

Tetrandrine vs nicardipine in cerebral ischemia-reperfusion damages in gerbils

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AIM: To study the effects of tetrandrine (Tet) vs nicardipine (Nic) on cerebral ischemia-reperfusion damages. **METHODS:** Cerebral ischemia was produced by 10-min occlusion of bilateral carotid arteries followed by 5-min reperfusion in gerbils. The changes in electroencephalogram (EEG), calcium and water contents, lipid peroxide (LPO) content and ultrastructure in gerbil brains were compared. **RESULTS:** Pretreatment with Tet ($15 \text{ mg} \cdot \text{kg}^{-1}$, iv) and Nic ($0.25 \text{ mg} \cdot \text{kg}^{-1}$, iv) enhanced the recovery of EEG amplitude, reduced the calcium (151.2 ± 1.1 and $155.3 \pm 2.4 \text{ mg/kg dry wt}$ in Tet and Nic groups vs $193 \pm 8 \text{ mg/kg dry wt}$ in ischemia-reperfusion group, $P < 0.05$) and water contents, attenuated the increase in LPO content (293 ± 29 and $276 \pm 23 \mu\text{mol} \cdot \text{kg}^{-1}$ wet wt in Tet and Nic groups vs $427 \pm 24 \mu\text{mol} \cdot \text{kg}^{-1}$ wet wt in ischemia-reperfusion group, $P < 0.01$), and diminished the ultrastructural abnormalities of cortex and hippocampus in gerbil brain during ischemia and reperfusion. **CONCLUSION:** Tet and Nic had protective effects against ischemia-reperfusion brain damages in gerbils. The effects of Tet were similar to, but less potent than those of Nic.

KEY WORDS tetrandrine; nicardipine; cerebral ischemia; Gerbillinae; calcium; lipid peroxides; electroencephalography; electron microscopy

There has been an increasing interest in the pathophysiologic role played by calcium

and oxygen free radical-mediated lipid peroxidation in ischemia-reperfusion brain injury^(1,2). Calcium channel blockers are beneficial in cerebral ischemia⁽³⁾.

Tetrandrine (Tet) is extracted from a Chinese medicinal herb *Stephania tetrandra* S Moore. We have found that Tet had protective effects against ischemic brain damage in the rat model of global cerebral ischemia (to be published). The Mongolian gerbil, which lacks a functional connection between the carotid and vertebrobasilar arterial circulation, offers advantages for the screening of compounds as protective agents against brain ischemia⁽⁴⁾. In this paper, the effects of Tet on cerebral ischemia followed by reperfusion in gerbils were studied by assessing the changes in electroencephalogram (EEG), brain tissue calcium and water content, lipid peroxide (LPO) content, and ultrastructure in order to evaluate the therapeutic role of Tet in the acute phase of brain ischemia compared with nicardipine (Nic).

METHODS

Cerebral ischemia Mongolian gerbils of either sex ($n = 20$) weighing $68 \pm 10 \text{ g}$ were anesthetized with ip a mixture of urethane ($0.5 \text{ g} \cdot \text{kg}^{-1}$) and α -chloralose ($50 \text{ mg} \cdot \text{kg}^{-1}$). Bilateral common carotid arteries were occluded using atraumatic arterial clips. Reperfusion following 10-min ischemia was performed by removing the clips for 5 min. Then the gerbil brains were taken out rapidly. EEG was recorded on a polygraph connected to the needle electrodes inserted sc in the frontoparietal region. The gerbils were divided into 4 groups: (1) sham-operation (control); (2) ischemia-reperfusion (Isch-Rep); (3) Tet (15 mg

•kg⁻¹); (4) Nic (0.25 mg•kg⁻¹). The drugs were iv 15 min prior to ischemia.

Determination of brain calcium and water contents⁵² After reperfusion the gerbils were decapitated and the brain hemispheres were taken out quickly, washed with 0.9 % NaCl, then placed in a ceramic crucible, weighed, dried at 105 °C for 48 h and reweighed to determine the water content (wet wt - dry wt). The dry tissue was heated at 550 °C for 4 h in a muffle oven. The ash was dissolved in HNO₃ 0.75 mol•L⁻¹ containing 0.5 % LaCl₃. The calcium concentration of ashed samples was determined by an atomic absorption spectrophotometer (Varian AA-1475, Australia) at 422.7 nm.

Estimation of LPO After reperfusion the brain was weighed, mixed with double redistilled water (10:1, vol/wt) and then homogenized. The homogenate was centrifuged at 1073 × g for 10 min. LPO in the supernatant was collected and LPO was determined with thiobarbituric acid (TBA)⁵³. The TBA-reaction substance was measured by a spectrophotometer at 532 nm. 1,1,3,3-Tetramethoxypropane (Fluka Ltd) was used as an external standard, and the level of LPO was expressed as nmol of malondialdehyde (MDA).

Examination of brain ultrastructure The cortex and hippocampus were fixed in 5 % glutaraldehyde at 4 °C overnight, and trimmed into pieces (1 mm³), which were placed in 5 % glutaraldehyde at 4 °C for 2 h, washed with phosphate buffer, post-fixed with 1 % OsO₄ for 2 h, dehydrated in graded ethanol, and embedded in Epon 618. The epoxy blocks were sliced (0.06 μm) on a LKB-V ultratome. The ultrathin sections were stained with uranyl acetate and lead citrate, and examined under a Philips EM-450 transmission electron microscope.

RESULTS

The EEG amplitude was drastically reduced immediately after occlusion of bilateral carotid arteries and became isoelectric quickly in all groups (Fig 1). After reperfusion the EEG amplitude recovered gradually. At the end of 5-min reperfusion, the EEG amplitude was greater in Tet and Nic groups than that in Isc-Rep group (54±17 % and 50±19 % vs 11±4 % of control, P<0.01).

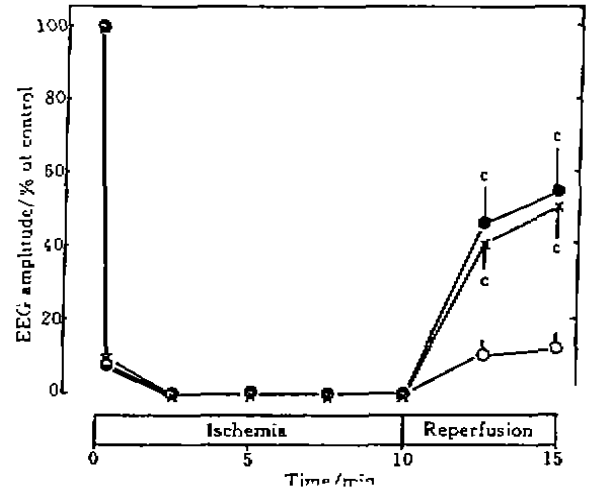


Fig 1. EEG amplitude during ischemia and reperfusion in Isc-Rep (○), Tet (●, 15 mg•kg⁻¹, iv), and Nic (×, 0.25 mg•kg⁻¹, iv) groups. n=5, x±s. *P<0.01 vs Isc-Rep group.

During ischemia and reperfusion the brain calcium and water contents in gerbils were higher in Isc-Rep group vs control (Tab 1), but lower in Tet and Nic groups vs the Isc-Rep group. These results showed that there was an increase in accumulation of calcium and water in the brain during ischemia and reperfusion, while depressed by Tet and Nic.

Tab 1. Effects of Tet (15 mg•kg⁻¹, iv) and Nic (0.25 mg•kg⁻¹, iv) on brain calcium, malondialdehyde (MDA) and water content in gerbils. n=5, x±s. ^bP<0.05, ^cP<0.01 vs Isc-Rep group. ^fP<0.01, vs sham-operation group.

Groups	Calcium, mg/kg dry wt	MDA, nmol/g wet wt	H ₂ O, %
Sham-operation	128±6	266±11	77.5±0.4
Isc-Rep	193±8 ^f	427±24 ^f	78.6±0.5 ^f
Tetrandrine	151.2±1.1 ^b	293±29 ^c	78.1±0.3 ^d
Nicardipine	155.3±2.4 ^b	276±23 ^c	78.0±0.4 ^b

During ischemia and reperfusion the brain MDA content was higher in Isc-Rep group than that in control, indicating a higher level

of lipid peroxidation. The brain MDA content was lower in Tet and Nic groups than that in Isc-Rep group (Tab 1), suggesting that Tet and Nic suppressed the rise in brain MDA content during ischemia and reperfusion.

The cortical and hippocampal neurons appeared to be normal in the control gerbils (Fig 2A, Plate 3). The cortical neurons in Isc-Rep group revealed ischemic alterations: the shrinkage of nuclear membrane, swelling of mitochondria, and slight distension of endoplasmic reticulum. The ischemic changes in hippocampal neurons were severer than those in cortical neurons. The nuclear membrane was loose and partially indiscernible, the chromatin was margined, the mitochondria became enlarged and vacuolated, and the endoplasmic reticula were distended (Fig 2B). In contrast, the cortical and hippocampal neurons showed no significant abnormalities in Tet and Nic groups (Fig 2C, D), ie, there was no significant difference between the control and the groups pretreated with Tet and Nic.

DISCUSSION

The increase of total brain calcium content during reperfusion after transient cerebral ischemia was found in gerbils^[7] and rats^[8,9]. In comparison with other published values in gerbils^[7] the brain calcium content in this study is similar to that in control, but lower in Isc-Rep group, which may be related to the shorter period of reperfusion in this model.

The brain MDA content in control is similar to that of other^[6]. Tet and Nic induced a reduction in brain calcium content accompanied by a decrease in brain MDA content suggesting that Tet and Nic prevented Ca^{2+} from entering into the neurons, thereby reducing the production of oxygen free radical, and consequently the level of lipid peroxidation.

The recovery of the brain electrical activities expressed as EEG amplitude during reperfusion has been shown to be correlated well with the reduction of cortical intracellular free Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$)^[11]. The favorable effect of Tet and Nic on the recovery of EEG amplitude may be related to their ability of reducing the calcium content in ischemic neurons.

After reperfusion following ischemia there were significant ischemic damages in brain tissue ultrastructure, especially in the hippocampus, a vulnerable region^[10]. The protective effects of Tet and Nic on ischemic neurons might be related to their ability of attenuating the increase of intraneuronal calcium content during ischemia and reperfusion.

In general, Tet exerts protective effects against ischemic and reperfusion damages in the model of global cerebral ischemia, which are similar to, but less potent than those of Nic.

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125-110

比较粉防己碱和尼卡地平对沙土鼠脑缺血再灌注损伤的影响

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目的: 比较粉防己碱 (Tet) 和尼卡地平 (Nic)

对沙土鼠脑缺血再灌注损伤的影响。方法: 沙土鼠双侧颈总动脉结扎10 min 后再灌注5 min, 造成脑缺血再灌注损伤。观察 Tet 和 Nic 对沙土鼠脑电图 (EEG), 脑组织钙、水和脂质过氧化物 (LPO) 含量以及脑组织超微结构的影响。结果: Tet (15 mg·kg⁻¹, iv) 和 Nic (0.25 mg·kg⁻¹, iv) 促进脑缺血再灌注沙土鼠 EEG 幅度的恢复; 减轻脑水肿和脑组织钙累积; 降低脑组织 LPO 含量; 改善脑组织超微结构。结论: Tet 对缺血再灌注脑组织损伤有明显拮抗作用, 其作用与 Nic 类似, 但较弱。

关键词 粉防己碱; 尼卡地平; 脑缺血; 沙土鼠科; 钙; 脂质过氧化物; 脑电图描记术; 电子显微镜检查

《中国药理学报》1994年优秀论文

1994年《中国药理学报》所载论文136篇, 根据研究思路新颖, 科研有创造性, 技术水平先进等综合因素, 经我刊编委投票选出1994年优秀论文如下(按得票数前10名排次), 特予以刊登表扬。

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