point scale where 0 indicated no neurological deficit, 1 (failure to extend left forepaw fully) indicated mild focal neurological deficit, 2 (circling to the left) indicated moderate focal neurological deficit, 3 (falling to the left) indicated severe focal deficit, and 4 (did not walk spontaneously) indicated a depressed level of consciousness. Rats that scored ≥ 3 points were selected for inclusion in groups C and D. Rats in group D received 20 mg/kg nimodipine (Nimotop®; Bayer AG, Leverkusen, Germany) by gastric perfusion on day 1 after surgery and once a day thereafter for the duration of the experimental period. Animals in the other three groups received an equivalent volume of normal saline by gastric perfusion, to the same schedule.

Sham operated control animals (group A) underwent surgery to expose but not occlude the middle cerebral artery. Rats with no postoperative neurological deficit (neurological function score 0) were included in group A. Focal cerebral ischaemia was induced in group B by 15 min treatment with a short intraluminal filament (10 mm) that did not make contact with the middle cerebral artery, as described previously. Animals with postoperative neurological function scores of 1 – 2 were included in group B. In total, 10 rats were excluded from the study on the basis of postoperative neurological testing. After surgery and exclusions, each experimental group comprised 15 rats.

MORRIS WATER MAZE TEST
Each experimental group was divided into two subgroups: animals that were trained in the Morris water maze test at 2 months after surgery (n = 7 from each group); and animals that were trained at 4 months after surgery (n = 8 from each group). The Morris water maze test placed the animal in a large pool of water with a submerged escape platform, the position of which was kept unaltered throughout the training sessions. Each animal was subjected to acquisition trials on four consecutive days, with four consecutive trials on each day and a gap of 5 min between trials. The rat was gently placed in the water facing the wall of the pool, with the drop location changing for each trial, and allowed 120 s to locate the submerged platform, where it was allowed to remain for 20 s. If the animal failed to find the platform within 120 s it was guided gently onto the platform and allowed to stay there for 20 s. The time taken to locate the platform (escape latency time [ELT]) was noted as the index of acquisition or learning.

PWI
After completion of the Morris water maze test, magnetic resonance perfusion-weighted imaging (PWI) was performed using a 1.5-T magnetic resonance scanner (Intera®; Philips Healthcare, Best, The Netherlands). A 47-mm surface coil was placed on the head region and a bolus injection of gadolinium diethylenetriaminepenta-acetic acid (0.2 mmol/kg) was administered through a tail vein cannula. A dynamic series of 30 images was obtained from each selected slice using single-shot gradient echo–echo planar sequence (repetition time, 1500 ms; echo time, 28 ms; flip angle, 10°; image acquisition matrix, 64 × 128; field of view, 156 × 43.5 mm; number of excitations, 2). Regional cerebral blood volume (rCBV), rCBF, and mean transit time (MTT) were measured in two regions of interest in the bilateral hippocampus, and mean values were calculated.
PREPARATION OF HIPPOCAMPAL TISSUE
Rats were sacrificed after completion of the Morris water maze test and PWI, and the hippocampus was dissected according to an atlas of the rat brain (at the coronal plane at interaural 7.7 mm and interaural 15.7 mm). The hippocampi were fixed in 4% paraformaldehyde for 2 days and then placed in 0.1 M phosphate buffered saline (PBS), pH 7.4, containing 30% sucrose for 1 day until the tissues sank. Tissues were then subjected to routine paraffin-embedding, serially sectioned (5 µm), stained with haematoxylin and eosin, mounted in buffered glycerine and observed with a light microscope.

IMMUNOHISTOCHEMISTRY FOR NF-κB
Hippocampal tissues were fixed as described above, embedded in optimal cutting temperature compound (Sakura Finetek, Torrance, CA, USA) and serially sectioned (5 µm) using a cryostat. Nuclear factor κB (NF-κB) was detected by streptavidin–biotin complex immunohistochemistry. Antigen retrieval was carried out by incubation in 10 mM citrate buffer (pH 6.0; Sigma-Aldrich) for 5 min at 98 °C. Sections were then cooled to room temperature, washed twice with PBS (0.1 M, pH 7.4), and endogenous peroxidase activity was quenched with 3% hydrogen peroxide in PBS for 5 min at 4 °C. Sections were incubated in rabbit antirat NF-κB polyclonal antibody (1 : 100 dilution; Boster Biological Technology, Wuhan, China) overnight at 4 °C, then washed three times with PBS for 5 min. Slides were then incubated with biotinylated mouse antirabbit immunoglobulin G (Boster Biological Technology) at 37 °C for 1 h and washed three times with PBS for 5 min. PBS was used in place of the primary antibody as a negative control. The signal was visualized using a streptavidin–biotin complex kit (Boster Biological Technology), according to the manufacturer’s instructions, and slides were counterstained using haematoxylin. The total number of NF-κB-positive cells was counted in five randomly chosen high-power fields (× 400 magnification) from each slide.

RADIOIMMUNOASSAY FOR TNF-α AND IL-1β
Hippocampal TNF-α and interleukin 1β (IL-1β) were quantified using radioimmunoassay. Hippocampal tissue (50 mg) was homogenized (Glas-Col LLC, Terre Haute, IN, USA) with 50 mM acetic acid (pH 4.75), centrifuged at 12 000 g for 15 min at 4 °C, and the resulting supernatant was stored at −20 °C. Protein levels in the supernatant were measured using specific TNF-α and IL-1β radioimmunoassay kits (Sigma-Aldrich) according to the manufacturer’s instructions. Radioactivity was detected using a gamma counter (BH6018-type four-probe autogamma counter; Beijing Nuclear Instrument Factory, Beijing, China) and the counts per minute (cpm) were used to construct a standard curve for cytokine quantification. The levels of TNF-α and IL-1β were described as ng/ml and pg/ml, respectively.

STATISTICAL ANALYSES
All statistical analyses were performed using the SPSS® statistical package, version 16.0 (SPSS Inc., Chicago, IL, USA) for Windows®. The mean ± SD of the data were calculated and compared using one-way analysis of variance followed by a post-hoc least significant difference test. A P-value < 0.05 was considered to be statistically significant.

Results
The ELT decreased from day 1 to day 4 in groups A, B and D, at both 2 and 4 months after surgery (Fig. 1), reflecting normal
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learning ability. This decrease in ELT was much smaller in group C at 2 months after surgery, indicating an impairment of learning ability. At 4 months, however, rats in group C demonstrated a similar degree of decrease in ELT to that in the other three groups (Fig. 1B). Day 4 ELTs were significantly higher in group C at both 2 and 4 months than in groups A, B and D (P < 0.01 for all comparisons; Fig. 1), again indicating impaired learning ability. At 4 months, day 1 – 4 ELTs were significantly higher in group C than in groups A, B and D (P < 0.01 for all comparisons; Fig. 1).

Data regarding rCBF, rCBV and MTT are presented in Table 1. Sham operated control animals (group A) had significantly higher rCBF and rCBV and significantly lower MTT compared with all other groups at both 2 and 4 months after surgery (P < 0.01 for all comparisons). In addition, rCBF and rCBV were significantly lower and MTT was significantly higher in group C compared with all other groups at both 2 and 4 months after surgery (P < 0.01 for all comparisons). The rCBF and rCBV were also significantly lower and MTT significantly higher in group D than group B at both time points (P < 0.01 for all comparisons).

Levels of NF-κB, TNF-α and IL-1β were significantly higher in group C than in all other groups at both 2 and 4 months after surgery (P < 0.01 for all comparisons; Table 2).

Both normal and abnormal nerve cells were visible in the CA1 region of the hippocampus in all four groups at 4 months after surgery. The normal nerve cells were relatively round with a clear boundary (Fig. 2A), while damaged nerve cells were swollen with altered symmetry or were concentrated and trachychromatic (Fig. 2B). The damaged nerve cells were triangular, signifying ischaemic necrosis. The characteristics of damaged nerve cells were particularly evident in group C (Fig. 2C). Nerve cell morphology in tissue from group D rats appeared near normal (Fig. 2D).

Discussion

Rats with untreated vascular dementia in the present study were found to have abnormal cognitive function, as demonstrated by their

![FIGURE 1](image-url): Escape latency time (ELT) in the Morris water maze test on days 1 – 4 of testing at (A) 2 months (n = 7/group) and (B) 4 months (n = 8/group) after surgery. Group A, sham operated control rats; group B, rats with focal cerebral ischaemia; group C, rats with vascular dementia; and group D, rats with vascular dementia treated with 20 mg/kg nimodipine daily. Data presented as mean ± SD. **P < 0.01 versus all other groups at same time point; one-way analysis of variance followed by post-hoc least significant difference test.
**TABLE 1:**
Regional cerebral blood volume (rCBV), regional cerebral blood flow (rCBF) and mean transit time (MTT) in the hippocampus of sham operated control rats (group A), rats with focal cerebral ischaemia (group B), vascular dementia (group C), and vascular dementia treated with 20 mg/kg nimodipine daily (group D), at 2 and 4 months after surgery.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>2 months after surgery</th>
<th>4 months after surgery</th>
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<tbody>
<tr>
<td></td>
<td>Group A</td>
<td>Group B</td>
</tr>
<tr>
<td>rCBV, ml/100 g</td>
<td>11.58 ± 2.36</td>
<td>9.26 ± 1.85a</td>
</tr>
<tr>
<td>rCBF, ml/100 g per min</td>
<td>2.64 ± 0.41</td>
<td>2.15 ± 0.38a</td>
</tr>
<tr>
<td>MTT, s</td>
<td>4.06 ± 0.58</td>
<td>5.04 ± 0.98a</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SD.

*P* < 0.01 versus group A; *P* < 0.01 versus group B; *P* < 0.01 versus group C; one-way analysis of variance followed by post-hoc least significant difference test.

**TABLE 2:**
Levels of nuclear factor-κB (NF-κB), tumour necrosis factor-α (TNF-α) and interleukin 1β (IL-1β) in the hippocampus of sham operated control rats (group A), rats with focal cerebral ischaemia (group B), vascular dementia (group C), and vascular dementia treated with 20 mg/kg nimodipine daily (group D), at 2 and 4 months after surgery.

<table>
<thead>
<tr>
<th>Protein</th>
<th>2 months after surgery</th>
<th>4 months after surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A</td>
<td>Group B</td>
</tr>
<tr>
<td>NF-κB-positive cellsa</td>
<td>7.0 ± 1.2</td>
<td>9.3 ± 1.8b</td>
</tr>
<tr>
<td>TNF-α, ng/ml</td>
<td>0.7 ± 0.1</td>
<td>0.9 ± 0.1b</td>
</tr>
<tr>
<td>IL-1β, pg/ml</td>
<td>0.6 ± 0.1</td>
<td>0.9 ± 0.1b</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SD.

*Total NF-κB-positive cells in five randomly chosen high power fields (× 400 magnification).

*P* < 0.01, versus group A; *P* < 0.01, versus group B; *P* < 0.01, versus group C; one-way analysis of variance followed by post-hoc least significant difference test.
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Impaired learning ability in the Morris water maze test. This learning ability was significantly better in rats with vascular dementia that were treated with nimodipine. It has been shown that a relationship exists between changes in cognitive function and hippocampal damage, and that nimodipine treatment reversed the impairment of short-term working memory caused by moderate hypoxia in mice. Studies on the effect of nimodipine on rats with vascular dementia are, however, scarce.

The present study evaluated the effect of nimodipine treatment on levels of NF-κB, TNF-α, and IL-1β in the hippocampus of rats with vascular dementia. Levels of all three of these proteins were significantly higher in rats with untreated vascular dementia than in those with focal cerebral ischaemia or nimodipine-treated vascular dementia. This is in accordance with the findings of others who showed that nimodipine significantly inhibited the production of TNF-α and IL-1β, and also of nitric oxide and prostaglandin E2 from lipopolysaccharide-stimulated microglia.

Histopathological findings in the present study showed that the hippocampi of rats with vascular dementia demonstrated...
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typical characteristics of damaged nerve cells. In contrast, nerve cells were of near normal morphology in nimodipine-treated rats with vascular dementia, suggesting that nimodipine has neuroprotective effects in the hippocampus. Hippocampal damage leads to cognitive impairment and has an important role in the development of vascular dementia. The mechanism of hippocampal degeneration and atrophy has been described in a longer-term mouse model of vascular dementia. The reduction in CBF occurs in the early stages of vascular disease and it is, therefore, possible for a variable period of chronic hypoxia to precede a major vascular event. This can result in changes to the brain’s cellular microenvironment and adaptive processes that may lead to cellular malfunction and degeneration and consequent significant hippocampal degeneration and neuronal loss. Hippocampal neurons, particularly those in the CA1 region, are very sensitive to ischaemia. Studies on the mechanism of hippocampal damage and the expression of cytokines in vascular dementia are rare, however.

The pathogenesis of vascular dementia is unclear, with many theories having been proposed to explain its aetiology. It is generally accepted that vascular dementia is closely associated with neuroanatomy (especially in hippocampal neuronal injury) and dysfunctional cytokine networks, but no direct correlations have been found between the various hypotheses for the aetiology of vascular dementia. Hippocampal levels of the proinflammatory cytokines TNF-α and IL-1β were significantly higher in rats with experimental vascular dementia compared with control animals in the present study. In addition, hippocampal nerve cells showed signs of damage in animals with vascular dementia, suggesting a relationship between dysfunctional cytokine networks and the neuroanatomical mechanisms underlying vascular dementia. Lesions in the hippocampus have been shown to be important for the onset of vascular dementia. Administration of cytokines in mice found that TNF-α reduced and IL-1β increased hippocampal cell proliferation. The apparent difference from the findings of the present study in the effect of these cytokines on hippocampal neurons may be a reflection of different disease states and/or different physiological status (in vitro versus in vivo), suggesting that cytokines may differentially regulate hippocampal plasticity in different neuropsychiatric conditions. The potential relationship between these two important factors in the development of vascular dementia might lead to new theories regarding pathogenesis as well as new therapeutic approaches. Detecting levels of cytokines and NF-κB in hippocampal tissues seems to be important when investigating the pathological mechanisms underlying vascular dementia and for evaluating different treatment interventions.

Treatment with nimodipine increased rCBF and rCBV and improved regional tissue perfusion in the hippocampus of rats with experimental vascular dementia in the present study. Nimodipine reduces intracellular calcium concentrations by suppressing transmembrane calcium flux in vascular smooth muscle cells in the injured area, selectively improving CBF in the region of brain injury and protecting neurons from apoptosis and necrosis. Nimodipine was found to improve CBF in an experimental global ischaemic rat model. Although nimodipine can improve rCBF, it cannot directly suppress cytokines. Cytokine levels in nimodipine-treated rats were lower than in untreated rats with vascular dementia in the present study, suggesting that the
mechanism of cytokine suppression may involve improvement in the rCBF in these animals. Use of PWI provides a noninvasive approach for the evaluation and analysis of cerebral perfusion and could be used to verify the effects of therapy on CBF in patients with vascular dementia. A literature search failed to identify any studies that have evaluated cerebral perfusion using PWI in patients with vascular dementia.

The relatively short follow-up period (4 months) limited the findings of the present study, as it was not possible to determine the long-term effects of nimodipine treatment on cognitive function, cytokine levels or hippocampal morphology. In addition, future studies should examine other parts of the brain.

In conclusion, nimodipine improved the symptoms of cognitive impairment, increased rCBF, reduced hippocampal cytokine levels and alleviated neuronal injury in the hippocampus of rats with experimental vascular dementia. These findings provide a new avenue for exploring the pathogenesis of vascular dementia and may lead to the selection of new therapeutic targets.

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Conflicts of interest
The authors had no conflicts of interest to declare in relation to this article.

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