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Neuroprotective action of dextromethorphan in rat photochemically-induced focal cerebral ischemia[1]

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KEY WORDS dextromethorphan; cerebral blood flow; cerebral ischemia; *bcl*-2 genes; *bax* genes; neuroprotective agents; immunohistochemistry

AIM: To study the effects of dextromethorphan (Dex) on photochemically-induced focal cerebral ischemia in rats. **METHODS:** Anesthetized rats undergone 10-min light irradiation on exposed skull after rose bengal injection were pretreated with saline and Dex at 3 doses (12.5, 25, and 50 mg · kg^{-1}, ip, 15 min before ischemia). The alteration of volume of lesioned cortical region, regional cerebral blood flow (CBF), *bcl*-2 and *bax* expression at penumbra area were studied. **RESULTS:** Dex dose-dependently decreased the infarcted volume (17 %, 26 %, and 50 % reduction, respectively). Pretreatment with Dex at a dose of 50 mg · kg^{-1} improved the postischemic hypoperfusion compared with the control at 20 and 30 min after lesion (both 31 % increase), and also upregulated the expression of anti-apoptosis gene *bcl*-2. **CONCLUSION:** Dex protects against ischemic neuronal damage in this model and its effects on CBF and *bcl*-2 expression may contribute to its neuroprotective action.

The excitatory amino acids (EAA) glutamate and aspartate are the predominant excitatory neurotransmitters in the mammalian brain. Normally, they activate postsynaptic receptors, one of which is coupled to ion channels, including NMDA and non-NMDA subtypes. However, excessive release of EAA and overstimulation of their receptors lead to postsynaptic overexcitation and eventual cell death of surrounding neurons in some pathological states such as cerebral ischemia, which has been called as excitotoxicity of glutamate[1,2]. Although several noncompetitive NMDA antagonists have been successful in attenuating various pathological changes following focal cerebral ischemia, their side effects limit their clinical uses[3]. Dextromethorphan, a widely used antitussive drug, is a NMDA antagonist and prevents neuronal death induced by focal or global cerebral ischemia[4,5].

Apoptosis may be involved in the delayed neuronal death induced by cerebral ischemia[6]. Apoptotic neuronal death is an active process which is regulated by specific genes[7-9]. The purpose of this study was to investigate whether Dex had the same neuroprotective effect on photochemically-induced cortical focal ischemia and whether this role correlated with the changes in cerebral blood flow (CBF) and apoptosis-related genes *bcl*-2/*bax* expression.

MATERIALS AND METHODS

Rats Sprague-Dawley rats (♂, $n = 28$, weighing 250 ± s 20 g, from Dept of Experimental Animals, Shanghai Medical University) were equally divided into 4 groups: 1) ischemic group with saline, 2) ischemic group with Dex 12.5 mg · kg^{-1}, 3) ischemic group with Dex 25 mg · kg^{-1}, and 4) ischemic group with Dex 50 mg · kg^{-1}.

Chemicals Dex and rose bengal were purchased from Sigma Chemical Co, USA. Antibodies against *bcl*-2 and *bax* were purchased from Oncogene Science, USA. ABC kit was purchased from Vector Lab, USA.

Ischemia model[10] Rats were anesthetized with chloral hydrate 360 mg · kg^{-1} ip and fixed on a stereotaxic apparatus (Narishige Scientific Instrument Lab, Japan). After injecting rose bengal 80 mg · kg^{-1} into the femoral vein, a beam of cold light (Olympus, Danmark) was finely focused on the exposed flat skull at 1.8 mm posterior to the bregma and 2.8 mm lateral to the midline. The irradiated light was persisted for 10 min and sensitive light wave was 550 - 1000 nm. Meanwhile, Dex (12.5, 25,

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and 50 mg·kg^{-1}) and saline were injected ip at 15 min before light irradiation.

CBF measurement CBF was measured by using a Laser-Doppler flow monitor (Medical System Corp, USA). The probe was implanted on the dura mater 4.8 mm posterior to the bregma and 2.8 mm lateral to the midline. CBF measurement was started at 30 min before ischemia and stopped at 30 min after ischemia and recorded every 5 min.

Stain and immunohistochemistry All rats were killed at 12 h after ischemia, and then fixed through intracardial perfusion with 4 % paraformaldehyde. Coronal 30 μm serial sections were cut on a freezing microtome. The sections were divided into 3 sets. One set was stained with 1 % cresyl violet (CV), while the other 2 sets were processed for *bcl*-2 and *bax* immunohistochemistry according to avidin-biotin-peroxidase complex method.

Data analysis Six coronal planes were selected from the CV stained sections through the rostrocaudal extent of the lesion in each rat. The area of ischemic region at each section was detected by Q570 image analysis system (Leica, Germany), and then the infarction volume was calculated according to the trapezoidal rule: $V = t_1(A_1 + A_2)/2 + t_2(A_2 + A_3)/2 + \cdots\cdots t_{n-1}(A_{n-1} + A_n)/2$ (V = volume, t = distance of between 2 adjacent section, A = infarction area)[11]. CBF amounts during and after ischemia were compared to preischemic values. *Bcl*-2 and *bax* immunoreactive positive cells in penumbra area per section were counted by employing Q570 image analysis system. Differences between groups were analyzed by one-way ANOVA followed by student Newman-Keuls test.

RESULTS

Infarcted volume In CV-stained section, the lesioned cortical region appeared to be pale and included 2 parts, the infarcted core and the penumbra area. The infarcted core was characterized by complete neuronal death, thrombotic vessels, and widespread inflammatory cell infiltration, while the area penumbra appeared to be a distored cell profile and a decreased number of CV stained neurons. Pretreatment with Dex 12.5 or 25 mg·kg^{-1}, the volume of lesioned cortical region showed 17 % and 26 % reduction respectively as compared with vehicle-treated rats ($P > 0.05$). However, administration of Dex 50 mg·kg^{-1} 15 min preischemia declined the lesioned volume by 50 % (Tab 1, Fig 1A – B).

Tab 1. Effects of Dex on infarction volume after photochemically-induced focal ischemia. $\bar{x} \pm s$. **$^aP > 0.05$, $^bP < 0.05$ *vs* vehicle.**

Group	Rats	Infarction volume/mm^3
Isch + vehicle	7	6.0 ± 0.5
Isch + Dex 12.5 mg	7	5.0 ± 0.5^a
Isch + Dex 25 mg	7	4.4 ± 0.9^a
Isch + Dex 50 mg	7	3.0 ± 0.4^b

CBF Compared to the control, postischemic CBF tended to improve in ischemic rats treated with Dex 50 mg·kg^{-1} at 20 min and 30 min after lesion, which increased both by 31 %. Pretreatment with Dex 12.5 or 25 mg·kg^{-1} did not show significant improvement on regional CBF after ischemia (Tab 2).

Tab 2. Effects of Dex on CBF after photochemically-induced focal ischemia (ip, 15 min before ischemia). Data were presented as ratios of CBF to pre-injection. $^aP > 0.05$, $^bP < 0.05$ *vs* vehicle. * 5 min preischemia.

Dex Rats	0 7	12.5 mg 6	25 mg 6	50 mg 7
Pre-injection	100 (123 ± 27)	100 (118 ± 19)	100 (116 ± 21)	100 (106 ± 20)
Post-injection*	72 ± 10	70 ± 15^a	78 ± 12^a	109 ± 23^a
Ischemia				
0 min	71 ± 12	71 ± 14^a	82 ± 18^a	89 ± 19^a
10 min	58 ± 10	42 ± 8^a	56 ± 11^a	85 ± 21^a
20 min	38 ± 6	54 ± 14^a	81 ± 29^a	69 ± 12^b
30 min	34 ± 5	35 ± 6^a	58 ± 15^a	65 ± 12^b

Genes *bcl*-2 and *bax* expression Although *bcl*-2 protein also expressed in penumbra area in ischemic group with vehicle treatment, an apparent upregulation of *bcl*-2 expression was found in Dex 50 mg·kg^{-1} treated rats following photochemically-induced focal cerebral ischemia (Fig 1 C – F). No effects of Dex on *bax* protein expression were seen (Fig 1 G – J).

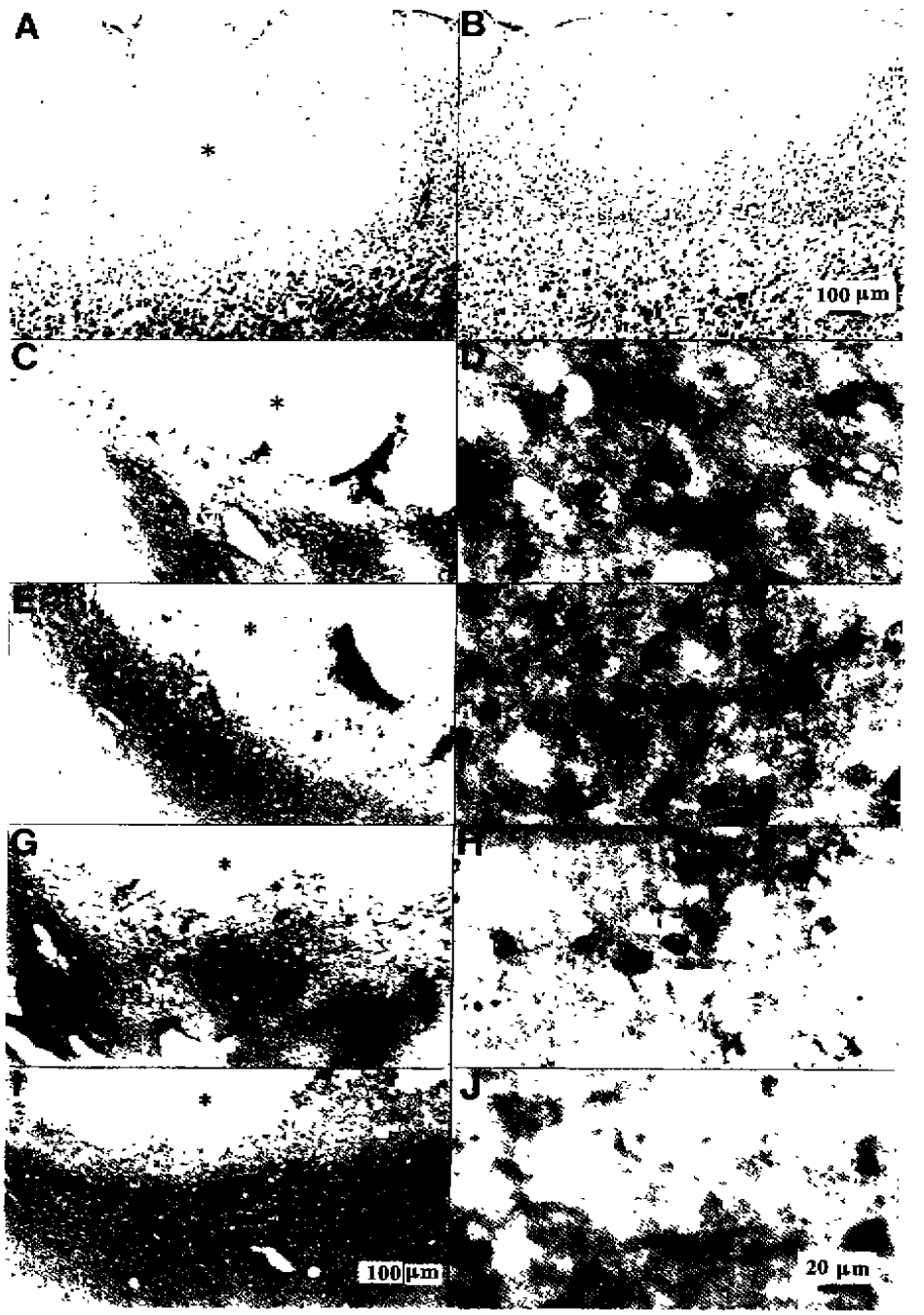

Fig 1. Effects of dextromethorphan on infarction volume and *bcl-2/bax* expression. A – B: cresyl violet staining C – F: *bcl-2* immunohistrochemistry G – J: *bax* immunohistrochemistry. Ischemia group with vehicle: A, C – D, G – H. Ischemia group with Dex 50 mg·kg^{-1} ip: B, E – F I – J. * Infarction core. Bar: A – C, E, G, I 100 μm; D, F, H, J 20 μm.

DISCUSSION

Dextromethorphan, a non-competitive NMDA antagonist[12], prevents glutamate excitotoxicity *in vivo*[1] and neuronal death in the cerebral ischemia[4,5,13]. In the present study, we found, for the first time, that pretreatment with Dex also had a significant neuroprotection on the photochemical thrombotic cerebral ischemia model, which is consistant with our previous observation on the MCAO model[4].

It has been reported that the neuroprotective action of Dex was considered associating with blockade of neuronal NMDA receptors[13]. However, our previous study showed that PCP, an also noncompetitive antagonist for NMDA receptors, could induce cerebral vasoconstriction *in vitro* and change the cerebral blood flow (CBF)[14]. Therefore, we speculate that neuroprotectetive effect of Dex may relate to the changes of CBF during the ischemic pathogenesis. In the present study, we did observe that pretreatment with Dex significantly prevented the decrease in CBF at penumbra area following the cerebral ischemia, which was consistent with the previous results[15].

Apoptosis is an active cell death process and has been considered implicating in the pathogenesis of ischemic secondery neuronal damage[6]. It associates with the activation of a genetic program in which apoptotic effector genes promote cell death, while repressor genes enhance cell survival. Several recent researches on apoptosis-related genes in mammalian cells focused on two proto-oncogenes, *bcl*-2 and *bax*, which have an opposite role in controlling apoptosis. *Bcl*-2 protein can block apoptosis and promote cell survival, while *bax* protein heterodimerized with *bcl*-2 accelerates apoptosis. The postischemic infarct volume was significantly decreased when *bcl*-2 was overexpressed using molecular biological techniques[9]. Meanwhile, *bcl*-2 gene expression was upregulated in the survival neurons in the rat brain following the cerebral focal ischemia[8]. Moreover, it has been suggested that the ratio value of *bcl*-2 to *bax* is an indicator for cell death or survival[7]. In this study, we further found that adexinistration with Dex 50 $mg \cdot kg^{-1}$ increased the expression of apoptosis-related gene *bcl*-2. Taken together, the increased postischemic expression of *bcl*-2 protein may be involved in the mechanisms of neuroprotective action of Dex.

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右美沙芬在大鼠光化学脑缺血中的神经保护作用[1]

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关键词 右美沙芬；脑血流量；脑缺血；*bcl*-2 基因；*bax* 基因；神经保护剂；免疫组织化学

目的：研究右美沙芬在脑缺血中的作用和机制。**方法**：采用光化学脑缺血模型，在缺血前 15 分钟，腹腔注射 12.5，25，50 $mg \cdot kg^{-1}$ 的右美沙芬，观察对损伤区体积、局部脑血流量和 *bcl*-2/*bax* 表达的影响。**结果**：右美沙芬呈剂量依赖性地减轻损伤区体积，梗死体积分别下降了 17 %，26 % 和 50 %；在缺血后 20 分钟及 30 分钟，右美沙芬（50 $mg \cdot kg^{-1}$）可明显改善缺血后的低灌流状态；该剂量的右美沙芬还可以上调缺血后 *bcl*-2 的表达。**结论**：右美沙芬在光化学脑缺血性损伤中有神经保护作用，其机制与改善脑血流、增加 *bcl*-2 的表达有关。