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Antagonistic effect of 3，6-dimethamidodibenzopyriodonium gluconate on lipid peroxidation in cerebral cortical neuronal cultures and rat brains during focal cerebral ischemia reperfusion

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KEY WORDS 3，6-dimethamidodibenzopyriodonium gluconate；lipid peroxides；cultured cells；neurons；cerebral cortex；cerebral ischemia；reperfusion injury

ABSTRACT

AIM：To study 3，6-dimethamidodibenzopyriodonium gluconate (Ⅰ-93) antagonistic effects on lipid peroxidation in cerebral cortical neuronal cultures and rat brains during focal cerebral ischemia-reperfusion. **METHODS**：Cerebral cortical neurons were cultured and rat focal cerebral ischemia-reperfusion model was established by reversible middle cerebral artery occlusion (MCAO) without craniectomy. The efflux of lactate dehydrogenase (LDH) from neurons，content of malondialdehyde (MDA) in neurons and brain homogenate，activity of superoxide dismutase (SOD) in brain homogenate，and index of cerebral edema as well as brain morphology were investigated. **RESULTS**：Ⅰ-93 10 – 40 $\mu mol \cdot L^{-1}$ concentration-dependently inhibited efflux of LDH and elevated levels of MDA induced by addition of H_2O_2 (10 $\mu mol \cdot L^{-1}$) *in vitro*. Ⅰ-93 0.5 $mg \cdot kg^{-1}$ improved the cerebral morphology，reduced brain edema，decreased MDA content，and enhanced SOD activity in brain homogenate. **CONCLUSION**：Ⅰ-93 protects neurons from H_2O_2-induced neurotoxicity and ischemia-reperfusion mediated damage by increasing the activity of antioxidant enzymes and suppressing the generation of lipid peroxides.

INTRODUCTION

The central nervous system is susceptible to oxidative damage due to its high oxygen consumption，low level of antioxidant enzymes，and high concentration of unsaturated lipids which are highly oxidizable substances. 3，6-Dimethamidodibenzopyriodonium gluconate (Ⅰ-93) is the newest product of iodoniumheterocycle compound series. As its antioxidative potential has already been proven in our lab[1]，in this study，we observed the influence of Ⅰ-93 on lipid peroxide production in cerebral cortical neuronal cultures and rat brains during focal cerebral ischmia-reperfusion.

MATERIAL AND METHODS

Reagent Ⅰ-93，purity > 95 %，was synthesized by Professor HOU Zi-Jie and dissolved in 5 % glucose solution. Malondialdehyde was purchased from Merck. Dulbecco's modified Eagle medium (DMEM) was a Gibco product. Other chemicals were of AR grade.

Primary cortical neuronal culture Rat cortical neurons were isolated from 16 – 18 d embryos according to the method as previously described[2]. The dissected hemispheres were rinsed with D-Hanks' solution (pH 7.2 – 7.4). Meninges and blood vessels were meticulously removed. Following a wash step with D-Hanks' solution，the cerebral cortex was minced，incubated in 0.25 % trypsin at 37 ℃ for 20 min，and incubation was stopped with Hanks' balanced salt solution. The cell pellet was resuspended in DMEM supplemented with 20 % fetal bovine serum. Cells were seeded onto *L*-polylysine-coated 24-well plates at a density of $10^9 \cdot L^{-1}$ and incubated at 37 ℃ in 5 % CO_2 atmosphere. After a 7-d culture，non-neuronal cell division was halted by a 3-d exposure to cytosine arabinoside (10 $\mu mol \cdot L^{-1}$). The culture medium was renewed every 3 – 4 d.

Treatment with H_2O_2 and Ⅰ-93 Experiments were performed on cell cultures after 14 d. H_2O_2 (10 $\mu mol \cdot L^{-1}$) was added to the DMEM (containing 5 % bovine serum and different concentration of Ⅰ-93) and

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incubated at 37 ℃ for 24 h. LDH in the supernatant medium was determined by automatic biochemical analyzer. Attached cells were washed with D-Hank's twice, then exposed to 0.25 % Triton-100 1.0 mL per well and vibrated gently on an oscillator for 15 min. The samples of splitting decomposition were centrifuged for 15 min at 4000 × *g*, and the supernatant was used for assaying MDA contents[3].

Rat model Reversible MCAO was performed following the procedure of Longa[4] *et al* with minor modifications. Sprague-Dawley rat either sex, obtained from the Animal Breeding Center of Xian Medical University, weighing (248 ± 31) g were anesthetized with ketamine hydrochloride 80 mg·kg^{-1} ip. The left external carotid artery (ECA) was separated and after the branches of ECA were coagulated with microbipolar cautery forceps and divided, a 5-cm length of 4 – 0 monofilament nylon suture, its tip rounded by heating near a flame, was inserted into ECA lumen, then gently advanced from the ECA to the internal carotid artery (ICA). After about 19 – 21 mm of suture (from the origin of ICA) was inserted into ICA, resistance was felt, indicating that the blunted tip of the suture had passed the MCA origin and had reached the proximal segment of the anterior cerebral artery. The nylon suture was pulled back after a 3-h MCAO to restore the blood flow to the MCA territory. In sham-operated group the suture reached pterygopalatine artery of the ICA but did not occlude MCA; Ⅰ-93 group was injected with I-93 0.5 mg·kg^{-1} ip 10 min before the onset of ischemia; Ischemia-reperfusion group was given equal volume of 5 % glucose solution.

HE staining After a 3-h reperfusion, rats were anesthetized with ketamine 80 mg·kg^{-1} ip and perfused intracardially with normal saline (containing heparin 100 kU·L^{-1}) 50 mL followed by 10 % buffered formalin 300 mL. Brains were fixed in formalin, then paraffin-embedded in the coronal plane, sectioned and stained with hematoxylin and eosin.

Determination of water, MDA content, and SOD activity After a 3-h reperfusion, rats were decapitated. The brain tissues separated from the left MCA territory were dried at 110 ℃ after taking their wet weight. The water content = (wet weight – dry weight)/(wet weight) × 100 %. Other brain tissues from left MCA territory were homogenized in normal saline at 0 ℃. The homogenates were centrifuged at 4000 × *g* for 15 min and the supernatant was used for assay of MDA[5] content and SOD activity[6].

RESULTS

H_2O_2 neurotoxicity *in vitro* Neurotoxicity was estimated by LDH activity released into media from damaged neurons and MDA content in neurons. H_2O_2 10 μmol·L^{-1} caused efflux of LDH and increased the MDA content. Addition of Ⅰ-93 10, 20, and 40 μmol·L^{-1} concentration-dependently inhibited H_2O_2-induced LDH efflux and MDA elevation (Tab 1).

Tab 1. Effects of Ⅰ-93 on LDH efflux and MDA content in cortical neuronal cultures after H_2O_2-induced toxicity. $n = 4$. $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* control. $^eP < 0.05$, $^fP < 0.01$ *vs* H_2O_2 group.

Group	LDH/ μmol·min^{-1}·g^{-1} protein	MDA/ nmol·g^{-1} protein
Control	12.25 ± 1.71	3.14 ± 0.68
H_2O_2	19.50 ± 1.65^c	9.83 ± 1.03^c
Mannitol (10 μmol·L^{-1})	12.75 ± 0.96^{af}	2.31 ± 0.31^{af}
Ⅰ-93 (10 μmol·L^{-1})	14.75 ± 0.96^{ae}	3.31 ± 0.67^{af}
Ⅰ-93 (20 μmol·L^{-1})	13.75 ± 0.50^{af}	2.50 ± 0.29^{af}
Ⅰ-93 (40 μmol·L^{-1})	11.50 ± 1.29^{af}	2.23 ± 0.09^{af}

Morphological changes in the brain There was no significant abnormality in neurons and nerve fiber structures of cerebral group cortex in sham-operated rats. In the group of 3-h MCAO followed by a 3-h reperfusion, the number of neurons decreased markedly and the outer spaces of neurons and gliocytes were extended. Degenerative changes and nuclear pyknosis were manifestated and dendrites and axons disappered. Ⅰ-93 reduced the brain damage and most neurons appeared normal. The degeneration was observed to be significantly improved.

Water content of brains At a 3-h reperfusion after a 3-h ischemia water accumulated in brain tissue. Ⅰ-93 0.5 mg·kg^{-1} ip 10 min before ischemia reduced the water content (Tab 2).

Tab 2. Effect of Ⅰ-93 on water content of brains in cerebral ischemia-reperfusion rat. $n = 11$. $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* sham-operated. $^fP < 0.01$ *vs* ischemia-reperfusion.

Group	wet weight/ g	dry weight/ g	water content
Sham operated	0.138 ± 0.034	0.029 ± 0.006	78.9 ± 1.1
Ischemia-reperfusion	0.141 ± 0.025	0.022 ± 0.005	84.2 ± 1.9^c
Ⅰ-93 (0.5 mg·kg^{-1})	0.122 ± 0.026	0.023 ± 0.004	80.6 ± 1.4^{af}

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MDA content and SOD activity of brain homogenate In sham-operated group，MDA content and SOD activity was (0.070 ± 0.024) μmol·g^{-1} protein and (0.793 ± 0.196) mmol·min·g^{-1} protein，respectively. Ischemia-reperfusion increased MDA content and inhibited the SOD activity. Ⅰ-93 0.5 mg·kg^{-1} attenuated elevation of MDA levels and reversed inhibition of SOD activity (Tab 3).

Tab 3. Effects of Ⅰ-93 on MDA content and SOD activity in brains of cerebral ischemia-reperfusion rats. $n = 10$. $\bar{x} \pm s$. $^{a}P > 0.05$，$^{c}P < 0.01$ *vs* sham-operated. $^{f}P < 0.01$ *vs* ischemia-reperfusion.

Group	MDA/ μmol·g^{-1} protein	SOD/ mmol·min^{-1}·g^{-1} protein
Sham operated	0.068 ± 0.024	0.793 ± 0.196
Ischemia-reperfusion	0.112 ± 0.021^{c}	0.459 ± 0.136^{c}
Ⅰ-93 (0.5 mg·kg^{-1})	0.072 ± 0.014^{af}	0.814 ± 0.146^{af}

DISCUSSION

Oxygen free radicals generated during aging，injury，and diseases have been postulated to be a major cause of neuronal cell death. In our study，exposure of cultured cortical neurons to H_2O_2，which produces hydroxyl free radical，resulted in release of LDH from neurons and accumulation of lipid peroxide. Ⅰ-93 concentration-dependently inhibited efflux of LDH and MDA elevation，indicating that Ⅰ-93 can protect cortical neurons against the free radical insult.

Elevation in free radicals and lipid peroxidation taking place after reperfusion plays a key role in the injury associated with ischemia-reperfusion. Depletion of antioxidants or deficit in antioxidant defense mechanism increases oxidant stress. Ⅰ-93 0.5 mg·kg^{-1} improved the brain damage，reduced brain edema，decreased MDA content of brain，and elevated activity of SOD in the brain. The results indicate that the beneficial effects of Ⅰ-93 on cultured cortical neurons and rat brain during cerebral ischemia-reperfusion are derived from its protective action on the antioxidant enzymes and by preventing membrane lipid peroxidation.

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3，6－(二甲氨基)－二苯骈碘杂六环葡萄糖酸盐对培养的脑皮质神经元和缺血再灌注大鼠脑脂质过氧化的拮抗作用

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关键词 3，6－(二甲氨基)－二苯骈碘杂六环葡萄糖酸盐；脂质过氧化物；培养的细胞；神经元；大脑皮质；脑缺血；再灌注损伤

目的：观察3，6－(二甲氨基)－二苯骈碘杂六环葡萄糖酸盐对培养的脑皮质神经元和缺血再灌注大鼠脑脂质过氧化的拮抗作用. **方法**：原代培养大鼠胎鼠脑皮质神经元并制做可逆性不开颅大鼠大脑中动脉梗塞模型，测定培养上清液中乳酸脱氢酶(LDH)的溢出，神经元内和脑匀浆中丙二醛(MDA)含量，脑匀浆超氧化物歧化酶(SOD)活性，脑水肿指数和形态学变化. **结果**：Ⅰ-93 10－40 μmol·L^{-1}剂量依赖性抑制 H_2O_2 引起的神经元 LDH 外漏和 MDA 升高. Ⅰ-93 0.5 mg·kg^{-1}改善脑组织结构损害，减轻脑水肿，降低脑匀浆中 MDA 含量并提高 SOD 活性. **结论**：Ⅰ-93 通过提高抗氧化酶活性和抑制脂质过氧化对 H_2O_2 引起的神经毒性和缺血再灌注引起的脑损伤产生保护作用. (责任编辑 刘俊娥)