研究快报

帕金森病裸 DNA 法基因治疗的实验研究

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摘要　采用体内裸 DNA 基因治疗帕金森病（PD）取得显著疗效．将酪氨酸羟化酶（TH）基因表达质粒与 Lipofectin 形成的复合物立体定位注射于 PD 模型鼠纹状体，显著改善了 PD 鼠的不对称旋转行为．免疫组化证实神经细胞表达了外源 TH．

关键词　帕金森病，基因治疗，酪氨酸羟化酶

帕金森病（PD）是中老年期常见的神经系统变性疾病，也是最常见的致残疾病之一．主要病因是患者中脑黑质多巴胺能神经元损伤，表达的酪氨酸羟化酶（TH）减少或活性下降，造成脑内多巴胺含量明显减少．目前，外源性左旋多巴是最有效的抗 PD 药物，但只能控制症状而无法阻止病情发展，并有难以耐受的副作用．基因治疗 PD 的可能性已得到一些实验支持．将转染了 TH 基因的细胞移植于 PD 鼠脑尾核，发现移植细胞表达的 TH 可将酪氨酸转化为多巴[1—4]．我们将 TH 基因直接转染 PD 鼠纹状体，对 PD 进行实验性基因治疗，获得初步成功．

1　材料与方法

1.1　PD模型鼠制备　用6-羟基多巴胺（6-OHDA）损毁 SD 大鼠单侧黑质制成 PD 鼠．在阿朴吗啡（APO）诱导下 PD 大鼠将会向未损伤侧旋转显示模型制备成功．长期稳定（2个月以上）的模型用于实验．

1.2　TH 表达质粒构建　将大鼠 TH cDNA 编码区与真核基因表达载体 pSVK3（含 SV40 早期启动子和加 poly A 位点）重组构建治疗用表达质粒 pSVK3-TH．

1.3　基因转染　pSVK3-TH（高剂量5μg/只、低剂量1μg/只）与 Lipofectin（BRL）以1:3比例混合制成基因转染复合物，立体定向多点注射于 PD 鼠损毁侧的纹状体．

1.4　行为检测　治疗前后肌注 APO 诱导旋转，记数30min 内旋转次数进行比较．

1.5　免疫组化检测　治疗后第12天处死动物，分离双侧纹状体，用抗 TH 单抗进行免疫组化染色．

2　结果与讨论

治疗后第3天，PD 鼠 APO 诱发的旋转比治疗前明显减少，高、低剂量组分别减少50%和40%．免疫组化显示，治疗侧（即黑质损毁侧）纹状体内可见 TH 阳性神经细胞，其胞体

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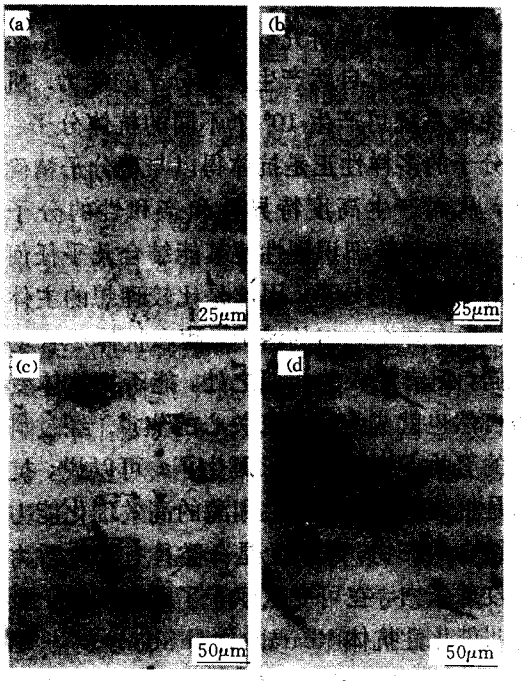

图 1　PD 鼠纹状体神经细胞 TH 免疫组化检测

(a) 注射基因侧纹状体，(b) 未注射基因侧纹状体，(c)，(d) 注射侧纹状体内 TH 阳性神经细胞. 实心箭头所示为 TH 阳性胞体，空心箭头为阳性轴突.

和轴突呈棕色，多集中于注射部位附近，而对照侧（即黑质正常侧）纹状体内无 TH 阳性细胞（图 1），证明外源 TH 基因确已进入纹状体细胞并表达了有生物活性的 TH，将酪氨酸转变为多巴从而产生疗效.

以上结果从细胞水平和动物整体水平初步证明脂质体可介导 TH 基因在体内直接转染神经细胞并成功表达，预示采用裸 DNA 直接转染方法治疗 PD 是可能的. 目前，用这一新方法治疗 PD 国内外尚未见报导. 进一步全面深入研究 TH 基因转移和表达，包括核酸分子水平检测和动物长期疗效观察，正在进行中.

参 考 文 献

1　Wolff, J A, Fisher L J, Xu L *et al*. Proc Natl Acad Sci USA, 1989; **86**: 9011

2　Horellou P, Brundin P, Kalen P *et al*. Neuroendocrinology, 1990; **5**: 393

3　Fisher L J, Jinnah H A, Kale L C *et al*. Neuroendocrinology, 1991; **6**: 371

4　Jiao S, Gurevich V, Wolff J A. Nature, 1993; **362**: 450

Zhengxing. Liu Huizhong. Bao Jingqi. Chen Hongzhuan. Sun Yuyan. Sun Chen. (*Dpartment of Pharmacology, Shanghai 2nd Medical University, Shanghai* 200025). *Prog. Biochem. Biophys.* (China). 1994: **21** (4): 362—366

A direct micro determination of glutathione peroxidase (GSH-PX) activity with spectrophotometry was developed. Conditions of the assay were studied in detail. 10μl blood sample was diluted with distilled water and treated with 10% TCA to remove protein. There was a good linearity between DTNB product and concentration of GSH after 3 minutes enzymatic reaction at the temperature of 37℃ in pH 6.5 solution. The method had higher sensitivity, better reproducibility. It may became useful tool for analyzing GSH-PX in scientific research and clinical work.

Key words glutathione peroxidase (GSH-PX), spectrophotometry, assay

Production of Specific Antisera to Thyroid-stimulating Hormone (TSH). Zhou Ling. (*Deparment of Isotope, China Institute of Atomic Energy, Beijing* 102413). *Prog. Biochem. Biophys.* (China). 1994: **21** (4): 366—368

Specific high titre antisera to TSH were raised in two sheeps injected with 100μg (booster injection, 50μg) highly purified TSH preparation by the multi-site intradermal immunization technique. Blood were bled at two week intervals by cardiac puncture without killing the animals and solution of anti-anemia drug was given to sheeps after each leting blood. The antisera were monitored by TSH RIA Kit. Titres were range from 28×10^4 to 205×10^4 and no cross-reaction occured between TSH antisera and human LH, FSH, HCG and all antisera have the avidity more than 10^{10}L/mol.

Key words TSH, antiserum, RIA

Experimental Research on Naked DNA Gene Therapy of Parkinson's Disease. Cao Lei, Zheng Zhongcheng, Liu Xinyuan, Liu Zhenguo, Zhao Yingchun, Chen Shengdi, Jiang Zhihua, Zhou Changfu. (*Shanghai Institute of Biochemistry, Academia Sinica, Shanghai* 200031). *Prog. Biochem. Biophys.* (China), 1994; **21** (4): 369, 289

In vivo naked DNA gene transfer method was used in gene therapy of Parkinson's disease (PD). The complex of rat tyrosine hydroxylase (TH) expression plasmid and Lipofectin was injected stereotaxically into striatum of PD rat model. The asymmetric rotational behavior was reduced substantially and quickly. On the third day after injection, drug-induced rotation decreased 50% compared with pretreatment scores. Immunohistochemical staining showed TH-positive nerve cells in striatum of injection side, which indicated that TH gene was uptaked and expressed by nerve cells. These preliminary results have general implications for the application of naked DNA transfer technique in gene therapy of human neurological disease and specific implications for PD.

Key words Parkinson's disease, gene therapy, tyrosine hydroxylase

Production of TSH-Free Thyroid Stimulating Hormone Serum. Zhou Ling. (*Department of Isotope, China Institute of Atomic Energy, Beijing* 102413). *Prog. Biochem. Biophys.* (China), 1994; **21** (4): 370—371

Using affinity chromatography to eliminate TSH from normal human serum. Preliminary treatment to TSH with 33% saturated ammonium sulfate were coupled to agarose in alkaline condition and packed in column (1.2cm×