ISSN 1671-4083 Acta Pharmacol Sin 中国药理学报 2002 Jul; 23 (7): 609-611
http://www.chinaphar.com

Safety and bioactivity of intracoronary delivery of naked plasmid DNA encoding human atrial natriuretic factor

HUANG Guo-Ming[1], LI Geng-Shan[2], ZHU Gang-Yan[2], LAI Yi-Mu[2], ZHANG Hong-Xiang[2], WANG Jing[2], WANG Hong-Ru (*Department of Cardiology, 94th Hospital of PLA, Nanchang 330002, [2]Department of Cardiology, People's Hospital of Wuhan University, Wuhan 430060, China*)

KEY WORDS atrial natriuretic factor; plasmids; heart catheterization; gene expression; myocardium; dogs

ABSTRACT

AIM: To evaluate the safety and bioactivity of catheter-mediated intracoronary gene delivery of naked plasmid DNA encoding human atrial natriuretic factor (hANF). **METHODS**: hANF gene delivery was performed in 12 canines. For each canine, 4 mg of reconstructed naked plasmid DNA encoding hANF (pCR3.hANF, $n=6$) or pCR3 ($n=6$, control) in 2 mL normal saline was injected into left coronary artery *via* a coronary angiographic catheter. The expression of hANF mRNA in left ventricular myocardium, liver, lung, and kidney was identified by reverse transcription polymerase chain reaction and Southern blot analysis 40 d after gene delivery. Plasma ANF levels were determined by radioimmunoassay. **RESULTS**: The naked pCR3.hANF caused significant expression of hANF mRNA in ventricular myocardium ($P<0.01$). No hANF mRNA was detected in distal tissues, including liver, lung, and kidney ($P>0.05$). ANF levels were significantly higher in pCR3.hANF group than those in control group. **CONCLUSION**: These facts firstly demonstrate that intracoronary delivery of naked pCR3.hANF is probably a safe and feasible method for gene delivery by which hANF gene can be expressed in ventricular myocardium effectively.

INTRODUCTION

Atrial natriuretic factor (ANF) is a 28-amino acid peptide hormone with natriuretic/diuretic and vasorelaxant actions. Applied clinically from the early 1990s, ANF has been shown to be effective in the treatment of hypertension and congestive heart failure[1]. In recent years, researchers have paid great attention to gene therapy for heart diseases. Gene therapy is beneficial for the patients who need long-term medication such as chronic heart failure, because gene expression can keep several weeks to several months. Is catheter-mediated intracoronary delivery of naked hANF plasmid DNA a safe and effective method? Finding the answer of the question was the purpose of this paper.

MATERIALS AND METHODS

Construction and purification of plasmid DNA (pCR3.hANF) The hANF cDNA insert was released from pT73D.hANF by *Eco*R Ⅰ and *Not* Ⅰ digestion and cloned into the plasmid pCR3 (from invitrogen) at *Eco*R Ⅰ and *Not* Ⅰ sites. After identified by restriction endonucleases, pCR3.hANF was purified with polyethylene glycol.

Catheter-mediated intracoronary gene delivery hANF gene delivery was performed in 12 canines (Grade Ⅰ, from Medical Experimental Animal Center, the First Clinical College of Wuhan University, Certificate No 19-036). For each canine (20-25 kg), 4 mg of pCR3.hANF ($n=6$) or pCR3 ($n=6$, control) in 2 mL normal saline was injected into left coronary artery *via* the coronary angiographic catheter.

Total RNA extraction All canines were killed 40 d after gene delivery. The total RNA was extracted from left ventricular myocardium, liver, lung, and kidney tissues with TRIZOL reagent (Gibco, USA) according to the manufacturer's instructions.

Reverse transcription polymerase chain reaction (RT-PCR) and Southern blot analysis The upstream and downstream primers for RT-PCR were 5′-ACGCAGACCTGATGGATTTC-3′ and 5′-TTGCTT-TTTAGGAGGGCAGA-3′ respectively. RT-PCR was performed with access RT-PCR system (Promega, USA)

[1]Correspondence to HUANG Guo-Ming, PhD.
Phn 86-791-649-1623. E-mail huangguoming@hotmail.com
Received 2001-10-08 Accepted 2002-04-03

ISSN 1671-4083 Acta Pharmacol Sin 中国药理学报 2002 Jul; 23 (7)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

according to its instructions. The RT-PCR products were analyzed with agarose gel electrophoresis and the optical density of every electrophoresis lane was measured with ImageMaster VDS (Pharmacia Co, USA). The RT-PCR products 20 μL was subjected to a Southern blot analysis. A DIG DNA labeling and detection kit (Boehringer Mannheim, Germany) was used for probe labeling, prehybridization, hybridization, and color reaction. The blot was washed twice in 2 × SSC (sodium chloride/sodium citrate), 0.1 % SDS (sodium dodecyl sulfate) at room temperature for 5 min and then washed twice in 0.1 × SSC, 0.1 % SDS at 68 ℃ for 15 min.

Radioimmunoassay for ANF Forty days after gene delivery, 2 mL blood was withdrawn from the femoral vein for each canine. The samples were immediately transferred to chilled glass tubes containing 30 μL 10 % Na_2-edetic acid and 40 μL aprotinin, and then centrifuged at 4 ℃. Plasma was frozen immediately and stored at −70 ℃ until assay. Plasma ANF concentration was measured with commercially available atrial natriuretic polypeptide radioimmunoassay kit (Beijing Dongya Biotechnical Institute, China) according to its instructions. The radioactivity of the precipitate was detected in a gamma counter.

Statistical analysis Data were presented as $\bar{x} \pm s$ and analyzed by Student's t test. $P < 0.05$ was considered statistical significance.

RESULTS

RT-PCR and Southern blot The results of electrophoresis of RT-PCR products showed that clear lanes could be seen at the site of 235 bp (Fig 1), which were confirmed as ANF gene by Southern blot. The optical density of RT-PCR products in ventricular myocardium was significantly higher in pCR3.hANF group than that in control group. There were no marked changes of optical density in liver, lung, and kidney between two groups (Tab 1).

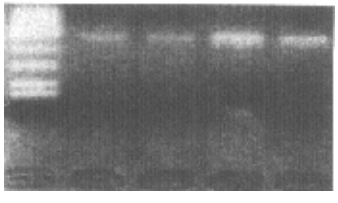

Fig 1. Electrophoresis of RT-PCR products from heart. Lane 1, 2: pCR3.hANF group; Lane 3, 4: control group; Lane 5: pBR322/Msp I DNA Markers.

Tab 1. Comparison of optical density of RT-PCR products between pCR3.hANF group and control group. $n = 12$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* control.

Groups	Optical density			
	Heart	Liver	Lung	Kidney
pCR3.hANF	183 ± 14[c]	121 ± 8	177 ± 8	189 ± 8
Control	139 ± 10	121 ± 5	176 ± 4	186 ± 8

Radioimmunoassay The plasma ANF levels were significantly higher in pCR3.hANF group than those in control group 40 d after injection (429 ± 56 *vs* 188 ± 30, $P < 0.01$).

DISCUSSION

There are many pathways for gene delivery into heart, including direct intramyocardial injection, catheter-based pericardial gene transfer, and intracoronary delivery and so on[2-4]. Direct intramyocardial injection of naked plasmid DNA is confirmed as a safe and effective method for gene therapy, but thoracotomy is needed for gene injection. Pericardial cavity delivery also is not a good method because the catheter must puncture the right atrium into pericardial cavity. Previous study indicated that catheter-mediated intracoronary gene transfer of replication-defective adenovirus was efficient. Using the same method, we evaluated the safety and bioactivity of delivery of naked hANF plasmid DNA. In our study, hANF gene could be expressed efficiently in ventricular myocardium and the expression could last 40 d or more. Furthermore, there was no marked difference of optical density in liver, lung, and kidney between two groups.

In our opinions, there are at least 3 possible factors by which naked hANF gene with catheter-mediated intracoronary delivery can be expressed efficiently in ventricular myocardium. Firstly, as blood supply in myocardium is very rich, hANF gene delivered through the coronary artery can fully contact with the membranes of ventricular myocytes and can be ingested easily by myocytes. Secondly, delivery of high-dose hANF gene may have contributed to increase the contacting sites between hANF gene and ventricular myocytes. As a result, the probability that hANF gene is ingested by

myocytes increases. Lastly, ventricular myocytes are rich in sarcoplasmic reticula, which make the intake of hANF gene easier.

Although no expression of hANF gene was observed in liver, lung, and kidney in the study, whether or not hANF gene had entered the blood circulation and expressed in other tissues still keeps unknown. Further researches will uncover the puzzle.

In summary, our study demonstrates that intracoronary delivery of naked hANF plasmid DNA is probably a safe and effective method, which provides a new and convenient pathway for clinical gene therapy.

REFERENCES

1 Wilkins MR, Redondo J, Brown LA. The natriuretic peptide family. Lancet 1997; 349: 1307 - 10.

2 Losordo DW, Vale PR, Symes JF, Dunnington CH, Esakof DD, Maysky M, *et al*. Gene therapy for myocardial angiogenesis initial clinical results with direct myocardial injection of phVEGF165 as sole therapy for myocardial ischemia. Circulation 1998; 98: 2800 - 4.

3 March KL, Woody M, Mehdi K, Zipes DP, Brantly M, Trapnell BC. Efficient *in vivo* catheter-based pericardial gene transfer mediated by adenoviral vectors. Clin Cardiol 1999; 22 (1 Suppl 1): I 23 - 9.

4 Barr E, Carroll J, Kalynych AM, Tripathy SK, Kozarsky K, Wilson JM, *et al*. Efficient catheter-mediated gene transfer into the heart using replication-defective adenovirus. Gene Ther 1994; 1: 51 - 8.

冠状动脉内导入裸人心钠素质粒 DNA 的安全性和生物活性

黄国明[1], 李庚山[2], 朱刚艳[2], 赖亦木[2], 张宏祥[2], 王 晶[2], 王洪如
(解放军 94 医院心内科, 南昌 330002, [2]武汉大学人民医院心内科, 武汉 430060, 中国)

关键词 心钠素; 质粒; 心脏导管插入术; 基因表达; 心肌; 犬

目的: 评价冠状动脉内导入裸人心钠素(hANF)质粒 DNA(pCR3.hANF)的安全性和生物活性. **方法**: 经冠状动脉造影导管, 将 4 mg 的裸 pCR3.hANF (n = 6)或 pCR3 (对照组, n = 6)注入犬的左冠状动脉. 基因导入 40 天后, 通过聚合酶链式反应和 Southern 杂交检测 hANF mRNA 在左心室肌、肝、肺、肾的表达情况, 且采用放射性免疫测定法测定血浆 ANF 的水平. **结果**: pCR3.hANF 组中, hANF mRNA 在心室肌中得到了较高的表达($P < 0.01$), 血浆 ANF 水平明显高于对照组($P < 0.01$), 且未发现 hANF mRNA 在肝、肺、肾中表达($P > 0.05$). **结论**: 经冠状动脉导入裸 pCR3.hANF 可能是一安全、有效的基因导入途径.

(责任编辑 韩向晖)