

The World's First Myoblast Study of Type II Diabetic Patients

a report by

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Introduction

Type II diabetes, also called non-insulin-dependent diabetes mellitus (NIDDM), is characterized by high blood glucose resulting from the genetic defect of the GLUT4 genome. The latter is manifested in the diminished glucose uptake into skeletal muscles. In normal human beings, insulin combines with insulin receptors to change the membrane conformation of skeletal muscle fibers, allowing blood glucose to move down its concentration gradient into the fibers for metabolism. A disorder called 'insulin resistance' exists in Type II diabetic patients in which normal or even elevated levels of plasma insulin would not elicit normal glucose uptake into the muscle fibers. This article hypothesizes that these diabetic fibers exhibit less insulin receptors, or that these receptors exhibit abnormal molecular conformation, or both. Considering that highly metabolic muscle fibers constitute more than 50% of the human body by volume and weight, failure of blood glucose to gain entry would undoubtedly lead to high blood glucose and result in various organ failures sequentially. This is not the only defect, but it is likely the primary and significant one.

A potential genetic treatment of the disease involves myoblast transfer therapy (MTT) which is a platform technology of cell transplantation, genome therapy and tissue engineering.^{1,2} It consists of culturing immature muscle cells called myoblasts, derived originally from a 2g skeletal muscle biopsy from a healthy, young, male donor, and implanting them into the major muscle groups of the upper and lower extremities of the diabetic patients. The myoblasts exhibit natural cell fusion, and transfer their nuclei carrying the normal human genome into the host skeletal muscle fibers to effect genetic repair. Others fuse among themselves to form new myofibers that exhibit normal insulin receptors of donor origin. Through both mechanisms, new insulin receptors of donor origin that are genetically normal, will be

produced in the skeletal myofibers of the host.

The survival, development, and functioning of the implanted allogeneic myoblasts have previously been demonstrated in studies involving about 240 muscular dystrophy subjects and two chronically myocardial infarcted heart subjects with 100% safety and substantial efficacy results.^{3,4} Immunorejection was minimized using two months of cyclosporine following MTT. In addition, over 120 ischemic heart patients have received autologous myoblasts in their hearts in 10 countries. Mortality rate has been less than 10% traversing the last four years, with efficacy data being collected in Phase II clinical trials in Europe. Reported here are the world's first genetic transplants of two Type II diabetic patients using allogeneic myoblasts.

Materials and Methods

Myoblast Culture

Human myoblasts were manufactured according to the standard operating procedures (SOPs) of Cell Transplants Singapore Pte Ltd (CTS) with US patent no. 5,130,141 and Singapore patent no. 34490 (WO 96/18303) licenses. Cell production was in compliance with current good manufacturing practice (cGMP) and international organization for standardization (ISO) standard 9001 conditions. Around 2g of muscle biopsy was taken under local anesthesia from a 20-year-old, pathogen-free, male volunteer after he had met muscle donor criteria. Initial dissociation isolated approximately 10,000 satellite cells, which were then cultured accordingly to CTS's SOPs. The culture yielded 47.4×10^9 myoblasts that were 100% pure by positive desmin immunostain, and 92.8% viable according to vital dye exclusion tests. The cells were potent in myogenicity in that numerous myotubes were observed within four days in a fusion medium. Throughout the culture and for the final injectates, the myoblasts were free of endotoxin (<1.0 EU/ml) and

1. Law P K, et al. "Myoblast transfer as a platform technology of gene therapy", *Gene Ther. Mol. Biol.* 1 (1998), pp. 345–363.
2. Law P K, "Nuclear Transfer and Human Genome Therapy", *Business Briefing: Future Drug Discovery*, (2001), pp. 38–42.
3. Law P K, "The Regenerative Heart", *Business Briefing: PharmaTech*, (2002), pp. 65–70.
4. Law P K, et al. "Myoblast genome therapy and the regenerative heart", *Handbook of Cardiovascular Cell Transplantation*, Kipshidze N N and Serruys P W, (eds), Martin Dunitz, UK, (2004), Chapter 17, pp. 241–258.

mycoplasma, and negative for sterility (14-day test) and gram stain (absence of gram positive or negative bacteria) according to certified laboratory analyses outside CTS.

Subjects

Patient 1 is 42 years old, 157cm tall, and weighs 68kg. Patient 2 is 36 years old, 158cm tall, and also weighs 68kg. Diagnosis showed about two years history of Type II diabetes and hypertension but otherwise normal in heart, lung, kidney, and liver function without obesity. The laboratory report revealed tests results for syphilis, hepatitis B surface antigens, antibodies to HIV and hepatitis C virus to be negative.

Immunosuppression

Both patients had previously been enrolled as clinical trial subjects after qualifying for inclusion/exclusion criteria, and signing patients' informed consents with institutional approval. The subjects took two oral doses of cyclosporine totalling 5–7mg/kg body weight per day, beginning two days before grafting, weaning at half-dosage in the last two weeks, and off cyclosporine at eight weeks after grafting. The whole blood trough level of cyclosporine was monitored every two weeks. Doses were adjusted in an attempt to maintain the level at about 250ng/ml.

Myoblast Allograft

Myoblasts were harvested and processed under biological safety cabinets (Class 100) inside a cleanroom. Having been washed thoroughly, they were suspended in the injection medium. Quality assurance/quality control processes ensued, and the final quality control release test forms were issued for each of the two subjects. The myoblasts were then carried in syringes within sterile enclosures into two surgical suites for simultaneous implantation into both subjects. These were the world's first cases of allogeneic myoblasts being injected into Type II diabetic patients. The patients received 132 injections each and 24/23.4 billion myoblasts, respectively. The two-hour procedure was performed with the patients under general anesthesia. Cells were injected at 50x10⁶/ml and the injections were made under direct vision into 54 major muscle groups of each subject. The patients were transferred to the intensive care unit, where they recovered from the anesthesia and routine monitors were administered.

Results

The subjects recovered from the general anesthesia without rash or fever, and both patients were discharged at 48 hours post-operatively. Most pertinent to the specific goal of the this study was that, despite cyclosporine discontinuation at two months post-

Table 1: Plasma Glucose and Insulin Levels Before Versus after MTT for Patient 1

Date	Plasma Glucose			Plasma Insulin		
	Fasting	2 hrs after breakfast	2 hrs after lunch	2 hrs after dinner	Fasting	2 hrs after lunch
1 June	8.2					
2 June					12	76.3
3 June	6.3 (before MTT)			8.8 (after MTT)		
4 June	8.8	12.1	6.3	6.4		
5 June	6.6	13.3	7.8	6.3		
2 July	10.4	13.2				
9 July		8.3				
23 July		7.1				
10 August	6.18	7.94			13	57.5

Table 2. Plasma Glucose and Insulin Levels before Versus after MTT for Patient 2

Date	Plasma Glucose			Plasma Insulin		
	Fasting	2 hrs after breakfast	2 hrs after lunch	2 hrs after dinner	Fasting	2 hrs after lunch
1 June	7.4					
2 June					20.5	80.7
3 June	6.2 (before MTT)			5.0 (after MTT)		
4 June	7.2	8.4	7.1	6.2		
5 June	5.5	9.3	7.2	8.1		
28 June		11.2				
2 July	9.7	11.1				
9 July		12.2				
23 July		10.3				
10 August	7.99	7.74			25.2	83.3

For both patients, normal glucose levels are 3.9-6.1mmol/L, and normal insulin levels are 2.5-7.1mIU/L (Fasting), 8.7-29.7mIU/L (2hrs after meal).

operatively, no sign of rejection was observed. The patients appear to have good general health before and after MTT. Plasma glucose and insulin levels data are listed in Table 1 and Table 2 for the two subjects, respectively.

Discussion

This pioneering feasibility/safety study of myoblast allografts into the skeletal muscles of Type II diabetic patients leads the way in developing a genetic treatment for the disease. The immunosuppressant cyclosporine used in this study is known to increase plasma glucose. The subjects were weaned off cyclosporine only recently. They will be further monitored in the next six months to determine the preliminary efficacy, if any, of the 24-billion MTT. Meanwhile, the procedure appears to be safe for both subjects. ■