Original Article

A Study on Antitoxic Role of Vesicular Monoamine Transporter 2 in Transgenic Chinese Hamster Ovary Cells

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[**Abstract**] ***Objective***: *To study the antitoxic role of vesicular monoamine transporter 2 ($VMAT_2$) in transgenic Chinese Hamster ovary (CHO) cell.* ***Methods***: *With the technology of transgene from PC12 to CHO, MTT reduction assay was used to detect MPP^+ toxic effect on wild type CHO (wtCHO) and transgenic CHO. Meanwhile, the role of reserpine was also observed in MPP^+ toxic effects.* ***Results***: *The sensitivity of transgenic CHO to MPP^+ was much less than that of wtCHO with 0.5 mmol/L MPP^+. Transgenic CHO had the same sensitivity as wtCHO if rotenone was given. WtCHO, by given reserpine alone, didn't change its sensitivity to MPP^+.* ***Conclusions***: *$VMAT_2$ has protective effect on transgenic CHO by transporting MPP^+ to vesicles.*

[**Key words**] transgenic Chinese Hamster Ovary; Vesicular Monoamine Transporter 2; antitoxic; MPP^+; reserpine

Introduction

Studies on mechanisms of Parkinson's disease (PD) found that PC12 cells showed little susceptibility to the MPP^+, and that PC12 cells were dead by 4 d with 1 mmol/L MPP^+ and with 100 μmol/L MPP^+ by 2 weeks. However, dopaminergic neurons in the substantia nigra became dead with 1-10 μmol/L MPP^+ by 1-2 d[1,2]. It suggested that PC12 cells would have antitoxic component to MPP^+. Recently, vesicular monoamine transporter 2 ($VMAT_2$) was found in PC12 cells and dopaminergic neurons. It could carry MPP^+ and dopamine into subcellular structure (vesicule) and avoid toxicity entering mitochondria, in which respiration at the level of complex I may be inhibited[2,3].

In order to make certain about antitoxic effect on PC12 cells with $VMAT_2$, PC12 cells

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were transgened to Chinese Hamster Ovary (CHO) cells and MTT assay was used to measure dehydrogenase activity of wild type CHO (wtCHO) and transgenic CHO. Reserpine, the special inhibitor of $VMAT_2$, was given in order to observe its role in the toxicity of MPP^+.

Materials and Methods

Reagents

MPP^+, rotenone, MTT and DMSO were purchased from Sigma Company. Reserpine was obtained from Shanghai Pharmaceutical Factory. Culture medium DMEM was purchased from GIBICOL BRL Company.

Cell lines

WtCHO lines were purchased from Shanghai Institute of Cell Biology, Chinese Academy of Science and maintained in RPMI-1640 medium. Transgenic CHO lines were obtained from Dr Yongjian Liu (University of Pittsburgh ,US) and maintained in DMEM .

Methods

(1) WtCHO and transgenic CHO were placed in a 96-well plate with 2.5×10^5 cells per ml culture medium respectively. Every well had 200 μl confluent and was incubated at 37℃ with 5% vol CO_2 in culture case. One day later, the 96-well plate was divided into 4 groups. Group 1 was wtCHO, and Group 2 was transgenic CHO. These two groups were added with various concentration MPP^+ (0.1, 0.2, 0.25, 0.5, 1.0 mmol/L and blank control) to replace the former culture medium. The blank control was replaced by DMEM. Group 3 was wtCHO, and Group 4 was transgenic CHO. These groups were added with various concentration of rotenone (0.01, 0.1, 1.0, 10, 100 μmol/L and blank control) to replace the former culture medium. The blank control was also replaced by DMEM. MPP^+ of each concentration was placed in 3 wells. Incubation lasted 3 days.

(2) WtCHO and transgenic CHO were put in another 96-well plate with 2×10^5 cells per ml culture medium respectively. Every well had 200 μl confluent, incubated at 37 ℃ in 5% vol CO_2 culture case for 1 d. The 96-well plate was divided into 4 groups. Group 1 was wtCHO, and Group 2 was transgenic CHO. They were given MPP^+ (0.1, 0.2, 0.25, 0.5, 1.0 mmol/L and blank control), MPP^+ (0.1, 0.2, 0.25, 0.5, 1.0 mmol/L and blank control) and reserpine (1 μmol/L) was present in Group 3 and Group 4. The method was the same as the former mentioned above.

(3) Three days later, MTT assay was used to measure general cell dehydrogenase activity with the reduction of the tetrazolium dye MTT, according to Hansen[4]. The value of absorption D (λ) was detected by enzyme-linked immunosorbent assay meter (CliniBio 128C, sweden). D(λ)of the control group was rectified to zero. The rate of value increment R = test group D(λ)/ control group D(λ)×100%. The mean value was obtained from 3 wells of every concentration of MPP^+.

Statistical analysis

Experimental data were analyzed by SPSS 8.0 software. Statistical significance was accepted at a value of $P<0.05$. The Fig 1 and Fig 2 were taken by phase contrast microscope.

Results

The rate of value increment of wtCHO and transgenic CHO under various concentrations of MPP^+

Tab 1 and Tab 2 show that transgenic CHO cells can still live with 1 mmol/L MPP^+, however, wtCHO cells almost hardly survive this con-

centration of MPP^+. They have different sensitivities to MPP^+ ($P<0.05$).

Tab 1 D (λ) of wtCHO and Transgenic CHO under Various Concentrations of MPP^+ (mmol/L)

	MPP^+					
	0.00	0.10	0.20	0.25	0.50	1.00
wtCHO	0.47	0.35	0.30	0.22	0.16*	0.07*
Transgenic CHO	0.58	0.35	0.32	0.29	0.26	0.21

Compared with transgenic CHO group, * $P<0.05$; $n=3$.

Tab 2 D (λ) of wtCHO and Transgenic CHO under Various Concentration of MPP^+ and 1 μmol/L Reserpine (mmol/L)

	MPP^+					
	0.00	0.10	0.20	0.25	0.5	1.00
wtCHO	0.61	0.39	0.26	0.19	0.13	0.05
Transgenic CHO	0.59	0.52*	0.43*	0.38*	0.37*	0.30*
wtCHO+R	0.54	0.36	0.23	0.16	0.10	0.05
Transgenic CHO+R	0.57	0.41	0.33	0.19	0.10	0.04

Transgenic CHO Group compared with each of other group, * $P<0.05$; the other groups compared with each other, $P>0.05$; $n=3$. R means reserpine.

Tab 3 D (λ) of wtCHO and Transgenic CHO under Various Concentrations of Rotenone (μmol/L)

	Rotenone					
	0.00	0.01	0.10	1.00	10.00	100.00
wtCHO	0.61	0.56	0.41	0.34	0.33	0.18
Transgenic CHO	0.53	0.45	0.46	0.36	0.26	0.18

Compared wtCHO with transgenic CHO group, $P>0.05$; $n=3$.

There was difference in cell morphology at 1.0 mmol/L MPP^+ by high power objective of microscope (×400). The transgenic CHO cells were fusiform with a larger volume, however, wtCHO cells were round with a small volume, and some of them were ruptured. WtCHO cells showed greater sensitivity to MPP^+ than transgenic CHO cells. Tab 3 showed that transgenic CHO cells have no difference from wtCHO cells in susceptibility to rotenone ($P > 0.05$). Rotenone inhibited complex I of the respiratory chain and competes with MPP^+ for binding its presumed site of action. Thus if the antitoxic role of transgenic CHO cells was due to a change in the site of action, they should also show resistance to rotenone. Repeated experiments have demonstrated no substantial resistance to rotenone in the transfectant, suggesting the mechanism of resistance of the transfected cells seems somewhat specific to MPP^+. We examined the toxicity of MPP^+ on the transfected cells in the presence of 1 μmol/L reserpine and found dramatic reversion to wtCHO sensitivity (Fig 1,2). To demonstrate that reserpine does not affect the mechanism present in wtCHO cells, we tested its effect on wtCHO cells treated with the lower concentrations of MPP^+ to which they were normally sensitive. Reserpine shew very little reproducible effect on MPP^+ toxicity in wtCHO cells. The results suggested that the

transgenic CHO cells could survive in MPP+ because the cells express a vesicular amine-uptake activity, presumably derived from PC12 cells, which sequester the toxin effectively from its primary site of action in mitochondria.

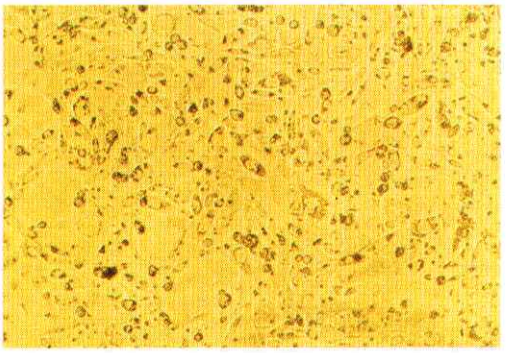

Fig 1 Transgenic CHO Cells with 1.0 mmol/L MPP+ (× 400)

Discussion

PD is a neurodegenerative disease. The relatively specific pathological features include an intraneuronal inclusion body - the Lewy body - and neuronal loss and depigmentation in the substantia nigra, particularly the zone compata. The etiology and pathogenesis of PD are still obscure. Several observations in idiopathic PD support the relevance of the MPTP model. The monoamine oxidase B expressed by glia converts MPTP to the active metabolite MPP+, which enters mitochondria of the cell and inhibits respiration[5]. But the features of the MPTP model were not understood such as the selective vulnerability of dopamine neurons in the substantia nigra. Although they also accumulate MPP+, multiple monoamine cell populations in the brain stem, sympathetic ganglia, and adrenal medulla showlittle toxicity. Some of these regions, such as the ventral tegmental area and locus coeruleus, show susceptibility to MPTP in pri-mates, but to a relatively minor degree. Since PD also affects these same populations to a lesser extent than the substantia nigra, the question of the susceptibility to toxin has considerable relevance to the idiopathic disorder.

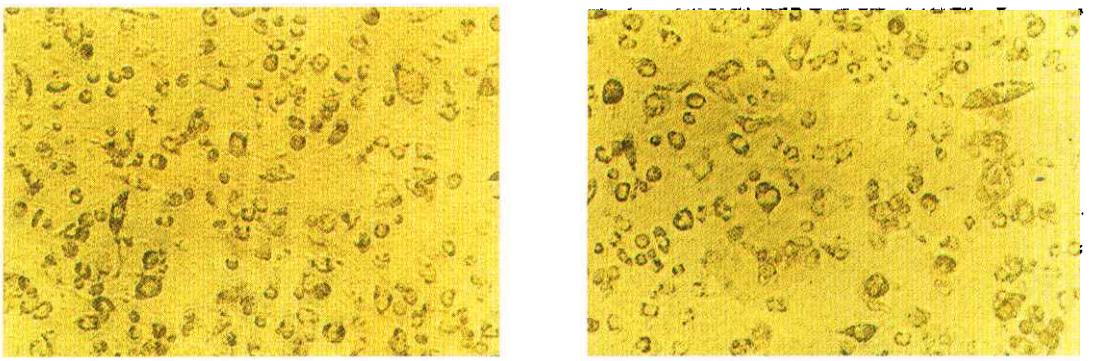

Fig 2 wtCHO Cells with 1.0 mmol/L MPP+(left) and Transgenic CHO Cells with 1.0 mmol/L MPP+ Plus Reserpine 1 μmol/L (right) (× 400)

To understand the differences in vulnerability to MPTP among cells that accumulate large amounts of toxin, we used transfected the MPP+-sensitive CHO cells with a cDNA library from PC12 cells. In contrast to wtCHO, the transgenic CHO cells can resist MPP+ at a high concentration (1 mmol/L MPP+). We then tested the mechanism that $VMAT_2$ conferred resis-

tance to the toxin by sequestering it in a vesicular compartment, away from the primary site of action in mitochondria. Indeed, the addition of reserpine, a potent inhibitor of $VMAT_2$, caused the sensitivity of the resistant cells to revert to wtCHO. Someone had done dopamine - loaded test[6], and found that wtCHO cells showed diffuse cytoplasmic staining, and transgenic CHO cells showed strong perinuclear and particulate cytoplasmic staining. This localized pattern reverts to wtCHO in the presence of 1 μmol/L reserpine, indicating that this mechanism is unique to the transfected cells. These results suggest that $VMAT_2$ may serve two functions in the nervous system, packaging transmitter for regulated release and neural protection.

The dopamine transporter (DAT) pumps MPP^+ and dopamine into dopaminergic neuronal cytoplasm, and the $VMAT_2$ can sequester MPP^+ and dopamine into the vesicles, and separate toxin from cytoplasm[7,8]. Thus DAT and $VMAT_2$ act to regulate the concentrations of these substrates in neuronal cytoplasm, where they can have more prominent toxicity, and in synaptic vesicles, where toxicities are less pronounced. The crucial role that different levels of expression of the transporter molecules can result in the pathophysiology of parkinsonian syndromes induced by experimental toxins is highlighted by recent studies of the molecular and cellular biology of DAT and $VMAT_2$. The levels of DAT and $VMAT_2$ expression in several dopaminergic neuronal groups correlate with the extent of their cell losses in PD brain[9,10]. In situ hybridization, studies on rats reveal significantly higher levels of DAT mRNA expression in cells of the substantia nigra pars compacta (SNc) than in those of the ventral tegmental area (VTA). However, $VMAT_2$ mRNA express in VAT more than in those of SNc. The ratio between the toxin accumulating (DAT) and toxin sequestering ($VMAT_2$) may also show greater differences between the dopaminergic neurons residing in these different nuclei[11].

Transporter expression in living humans has been studied by radioligand binding with detection by positron emission tomography (PET) or single-photon emission computed tomography (SPECT). Results suggest that there is true and significant individual variation in the expression of DAT and $VMAT_2$. Thus differences can alter vulnerabilities to dopaminergic toxins in PD patients[12,13].

Identifying transport-associated mechanism that explains selective losses in PD dopaminergic neurons could be of great importance. On the one hand, individual differences in DAT and $VMAT_2$ expression, genetically or environmentally determined, could serve as markers for vulnerability to PD. On the other hand, drugs that upregulate or enhance the functions of $VMAT_2$ or those that inhibit intracellular uptake mediated by DAT would be helpful for therapeutic trials targeted toward reducing disease onset or slowing progression. The further studies should be necessary.

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转基因中国仓鼠卵细胞中单胺囊泡转运体抗毒性作用的研究

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[摘 要] **目的**：研究转基因中国仓鼠卵(CHO)细胞中单胺囊泡转运体($VMAT_2$)的抗毒性作用。**方法**：利用转 PC12细胞基因到 CHO 细胞中形成的转基因 CHO 细胞(cDNA-CHO)，采用 MTT 比色法检测1-甲基-4-苯基吡啶离子(MPP^+)对 CHO 细胞野生株(wtCHO)和 cDNA-CHO 细胞的毒性作用，并观察利血平——$VMAT_2$的特异性阻滞剂对 MPP^+毒性作用的影响。**结果**：在 MPP^+ 0.5 mmol/L 以上浓度 cDNA-CHO 细胞对 MPP^+敏感性比 wtCHO 低得多；cDNA-CHO 和 wtCHO 对鱼藤酮(rotenon)的敏感性无显著差异；加入利血平后，上述保护作用消失，cDNA-CHO 对 MPP^+敏感性与 wtCHO 细胞无差异，而单独予以 wtCHO 细胞利血平则不能改变它对低浓度 MPP^+的敏感性。**结论**：此保护机制是由转基因细胞中 $VMAT_2$引起的，$VMAT_2$在转基因的非神经细胞系(CHO 细胞系)中也能将 MPP^+转运至囊泡内，从而保护细胞，同时也提示 PC12细胞内具有抗毒性作用的成分。

[关键词] 转基因中国仓鼠卵细胞；单胺囊泡转运体；抗毒性；1-甲基-4-苯基吡啶离子；利血平