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Cyclosporin A enhanced protection of nimodipine against brain damage induced by hypoxia-ischemia in mice and rats[1]

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KEY WORDS nimodipine; P-glycoprotein; blood-brain barrier; cyclosporins; brain hypoxia-ischemia

ABSTRACT

AIM: To study whether P-glycoprotein (P-gp) inhibitor cyclosporin A (CsA) enhanced the protection of nimodipine (NMD) against brain damage. **METHOD**: (1) After mice were given ip NMD alone or co-adminstration of CsA, survival time of mice were recorded following decapitation and ip injection of $NaNO_2$, respectively. (2) After rats were given ip NMD alone or co-adminstration of CsA, 20 min forebrain ischemia induced by the technique of two-carotid occlusion plus hypovolemic hypotension. Following reperfusion of 1 h, the content of malondialdehyde (MDA), lactic acid (LA) and activity of lactic dehydrogenase (LDH) in cortex tissue were measured. (3) NMD level in brain was also determined after ip injection of NMD 2 mg/kg alone and co-administration of CsA, respectively. **RESULTS**: NMD showed potent pharmacological activity in the three models. The survival time of mice by decapitation and ip $NaNO_2$ were significantly ($P < 0.05$) prolonged after co-adminstration of CsA. In rat forebrain ischemia/reperfusion, levels of MDA, LA, and LDH by co-aminstration of CsA were greatly modified, compared with those of NMD alone group. The level of NMD in rat brain was increased markedly after co-adminstration of CsA. **CONCLUSION**: P-gp inhibitor CsA may enhance the protection of NMD against brain damage.

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INTRODUCTION

The P-glycoprotein (P-gp) is a major factor responsible for development of drug resistance in tumor cells. P-gp is an ATP dependent drug transporter that reduces the intracellular concentration of cytotoxic compounds, thereby conferring drug resistance. P-gp was also detected in brain capillary endothelial cells (BCEC)[1,2]. Moreover, it was found that P-gp located in the luminal plasma membrane of BCEC and transport vincristin, cyclosporin A, doxorubicin, some peptides, and some amino acid[2-6] out of the cells, resulting in decreased permeation of them into the brain. These results led to an ideal that P-gp in BCEC may act as a part of the blood brain barrier (BBB) against the transfer of toxic compounds from circulation blood into brain interstitial fluid. Several compounds such as verapamil, cyclosporin A, and SDZ PSC 833, have been reported to be capable of inhibiting P-gp, and reversing the resistance mechanism, thus resulting in increase of pharmacological activity or toxicity of drugs.

Nimodipine (NMD) was a calcium antagonist of dihydropyridine and was successfully used to treat central nervous system disorders such as multi-infaract dementia, stroke and subarachnoid hemorrhage. NMD is also a substrate of P-gp, which suggested that the transport across BBB may be modulated by P-gp.

In the present study, we investigated whether P-gp inhibitor CsA enhanced the pharmacological activity of NMD on CNS. We also hoped to explore possibility to improve therapies of drugs including NMD on CNS disorders by interaction of drugs and P-gp.

MATERIALS AND METHODS

Materials Cyclosporin A (CsA) and nimodipine (NMD) were presented by Department of Pharmaceutics, China Pharmaceutical University. The drug suspensions were made in 0.5 % of sodium carboxymethylcellulose (CMC-Na). Male Sprague-Dawley

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rats, weighing 200 – 250 g (Grade Ⅱ, № 98004) and female ICR mice (Grade Ⅲ, № 003), 18 – 21 g, were supplied by Center of Experimental Animal, China Pharmaceutical University and Center of Shanghai Experimental Animal, respectively. All other chemicals were of analytical grade and commercially available.

Mouse complete brain ischemia Based on preexperimental results, mice were randomly assigned to 8 groups in 10 mice of each group. The mice fasted, but were given free access to water 12 h prior to experiment. The mice in A group were injected intraperitoneally (ip) with 0.5 % CMC-Na. The mice in B group were treated with ip CsA 5 mg/kg. The mice in C – E groups were injected ip with NMD 4, 8, and 16 mg/kg, respectively. And, the mice in F – H groups were injected ip with NMD 2, 4, and 8 mg/kg at 45 min after ip CsA 5 mg/kg, respectively. Fifteen min after ip NMD, or 1 h after ip CsA, the mice were killed by decapitation and the time from decapitation to breath stop was recorded.

Mouse brain hypoxia induced by $NaNO_2$ Based on preexperimental results, mice were randomly assigned to 8 groups in 10 mice of each group. The mice fasted, but were given free access to water 12 h prior to experiment. The mice in A group were injected intraperitoneally (ip) with 0.5 % CMC-Na. The mice of B group were treated with ip CsA 5 mg/kg. The mice in C – E groups were injected ip with NMD 2, 4, and 8 mg/kg, respectively, and the mice in F – H groups were injected ip with NMD 0.5, 1, and 2 mg/kg at 45 min after ip CsA 5 mg/kg, respectively. Fifteen min after ip NMD, the mice were given $NaNO_2$ (250 mg/kg) ip to induce hypoxia and the survival time of the mouse was recorded.

Rat forebrain ischemia/reqerfusion Male rats were randomly grouped into (A) sham-operation, (B) ischemia/reperfusion, (C) ischemia/reperfusion treated with CsA, (D) ischemia/reperfusion treated with NMD and (E) ischemia/reperfusion treated with NMD and CsA. The rats fasted, but were free access to water 12 h prior to experiment. the rats of C and D groups were given CsA 5 mg/kg and NMD 2 mg/kg ip, respectively. The rats of E group were given NMD 2 mg/kg ip at 50 min following ip CsA 5 mg/kg. The rats of A and B groups were given 0.5 % CMC-Na ip. Ten min after ip NMD or 1 h after ip CsA, 20 min forebrain ischemia induced by the technique of two-carotid occlusion plus hypovolemic hypotension was made as previously described[7]. After reperfusion of 1 h, the rats were killed. Cortex tissues were removed, weighed, and homogenated. The content of malondialdehyde (MDA), lactic acid (LA), and activity of lactic dehydrogenase (LDH) were measured.

Concentration of NMD in rat brain Male rats were randomly classed into 2 groups. One group was given ip NMD 2 mg/kg. Other group was given NMD 2 mg/kg ip at 50 min following ip CsA 5 mg/kg. Ten min after ip NMD, the rats were killed and brain tissues were removed, weighed, and homogenated. The concentrations of NMD in brain tissues were determined by HPLC[8] with UV detection at 238 nm. The sensitivity of the assay was 70 ng/g wet brain, reproducibility were better than 15 % in tested ranges and a good linearity was obtained from 70 – 2800 ng/g wet brain.

Data analysis All results were represented as $\bar{x} \pm s$. Statistical analysis was made on comparison of sample means by use of two-tailed and unpaired t test.

RESULTS

Protection from mouse complete brain ischemia induced by decapitation CsA itself significantly prolonged the duration of breath of mouse ($P < 0.01$). NMD dose-dependently prolonged the duration of breath. Significant prolongations were found in NMD 8 and 16 mg/kg ($P < 0.01$). The effects of NMD were markedly enhanced by co-administration of CsA, compared with those of NMD and CsA alone ($P < 0.01$) (Fig 1). The equations between prolonged duration of breath of mouse y (compared with those of control) and NMD dosage for alone NMD and co-administration of CsA were: $y = -2.95 + 2.734 \lg D$ ($r = 0.993$) and $2.34 + 4.624 \lg D$ ($r = 0.994$), respectively.

Protection from mouse brain hypoxia induced by $NaNO_2$ In this model, NMD 8.0 mg/kg significantly ($P < 0.05$) prolonged the survival time of mouse, but CsA, NMD 2 and 4 mg/kg did not. After treatment with ip CsA 5 mg/kg, the effect of NMD 0.5 mg/kg were even similar to that of NMD 8 mg/kg alone. The effect of NMD 2 mg/kg with CsA was significantly ($P < 0.01$) higher than that of NMD 2 mg/kg alone. The equations between prolonged survival time of mouse y (compared with those of control) and NMD dosage for alone NMD and co-administration of CsA were: $y = 0.46 + 2.106 \lg D$ ($r = 0.998$) and $8.37 + 5.850 \lg D$ ($r = 0.996$), respectively.

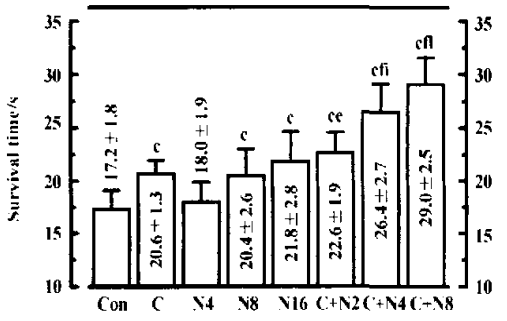

Fig 1. Effects of CsA and NMD on the breath duration of mouse after complete brain ischemia induced by decapitation. $n = 10$. $\bar{x} \pm s$. Con: control; C: CsA; N4, N8, and N16 represent NMD 4, 8, and 16 mg/kg, respectively; C + N2, C + N4, and C + N8 represent co-administration of CsA and NMD 2, 4 and 8 mg/kg, respectivly. $^cP < 0.01$ *vs* Con. $^eP < 0.05$, $^fP < 0.01$ *vs* CsA group. $^iP < 0.01$ *vs* N4. $^lP < 0.01$ *vs* N8.

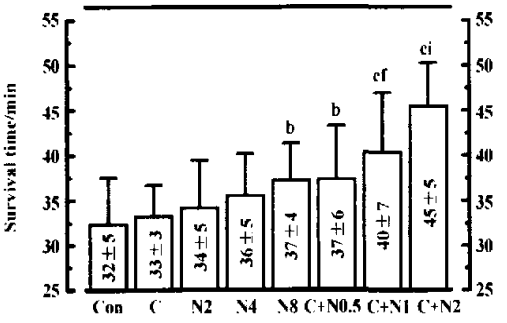

Fig 2. Effects of CsA and NMD on the survival time of mouse brain hypoxia induced by $NaNO_2$. $n = 10$. $\bar{x} \pm s$. Con: control; C: CsA; N2, N4, and N8 represent NMD 2, 4 and 8 mg/kg, respectively; C + N0.5, C + N1, and C + N2 represent co-administration of CsA and NMD 0.5, 1, and 2 mg/kg, respectivly. $^bP < 0.05$, $^cP < 0.01$ *vs* Con. $^fP < 0.01$ *vs* C. $^iP < 0.01$ *vs* N2.

Protection from rat brain damage induced by forebrain ischemia/reperfusion As demonstrated in Tab 1 that ischemia/reperfusion caused markedly ($P < 0.01$) decrease in the activity of LDH in brain tissues, to be compared with those of sham-operation. CsA had little effect on the activity of LDH. NMD 2 mg/kg made great improvement of the activity of LDH. The effect of NMD was further enhanced by co-administration of CsA and was significantly ($P < 0.01$) stronger than that of NMD alone.

Ischemia/reperfusion also made LA level in brain tissues significantly decrease ($P < 0.01$). NMD 2 mg/kg slightly increased the LA level ($P > 0.05$), and CsA significantly ($P < 0.05$) increased LA level to be compared with those of ischemia/reperfusion. After treatment with NMD and CsA, the LA level was further increased ($P < 0.01$) and was close to that of sham-operation rats.

Tab 1. Changes of LDH, LA, and MDA in rat cortex after ischemia/reperfusion. $\bar{x} \pm s$. $n = 6$. $^bP < 0.05$, $^cP < 0.01$ *vs* sham-operated. $^eP < 0.05$, $^fP < 0.01$ *vs* ischemia/reperfusion. $^hP < 0.05$, $^iP < 0.01$ *vs* NMD. $^kP < 0.05$, $^lP < 0.01$ *vs* CsA.

Groups	LDH/U·g^{-1} protein	LA/ nmol·g^{-1} protein	MDA/ nmol·g^{-1} protein
Sham-operation	6188 ± 341	10.1 ± 2.7	816 ± 69
Ischemia/reperfusion	4862 ± 347^c	3.3 ± 1.4^c	1254 ± 158^c
NMD 2.0 mg/kg	5319 ± 347^{cef}	4.2 ± 0.6^c	923 ± 86^{bf}
CsA 5 mg/kg	4890 ± 554^c	5.2 ± 1.1^{ce}	920 ± 154^l
NMD + CsA	5756 ± 280^{bfhl}	10.0 ± 0.9^{fil}	790 ± 86^{fhk}

Ischemia/reperfusion made level of MDA in brain tissues significantly ($P < 0.01$) increased, compared with those of sham-operation. Both NMD and CsA significantly ($P < 0.01$) reduced MDA level. Co-administration of NMD and CsA made the MDA level further reduced to that of sham-operation rats.

Concentration of NMD in brain tissues The NMD concentrations in brain tissues were determined 10 min after treatment of NMD 2 mg/kg. Pretreatment CsA 5 mg/kg significantly increased concentration of NMD in brain tissues ($P < 0.01$) [(603 ± 61) ng/g brain *vs* control (390 ± 96) ng/g brain].

DISCUSSION

The present study showed that CsA markedly enhanced effect of NMD on CNS in mice and rats. CsA itself had protection against brain damage induced by ischemia[9,10] by acting on mitochondrion respiration or via the suppression of some immunological reactions. The effect of CsA was also observed in mouse complete brain ischemia model and rat forebrain ischemia/reperfusion model. But, CsA did not prolong survival time of hypoxia mice induced by $NaNO_2$. NMD showed the potent protection against brain damage in the three models. These effects of NMD were markedly enhanced by co-administration of CsA. However, this mechanism was not clear.

CsA is a typical inhibitor of P-gp. CsA increased

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ivermetin neurotoxiciy in mice by increasing concentration of ivermetin[11] and also increased the permeability of doxorubicin[4] and rhodamine-123[12] to brain. NMD is also a substrate of P-gp, which suggested that the transport at BBB may be also involved in P-gp. Concentration of NMD in brain was lower than that in plasma, it was reported that the ratio of cerebrospinal fluid/serum concentration was only 0.01[13] and ratio of brain/plasma was below 0.2[14]. These results indicated that the enhancement of pharmacological activity of NMD on CNS was also likely to be related to the increase of NMD level in brain tissues, when given with CsA. In order to verify the hypothesis, we also determined the concentration of NMD in rat brain tissues alone or with administration of CsA. It was found that treatment with CsA made NMD concentration in brain tissues significantly increase. The result demonstrated that CsA maybe enhanced permeability of NMD to brain by inhibiting P-gp, thus resulting in enhancement of pharmacological activity of NMD on CNS.

About LA level in brain tissue, the result reported in the paper was in contrast to that of some reports. But there were also controversy reports. For example, Hetherington *et al*[15] found great accumulation of LA in rat brain after 30 min forebrain ischemia induced by four-vessel occlusion method and the LA concentration returned to normal values following 30 recirculation. However, Macmillan V[16] found that the level of LA in brain tissues was still higher than that of normal rat following 1 h reperfusion. But Pulsinelli *et al*[17] found that after 30 min forebrain ischemia following 1 h reperfusin, level of LA in paramedian neocortex was lower than that of normal rat, and the level of LA following 3 reperfusion was only 60 % of that of normal rats. These results indicated that the change of LA level in brain following ischemia/reperfusion may be depended on the method for ischemia, duration of ischemia, time of reperfusion and species of animals.

In summary, we first reported that CsA markedly enhanced pharmacological activity of NMD on CNS. These results demonstrated that it is possible to improve therapies of drugs on CNS disorders by use of interaction of drugs and P-gp.

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环胞素 A 加强尼莫地平对脑损伤保护作用[1]

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关键词 尼莫地平; P-糖蛋白; 血脑屏障; 环胞菌素类; 脑低氧-缺血

目的: 研究 P-糖蛋白(P-gp)抑制剂环胞素 A (CsA)是否加强尼莫地平(NMD)对脑损害的保护作用. **方法**: (1) 小鼠 ip NMD 或合用 CsA 后, 分别记录断头小鼠的喘气持续时间和 ip $NaNO_2$ 后的小鼠生存时间; (2) 大鼠 ip NMD 或合用 CsA 后, 用双侧颈总动脉结扎加低血压模型造成前脑缺血 20 min, 再灌 1 h 后, 测定皮层中丙二醛(MDA)、乳酸(LA)浓度和乳酸脱氢酸(LDH)活性. (3)测定单用 NMD 和合用 CsA 后, 脑内 NMD 的浓度. **结果**: NMD 对二种药理模型均显示一定的药理活性. 与单用 NMD 比较, 合用 CsA, 使断头小鼠和 ip $NaNO_2$ 后小鼠生存时间显著延长. 在脑缺血/再灌损伤模型大鼠中, 与单用 NMD 比较, 合用 CsA 后, 皮层中 MDA、LA 和 LDH 水平显著改善. 合用 CsA 使脑内 NMD 浓度显著增加. **结论**: P-gp 抑制剂 CsA 可加强 NMD 对脑损害的保护作用.

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