口服β-羧乙基倍半锗氧化物后锗在24名中国人体内的药物动力学

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关键词 锗；β-羧乙基倍半锗氧化物；药物动力学；原子吸收分光光度测定法

目的：比较口服不同剂量β-羧乙基倍半锗氧化物(Ge-132)的药物动力学的规律. 方法：三组健康志愿者(每组8人)分别一次口服Ge-132口服液1(低剂量，LD)、2.5(中剂量，MD)、4(高剂量，HD) $g \cdot m^{-2}$，用原子吸收光谱法测定24 h血浆锗和尿锗浓度，比较不同剂量Ge-132的人体药动学. 结果：用药后，$T_{\frac{1}{2}\alpha}$(LD，1.2±0.7 h；MD，1.1±0.6 h；HD，1.2±0.5 h)、$T_{\frac{1}{2}\beta}$(LD，5.2±1.2 h；MD，5.8±2.5 h；HD，5.5±1.4)和 Cl/F (LD，33±12 $L \cdot h^{-1}$；MD，35±10 $L \cdot h^{-1}$；HD，33±11 $L \cdot h^{-1}$)与剂量无关. T_{max}在0.75 h和2 h之间. C_{max}(LD，5.3±2.2 $mg \cdot L^{-1}$；MD，13±5 $mg \cdot L^{-1}$；HD，18±8 $mg \cdot L^{-1}$)和AUC (LD，31±13 $mg \cdot h \cdot L^{-1}$；MD，60±16 $mg \cdot h \cdot L^{-1}$；HD，79±42 $mg \cdot h \cdot L^{-1}$)与剂量呈正相关. 24 h尿锗排出量占所给低剂量的11±3 %、中剂量的9±3 %、高剂量的6±5 %(用实际尿锗排出百分数/F的公式计算)，与剂量呈负相关. 结论：口服Ge-132后符合一级吸收和一级消除二房室模式；经肾脏排泄的锗约占口服量的11 %以下.

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1996 Sep; 17 (5): 418－420

Inhibitory effects of dextromethorphan on c-*fos* protein expression during focal cerebral ischemia in rats[1]

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KEY WORDS cerebral ischemia; proto-oncogene proteins c-*fos*; immunohistochemistry; dextromethorphan

AIM: To study the effect of dextromethorphan (DM) in focal cerebral ischemia. **METHODS:** The c-*fos* protein was detected immunohistochemically in the brain of rats after focal cerebral ischemia (induced by placing a nylon thread in the lumen of the internal carotid artery) with and without treatment with DM. **RESULTS:** Focal cerebral ischemia induced c-*fos* protein expression outside the core territory of the middle cerebral artery (MCA) and neuronal damage in the core territory of the MCA. There was an evident expression of c-*fos* protein in the ipsilateral regions outside the MCA territory (eg cingulate cortices, piriform cortices and entorhinal cortices), and in the contralateral regions of hippocampus after 4-h reperfusion following 1-h MCA occlusion. But morphological results showed severe edema and neuronal damage in the core territory and the ipsilateral hippocampus. DM blocked both the c-*fos* protein induction and neuronal damage in all regions. **CONCLUSION:** DM reduced c-*fos* protein expression and blocked the neuronal damage after focal cerebral ischemia.

Proto-oncogene proteins c-*fos* can be induced by a variety of stimili, such as elevation of intracellular calcium level, exposure to excitatory amino acid *in vitro*, and glutamate receptor agonist *in vivo*[1-3]. Local devascularization of cerebral cortex and global ischemia result in transient c-*fos* expression which is blocked by *N*-methyl-*D*-aspartate (NMDA) receptor antagonist[4-6]. Focal

[1] Supported by Key Projects for the Eighth Five-year Plan, № 8591307.
Received 1994-05-03 Accepted 1995-12-29

ischemia differs from both brain injury and global ischemia in the ischemia core and 'penumbra' of partial ischemia surrounding the ischemia core depending on levels of blood flow. NMDA receptor-mediated injury is greater in focal ischemia than in global ischemia, and NMDA antagonist protects the brain better against the former than against the latter[7]. Dextromethorphan (DM), a common antitussive drug can act as an NMDA antagonist better than dizocilpine maleate and phencyclidine clinically, and protect the brain against neuronal injury [8] Because of the importance of c-*fos* as a marker of neuronal NMDA receptor activation, we examined the effect of DM in focal cerebral ischemia.

MATERIALS AND METHODS

Rats Sprague-Dawley rats ($n = 30$), ♀♂, weighing 241 ± s 32 g, were bred by Department of Experimental Animals, Shanghai Medical University.

MCA occlusion[9] Rats were divided into 3 groups: 1) sham-operated group ($n = 7$); 2) operated group ($n = 10$); 3) operated group with DM ($n = 13$). Rats were anesthetized with sodium pentobarbital 35 mg · kg^{-1} ip. With the aid of an operating microscope, the superior thyroid and pterygopalatine arteries were isolated and coagulated, and the occipital artery was ligated. A 4−0 monofilament nylon suture was introduced into the lumen of the internal carotid artery for a length about 22 mm. At the same time, NS or DM (20 mg·kg^{-1} and 15 mg·kg^{-1}) were injected ip at 20-min interval to the rats. Sham-operated group had no nylon suture introduced into the lumen of the carotid artery. The occlusion lasted 1 h.

Drug DM was purchased from Sigma Chemical Co. Antisera Ab-1 against c-*fos* protein was purchased from Oncogene Sciences. ABC kit was purchased from Vector Lab.

Immunohistochemistry After 4-h reperfusion, rats were deeply anesthetized with sodium pentobarbital 40 mg ·kg^{-1}, ip and perfused intracardically with normal saline 200 mL, followed by 4 % freshly prepared paraformaldehyde 300 mL dissolved in phosphate-buffered saline (PBS) 0.1 mol ·L^{-1} at pH = 7.4. The intact brain was then dissected. Serial coronal slices (35 μm) were cut on freezing microtome and processed for c-*fos* protein by avidin-biotin technique[10]. The section was examined under a light microscope (Nikon).

HE staining After 24-h reperfusion, rats were deeply anesthetized with sodium pentobarbital 40 mg · kg^{-1} ip and perfused intracardially with normal saline 200 mL, followed by 10 % buffered formalin 300 mL. Brains were stored in 10 % buffered formalin for 10 d, then blocked in the coronal plane, paraffin-embedded, sectioned at 7 μm, and stained with hematoxylin and eosin.

RESULTS

c-*fos* protein-like immunoreactivity (CFPLI) changes of neurons CFPLI was not observed in the sham-operated rats, but increased dramatically within ipsilateral cortex, particularly in the cingulate cortices, piriform cortices, and entorhinal cortices, outside the middle cerebral artery territory followed by 4-h reperfusion after 1-h MCA occlusion. The most intensely immunoreactive neurons were found in layers Ⅱ and Ⅳ, and to a lesser extent in layers Ⅰ, Ⅲ, Ⅴ. By contrast CFPLI induction in neurons within CA3 to CA4 and the dentate hilar region was observed in the contralateral hippocampus, while only a few pyramidal neurons in CA1 region were seen. In the rats pretreated with DM, c-*fos* protein induction was blocked (Fig 1, 2, Plate 1,2).

Pathological changes of neurons Severe brain damage was confined mainly to the cerebral cortex within MCA territory after 1-h MCA occlusion followed by 4-h reperfusion. Edematous degenerative changes and necrosis manifested as vacuolation shrinkage and triangulation of the nucleus and cytoplasma, and nuclear size and basophilia of nucleus were reduced. DM reduced the brain damage and most pyramidical cells showed a normal appearance. Same results were found in the hippocampus, though the degree of neuron damage was less (Fig 3, Plate 3).

DISCUSSION

CFPLI expression was induced in the zone surrounding the ischemic core territory[11]. But in the transient global ischemic model, CFPLI was largely confined to the dentate gyrus and CA3 region. This discrepancy was probably due to species and surgical differences. Expression of CFPLI in the ipsilateral cortex may be caused by spreading depression during focal cerebral ischemia[12]. In the core region of MCA, a little c-*fos* protein expression may be related to protein synthesis inhibition. Therefore, in the cortex

adjacent to MCA core territory but not within the core itself c-*fos* protein was readily expressed[13]. In the same way, neuronal damage was confined to core terriory of MCA. In our model of MCA occlusion, the choroidal artery was also occluded, thus CFPLI was detected in the hippocampus, little CFPLI in the ipsilatery hippocampus may be also associated with protein synthesis inhibition. In the contralatery of hippocampus, CFPLI was induced in neurons within CA3 to CA4 and dentate hilar regions, but the neurons in this region showed a normal appearance. In this study, we can not interpret these results.

DM blocked the c-*fos* protein induction in all regions, and reduced the neuronal edema and necrosis. DM inhibited the activation of the NMDA receptor and reduced the influx of calcium causing neuronal death. These results indicate that c-*fos* expression is coupled to activation of the NMDA receptor, possibly due to excessive release of excitotoxin amino acid. Therefore, it is concluded that systematic administration of DM protects the brain against focal cerebral ischemia.

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418-420

右美沙芬对局灶脑缺血所致的 c-*fos* 蛋白表达的抑制作用

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关键词 脑缺血；原癌基因蛋白 c-*fos*；免疫组织化学；右美沙芬

目的：观察右美沙芬对局灶脑缺血所致的 c-*fos* 蛋白表达的作用. **方法**：免疫组织化学及 HE 染色的方法. **结果**：在缺血侧远离缺血区（如扣带皮层，梨状皮层，嗅皮层）和对照侧海马有大量 c-*fos* 蛋白表达，而在缺血区则有较多细胞坏死给予 DM 后，所有区域的 c-*fos* 表达及坏死细胞均减少. **结论**：DM 对局灶脑缺血所致神经细胞坏死有拮抗作用

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