Retrovirus vector transfection of rat insulin gene into pancreas decrease blood glucose of diabetic rat

Min-Chuan Lai1,2, Chi Yang1*

1Department of Veterinary Medicine, College of Veterinary Medicine, National Chung Hsing University, Taichung, Chinese Taipei
2Bureau of Animal and Plant Health Inspection and Quarantine, Council of Agriculture, Executive Yuan, Chinese Taipei
Email: *cyang@dragon.nchu.edu.tw

Received 28 April 2013; revised 30 May 2013; accepted 10 June 2013

Copyright © 2013 Min-Chuan Lai, Chi Yang. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT

Human and animal diabetes mellitus were controlled by a dietary treatment supplemented with either a sulfonylurea drug or insulin injection. Insulin injections were inconvenient and the hypoglycemia induced by insulin-overdose could be fatal. Sulfonylurea drugs were administered orally, however, do not typically provide satisfactory control of blood glucose as a starting treatment in 25% - 30% patients. Therefore, it was imperative to develop a method for the control of human and animal diabetes mellitus. Recently, insulin gene transferred and expressed in non-pancreatic cells as a means for the treatment of diabetes was developed rapidly in the expanding gene therapy. Retrovirus, lentivirus, adenovirus, adeno-associated virus and herpes simplex had been used as viral vectors, and the constructed viral-insulin gene was successfully transferred into diabetic rat cells. A gene, containing promoter, enhancer and rat type I insulin gene (a-chain, b-chain and signal peptide), was constructed into a retrovirus vector in the study. The constructed viral-insulin gene was transferred into mouse fibroblast cell. The insulin concentration in 3-day cultured mouse fibroblast cells was 4806.35 ± 53.72 pg/ml. The insulin concentration for the viral vector containing enhancer and promoter of rat insulin gene was higher than the vector containing only insulin gene by a 61% increase in the cultured mouse fibroblast cells. The enhancer and promoter activity of rat insulin gene would be an important determinant for the expression of insulin gene. The secreted amount of insulin by retrovirus vector contained enhancer/promoter gene in this study could achieve as high concentrations (4806.35 ± 53.72 pg/ml) as the insulin injection therapy. Blood glucose decreased significantly for at last 10 days demonstrated that transfection, direction injection of viral-insulin gene into pancreas of diabetic rat, was successful. These studies suggest that the retrovirus vector might be used to transfer the insulin gene in vitro and in vivo.

Keywords: Enhancer; Diabetic Rat; Insulin Gene; Promoter; Viral Vector

1. INTRODUCTION

Human type I diabetes mellitus caused by the lack of insulin secretion secondary to the autoimmune destruction of pancreatic cells was usually treated by multiple injections of insulin [1,2]. Evidence also existed for a genetic basis and altered immune response in the pathogenesis of canine diabetes. In addition, at least 50% of diabetic dogs were type I diabetes based on the present evidence of immune destruction in beta-cells [3]. As a consequence, diabetic patients (including human and animal) experienced profound metabolic derangements (hyperglycemia, ketosis, and hyperlipemia) and developed vascular and neurological chronic complications. In type II diabetes mellitus, patients have functional cells, but these cells respond poorly to the stimulation of glucose. Therefore, the patients were treated with a dietary treatment supplemented with either a sulfonylurea drug or insulin injection to control type II diabetes mellitus. However, the insulin injections were inconvenient and the hypoglycemia induced by insulin-overdose would be fatal. Sulfonylurea drugs were administered orally; however, sulfonylurea drugs typically did not provide satisfactory control of blood glucose as a starting treatment in 25% - 30% patients [4]. Therefore, it is imperative to develop a new method for the control of human and animal diabetes mellitus. The use of rat in diabetes study is the most common mode as well as the one of the most feasible.
way. In addition, the molecular structure of the rat insulin is very similar to human and dog insulin molecular structure, both posed by the a-chain and b-chain. However, the thirtieth amino acid of the b chain in human, rat and dog are serine, threonine and alanine, respectively [5]. Recently, gene therapy has been rapidly expanding; insulin gene was transferred and expressed in non-pancreatic cells as a means for treating diabetes. Retrovirus, lentivirus, adenovirus, adeno-associated virus and herpes simplex had been used as a viral vector [6-9], the constructed viral-insulin gene was successfully transferred into diabetic rat cells [10-12], and the treatment temporarily decreased blood glucose concentration.

2. MATERIALS AND METHODS

2.1. Isolation of Pancreatic Islet

Five male Sprague-Dawley (SD) rats were anesthetized with pentobarbital sodium. Following a midline incision, the bile duct was cannulated with a polyvinyl tubing (0.625 mm, id) and injected with 15 - 20 ml of Krebs-Ringer bicarbonate (KRB) buffer. The pancreas tissues were cut into fine pieces, approximately 1 - 2 mm, and then digested with collagenase. Islets were individually hand-picked and collected by using a microscope. The animal use protocol was reviewed and approved by the Institutional Animal Care and Use Committee of National Chung Hsing University.

2.2. Preparation of DNA from Islet

The isolated islets were digested with proteinase K solution, then the rat genomic DNA was isolated and purified by using phenol/chloroform/isoamylalcohol method.

2.3. Polymerase Chain Reaction

Rat type I insulin gene (encoding a-chain, b-chain and signal peptide), enhancer and promoter gene (−346/+9 fragment) were synthesized by polymerase chain reaction (PCR).

The rat type I insulin gene used primers: CCTGTGGATCCGCTTCCTGC for the N-terminal primer and AGGCTTTATTCATTGCAGAG for the C-terminal primer.

The rat insulin enhancer and promoter gene used primers: TTTTAAGCTTCCCAACAACCT GCAACTTT CTGGGAAATGAG for the N-terminal primer and TGTAAGCTTCCCTTAAG GTGGGGAGTTACTGCGT CTC for the C-terminal primer. Each PCR cycle consisted of 1 min at 95°C, 1 min at 45°C and 2 min at 72°C and 30 cycle were performed in a GeneAmp PCR system 9600 (Perekin Elmer).

2.4. Cloning of Rat Insulin I Plasmid (pRat-Ins I)

PCR product of rat type I insulin gene (a-chain, b-chain and signal peptide) was ligated into pCR 2.1-TOPO (Invitrogen).

2.5. Cloning of Rat Enhancer/Promoter-Insulin I Plasmid (pEP-Rat-Ins I)

PCR product of rat insulin enhancer/promoter was cut by Hind III and Sac I to make a 361 bp fragment. pRat-Ins was also cut by Hind III and Sac I. The 361 bp enhancer/promoter fragment was ligated into pRat-Ins I.

2.6. Cloning of pLNCI2 Plasmid

PCR product of rat insulin enhancer/promoter was cut by Hind III and Sac I to make a 470 bp fragment. pLNCX2 (CLONTECH), retrovirus vector, was cut by Hind III and Xho I, then the 470 bp fragment was ligated into pLNCX2.

2.7. Cloning of pLNCP2 Plasmid

PCR product of rat insulin enhancer/promoter was cut by Hind III and Xho I to make an 815 bp fragment. pLNCX2 (CLONTECH), retrovirus vector, was cut with Hind III and Xho I, and then the 815 bp fragment was ligated into pLNCX2.

2.8. Transferring Retrovirus Vector

Packaging cells (mouse fibroblast cells), Packaging cells, mouse fibroblast cells, were cultured on a 60 mm plate for 12 - 24 hours, and then transferred with 10 μg plasmid DNA. After 24 hours, the culture medium was discarded and the transferred cells were washed with PBS. Another 5 ml of culture medium was added and cultured for an additional 12 - 48 hour to increase viral titer.

2.9. The Determination of Insulin Concentration

The expressed insulin concentrations were determined by using radioimmunoassay (RIA) as previously described by Hale and Randle in 1963. Rat insulin was used as standards for the RIA.

2.10. In Vivo Injection of pLNCI2 Plasmid and pLNCP2 Plasmid (in Mouse Fibroblast Cells) into Rat Pancreas

Ten adult SD rats (an average body weight 300 ± 50 g, including males and females) were used to induce diabetes. Rats were fasted for 12 hours before diabetes were induced by using streptozotocin (STZ). The dose of 150 mg/kg STZ (Sigma) was given to rats by intraperitoneal injection one time. The samplings had been made from the tail vein and measurements of the blood glucose were
made with Accu-Chek Go (Roche). One day after injecting STZ, rats with blood glucose more than 200 mg/dl were chosen for the in vivo transfection. Rats were divided into two groups (5 rats per group) and each group received one vector (500 μl), pLNCI2 and pLNCP2 (in mouse fibroblast cells), respectively.

2.11. Data Statistical Analysis

Data were analyzed by using analysis of variance (ANOVA) to determine the significance of treatment and time.

3. RESULTS

In this study, we constructed a gene containing rat type I insulin gene promoter, enhancer and insulin gene (a-chain, b-chain and signal peptide) into a retrovirus vector (Figures 1-3). The constructed viral-insulin gene was transfected into mouse fibroblast cells. The 3-day cultured mouse fibroblast cells were homogenized, and then the insulin concentration was determined by using RIA. The insulin concentration was 4806.35 pg/ml (Table 1). The insulin concentration for the viral vector containing enhancer and promoter of rat insulin gene was 61% higher than the vector containing only insulin gene. The activity of rat insulin enhancer and promoter gene would be an important determinant for the expression of insulin gene.

The constructed viral-insulin gene was transferred into rat pancreas, and the blood glucose concentrations were monitored to determine the effect of gene therapy in diabetic rats induced by STZ. Blood glucose concentration was significantly decreased in the diabetic rats from 1 to 10 days after exposure to pLNCP2 compared to pLNCI2, it showed that pLNCP2 transferred to pancreas was producing physiologically significant levels of insulin. One day after exposure plasmids, two groups of diabetic rats exhibited mean glucose levels of about 250 - 350 mg/dl. The blood glucose concentration in pLNCP2 transferred rats had fallen to within the normal range by 9 days after transfection. One to ten days transfection, blood glucose in the pLNCP2 injected diabetic rats lower than that in the pLNCI2 injected diabetic rats (Figure 4).

4. DISCUSSION

Diabetes was one of the important and serious human diseases, and was the fifth of the ten leading causes of death in Taiwan. In addition, diabetes was also closely related to the cerebrovascular disease, cardiovascular disease, and hypertension [13]. Insulin injections or oral

Table 1. The determination of insulin concentration by RIA.

<table>
<thead>
<tr>
<th>Packaging cell</th>
<th>RIA (counts)</th>
<th>Insulin (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vector A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 1</td>
<td>3062.5 ± 35.72</td>
<td>51.43 ± 0.69</td>
</tr>
<tr>
<td>Sample 2</td>
<td>1359.5 ± 26.36</td>
<td>1804.93 ± 1.55</td>
</tr>
<tr>
<td>Sample 3</td>
<td>1096.5 ± 33.19</td>
<td>2976.14 ± 51.10</td>
</tr>
<tr>
<td>Vector B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 4</td>
<td>3202.0 ± 37.81</td>
<td>25.37 ± 0.51</td>
</tr>
<tr>
<td>Sample 5</td>
<td>1062.5 ± 24.82</td>
<td>3255.80 ± 64.07</td>
</tr>
<tr>
<td>Sample 6</td>
<td>881.5 ± 3.86</td>
<td>4806.35 ± 53.72</td>
</tr>
</tbody>
</table>

1) Vector A: pLNCI2 transferred packaging cells (mouse fibroblast cells); 2) Vector B: pLNCP2 transferred packaging cells (mouse fibroblast cells); 3) Sample 1 and 4: packaging cells medium; 4) Sample 2 and 5: packaging cells; 5) Sample 3 and 6: packaging cells and medium; 6) The data of five independent experiments were used; Values were expressed as mean ± SE (n = 5).
are inconvenient and insulin overdose-induced hypoglycemia could be fatal, and the oral of sulfonylurea treatment typically do not provide satisfactory control of blood glucose.

Recently, due to the development in molecular biology, there are more in-depth understanding of the etiology and pathogenesis of diabetes, and new therapeutic method developed for the treatment of diabetes. Therefore, there are more researches trying to get through biotechnology to treat diabetes by using the viral vectors performance of insulin genetic in eukaryotic cells to proliferate insulin out in vivo [4,10,14]. Hopefully, there is no longer need the long-term insulin injections and also achieve the therapeutic effect. The study demonstrated that retrovirus vector was effectively for the transfection of insulin gene into mouse fibroblast cells.

The results in our study suggested that the activity of rat insulin enhancer/promoter gene was an important determinant for the expression of insulin gene. From blood glucose concentration of diabetic rats, with the retroviral vector transfection of the insulin gene into pancreas, in vivo, could demonstrate this gene therapy was effective.

Although the concentration of blood glucose decreased still was above the normal range between 2 and 8 days after transfection with pLNCP2 and pLNCI2, the concentration of blood glucose in pLNCP2 rats was always lower than in pLNCI2 rats. It should be emphasized that, the release of insulin by the insulin gene transferred did not regulate by glucose in the present study. In future studies will develop a mechanism to regulate insulin release. In addition, the pancreas of diabetic rat transferred with insulin gene will be perfused with glucose and determine the insulin concentrations to evaluate the effect of insulin gene therapy.

REFERENCES


