Reversal of type I diabetes via transplantation of insulin – producing cells by cord blood – derived multipotant stem cells in drug – induced diabetic rat

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ABSTRACT

Type 1 diabetes mellitus (DM) is an autoimmune disease that is characterized by inhibited insulin production as a result of T cell-mediated destruction of the pancreatic β cells in the islets of Langerhans. Although DM can be treated with islet transplantation which includes whole organ transplantation, transplantation of isolated islets and regeneration therapy, a shortage of donors limits this transplantation of islets cells. The present study will investigate for reversal of type I diabetes. The mesenchymal stem cells from the umbilical cord will differentiate efficiently into transplanted insulin – producing – cells in the liver that can be used to replace lost β – cells of islets of Langerhans through portal venous transplantation through cannulation of portal vein. The way will use for reversal of type 1 diabetes mellitus in drug induced diabetic rats.

LITERATURE SURVEY

Tissue repair and regeneration following injury or disease are often thought to recapitulate embryonic development by using similar molecular and cellular pathways. In addition, many embryonic tissues, such as the spinal cord, heart, and limbs, have some regenerative potential and may utilize mechanisms that can be exogenously activated in adult tissues. For example, BMP signaling regulates nervous system development, and SMAD reactivation plays a critical role in adult nerve regeneration and repair in animal models of spinal cord injury. While similar molecular pathways are utilized during embryogenesis and adult tissue regeneration, recent reports suggest the mechanisms by which these developmental programs are reactivated and maintained may vary in adult tissues **(Zhao et al., 2003)**. Adult fish and amphibians have a remarkable capacity for tissue regeneration, while mammals have a limited regenerative capacity. Given the shortage of organs available for transplant, tissue engineering may be an alternative. Recent studies have described the ability of tissue-resident adult (somatic) stem cells to repopulate organs such as the liver and pancreas. Additionally, induced pluripotent stem cells have demonstrated potential for repair and regeneration of diverse tissues including the heart following ischemia, retinal pigmented epithelium in studies of age-related macular degeneration, and pancreas following loss of insulin-secreting beta-cells **(Trucco, 2005)**. For decades, diabetes researchers have been searching for ways to replace the insulin-producing cells of the pancreas that are destroyed by a patient's own immune system. Now it appears that this may be possible. Each year, diabetes affects more people and causes more deaths than breast cancer and AIDS combined. Diabetes is the seventh leading cause of death in the United States today, with nearly 200,000 deaths reported each year. The American

Diabetes Association estimates that nearly 16 million people, or 5.9 percent of the United States population, Diabetes is actually a group of diseases characterized by abnormally high levels of the sugar glucose in the bloodstream. This excess glucose is responsible for most of the complications of diabetes, which include blindness, kidney failure, heart disease, stroke, neuropathy, and amputations. Type 1 diabetes, also known as juvenile-onset diabetes, typically affects children and young adults. Diabetes develops when the body's immune system sees its own cells as foreign and attacks and destroys them. As a result, the islet cells of the pancreas, which normally produce insulin, are destroyed. In the absence of insulin, glucose cannot enter the cell and glucose accumulates in the blood. Type 2 diabetes also called adult-onset diabetes, tends to affect older, sedentary, and overweight individuals with a family history of diabetes. Type 2 diabetes occurs when the body cannot use insulin effectively. This is called insulin resistance and the result is the same as with type 1 diabetes—a build up of glucose in the blood **(Van Belle et al., 2011)**.

There is currently no cure for diabetes. People with type 1 diabetes must take insulin several times a day and test their blood glucose concentration three to four times a day throughout their entire lives. Frequent monitoring is important because patients who keep their blood glucose concentrations as close to normal as possible can significantly reduce many of the complications of diabetes, such as retinopathy (a disease of the small blood vessels of the eye which can lead to blindness) and heart disease, which tend to develop over time. People with type 2 diabetes can often control their blood glucose concentrations through a combination of diet, exercise, and oral medication. Type 2 diabetes often progresses to the point where only insulin therapy will control blood glucose concentrations **(Cousin-Frankel, 2011)**.

currently have diabetes **(Bluestone et al.,2010)**.

Each year, approximately 1,300 people with type 1 diabetes receive whole-organ pancreas transplants. After a year, 83 percent of these patients, on average, have no symptoms of diabetes and do not have to take insulin to maintain normal glucose concentrations in the blood. However, the demand for transplantable pancreases outweighs their availability. To prevent the body from rejecting the transplanted pancreas, patients must take powerful drugs that suppress the immune system for their entire lives, a regimen that makes them susceptible to a host of other diseases. Many hospitals will not perform a pancreas transplant unless the patient also needs a kidney transplant. That is because the risk of infection due to immunosuppressant therapy can be a greater health threat than the diabetes itself. But if a patient is also receiving a new kidney and will require immunosuppressant drugs anyway, many hospitals will perform the pancreas transplant **(Bonner and Weir, 2005)**.

Over the past several years, doctors have attempted to cure diabetes by injecting patients with pancreatic islet cells—the cells of the pancreas that secrete insulin and other hormones. However, the requirement for steroid immunosuppressant therapy to prevent rejection of the cells increases the metabolic demand on insulin-producing cells and eventually they may exhaust their capacity to produce insulin. The deleterious effect of steroids is greater for islet cell transplants than for whole-organ transplants. As a result, less than 8 percent of islet cell transplants performed before last year had been successful **(Ricordi et al., 2008)**.

James Shapiro and his colleagues in Edmonton, Alberta, Canada, have developed an experimental protocol for transplanting islet cells that involves using a much larger amount of islet cells and a different type of immunosuppressant therapy. In this study, they report that seven of seven patients who received islet cell transplants no longer needed to take insulin, and their blood glucose concentrations were normal a year after surgery. The success of the Edmonton protocol is now being tested at 10 centers around the world. If the success of the Edmonton protocol can be duplicated, many hurdles still remain in using this approach on a wide scale to treat diabetes. First, donor tissue is not readily available, islet cells used in transplants are obtained from cadavers and the procedure requires at least two cadavers per transplant. The islet cells must be immunologically compatible, and the tissue must be freshly obtained—within eight hours of death. Because of the shortage of organ donors, these requirements are difficult to meet and the waiting list is expected to far exceed available tissue, especially if the procedure becomes widely accepted and available. Further, islet cell transplant recipients face a lifetime of immunosuppressant therapy, which makes them susceptible to other serious infections and diseases **(Shapiro et al., 2000)**.

Development of Cell-Based Therapies for Diabetes

In developing a potential therapy for patients with diabetes, researchers hope to develop a system that meets several criteria. Ideally, stem cells should be able to multiply in culture and reproduce themselves exactly. That is, the cells should be self-renewing. Stem cells should also be able to differentiate *in vivo* to produce the desired kind of cell. For diabetes therapy, it is not clear whether it will be desirable to produce only beta cells—the islet cells that manufacture insulin—or whether other types of pancreatic islet cells are also necessary. Studies by **Bernat Soria** and colleagues, for example, indicate that isolated beta cells—those cultured in the absence of the other types of islet cells—are less responsive to changes in glucose concentration than intact islet clusters made up of all islet cell types. Islet cell clusters typically respond to higher-than-normal concentrations of glucose by releasing insulin in two phases: a quick release of high concentrations of insulin and a slower release of lower concentrations of insulin. In this manner the beta cells can fine-tune their response to glucose. Extremely high concentrations of glucose may require that more insulin be released quickly, while intermediate concentrations of glucose can be handled by a balance of quickly and slowly released insulin **(Soria et al., 2000)**.

Isolated beta cells, as well as islet clusters with lower-than-normal amounts of non-beta cells, do not release insulin in this biphasic manner. Instead insulin is released in an all-or-nothing manner, with no fine-tuning for intermediate concentrations of glucose in the blood **(Soria et al., 1996)**. Therefore, many researchers believe that it will be preferable to develop a system in which stem or precursor cell types can be cultured to produce all the cells of the islet cluster in order to generate a population of cells that will be able to coordinate the release of the appropriate amount of insulin to the physiologically relevant concentrations of glucose in the blood **(Bosco and Medo, 1997)**.

Fetal Tissue as Source for Islet Cells

Several groups of researchers are investigating the use of fetal tissue as a potential source of islet progenitor cells. For example, using mice, researchers have compared the insulin content of implants from several sources of stem cells—fresh human fetal pancreatic tissue, purified human islets, and cultured islet tissue. They found that insulin content was initially higher in the fresh tissue and purified islets. However, with time, insulin concentration decreased in the whole tissue grafts, while it remained the same in the purified islet grafts. When cultured islets were implanted, however, their insulin content increased over the course of three months. The researchers concluded that precursor cells within the cultured islets were able to proliferate (continue to replicate) and differentiate (specialize) into functioning islet tissue, but that the purified islet cells (already differentiated) could not further proliferate when grafted. Importantly, the researchers found, however, that it was also difficult to expand cultures of fetal islet progenitor cells in culture (Beattie et al., 1997).

Adult Tissue as Source for Islet Cells

Many researchers have focused on culturing islet cells from human adult cadavers for use in developing transplantable material. Although differentiated beta cells are difficult to proliferate and culture, some researchers have had success in engineering such cells to do this. For example, Fred Levine and his colleagues at the University of California, San Diego, have engineered islet cells isolated from human cadavers by adding to the cells' DNA special genes that stimulate cell proliferation. However, because once such cell lines that can proliferate in culture are established, they no longer These investigators report that these cells do not produce as much insulin as normal islets, but it is within an order of magnitude. The major problem in dealing with these cells is maintaining the delicate balance between growth and differentiation. Cells that proliferate well do not produce insulin efficiently, and those that do produce insulin do not proliferate well. According to the produce insulin. The cell lines are further engineered to express the beta islet cell gene, PDX-1, which stimulates the expression of the insulin gene. Such cell lines have been shown to propagate in culture and can be induced to differentiate to cells, which produce insulin. When transplanted into immune-deficient mice, the cells secrete insulin in response to glucose. The researchers are currently investigating whether these cells will reverse diabetes in an experimental diabetes model in mice **(Dufaget et al., 2001 & Itkin-Ansari et al., 2001)**.

researchers, the major issue is developing the technology to be able to grow large numbers of these cells that will reproducibly produce normal amounts of insulin. Another promising source of islet progenitor cells lies in the cells that line the pancreatic ducts. Some researchers believe that multipotent (capable of forming cells from more than one germ layer) stem cells are intermingled with mature, differentiated duct cells, while others believe that the duct cells themselves can undergo a differentiation, or a reversal to a less mature type of cell, which can then differentiate into an insulin-producing islet cell.

Susan Bonner-Weir and her colleagues reported last year that when ductal cells isolated from adult human pancreatic tissue were cultured, they could be induced to differentiate into clusters that contained both ductal and endocrine cells. Over the course of three to four weeks in culture, the cells secreted low amounts of insulin when exposed to low concentrations of glucose, and higher amounts of insulin when exposed to higher glucose concentrations. The researchers have determined by immunochemistry and ultrastructural analysis that these clusters contain all of the endocrine cells of the islet **(Bonner-Weir et al., 2000)**.

Bonner-Weir and her colleagues are working with primary cell cultures from duct cells and have not established cells lines that can grow indefinitely. However the cells can be expanded. According to the researchers, it might be possible in principle to do a biopsy and remove duct cells from a patient and then proliferate the cells in culture and give the patient back his or her own islets. This would work with patients who have type 1 diabetes and who lack functioning beta cells, but their duct cells remain intact. However, the autoimmune destruction would still be a problem and potentially lead to destruction of these transplanted cells. Type 2 diabetes patients might benefit from the transplantation of cells expanded from their own duct cells since they would not need any immunosuppression. However, many researchers believe that if there is a genetic component to the death of beta cells, then beta cells derived from ductal cells of the same individual would also be susceptible to autoimmune attack **(Bonner-Weir et al., 2000)**.

Some researchers question whether the ductal cells are indeed undergoing a dedifferentiation or whether a subset of stem-like or islet progenitors populate the pancreatic ducts and may be co-cultured along with the ductal cells. If ductal cells die off but islet precursors proliferate, it is possible that the islet precursor cells may overtake the ductal cells in culture and make it appear that the ductal cells are dedifferentiating into stem cells. According to Bonner-Weir, both dedifferentiated ductal cells and islet progenitor cells may occur in pancreatic ducts. Some researchers reported that pancreatic ductal epithelial cells from adult mice could be cultured to yield islet-like structures similar to the cluster of cells found by Bonner-Weir. Using a host of islet-cell markers they identified cells that produced insulin, glucagon, somatostatin, and pancreatic polypeptide. When the cells were implanted into diabetic mice, the diabetes was reversed. They looked for islet-like stem cells from adult pancreatic tissue. They have discovered a population of stem-like cells within both the adult pancreas islets and pancreatic ducts. These cells do not express the marker typical of ductal cells, so they are unlikely to be ductal cells. Instead, they express a marker called nestin, which is typically found in developing neural cells. The nestin-positive cells do not express markers typically found in mature islet cells. However, depending upon the growth

factors added, the cells can differentiate into different types of cells, including liver, neural, exocrine pancreas, and endocrine pancreas, judged by the markers they express, and can be maintained in culture for up to eight months **(Zulewski et al., 2001)**.

Embryonic Stem Cells

The discovery of methods to isolate and grow human embryonic stem cells in 1998 renewed the hopes of doctors, researchers, and diabetes patients and their families that a cure for type 1 diabetes and perhaps type 2 diabetes as well, may be within striking distance. In theory, embryonic stem cells could be cultivated and coaxed into developing into the insulin-producing islet cells of the pancreas. With a ready supply of cultured stem cells at hand, the theory is that a line of embryonic stem cells could be grown up as needed for anyone requiring a transplant. The cells could be engineered to avoid immune rejection. Before transplantation, they could be placed into non-immunogenic material so that they would not be rejected and the patient would avoid the devastating effects of immunosuppressant drugs. There is also some evidence that differentiated cells derived from embryonic stem cells might be less likely to cause immune rejection. Although having a replenish able supply of insulin-producing cells for transplant into humans may be a long way off, researchers have been making remarkable progress in their quest for it. While some researchers have pursued the research on embryonic stem cells, other researchers have focused on insulin-producing precursor cells that occur naturally in adult and fetal tissues **(Kukreja et al., 2002)**.

Several teams of researchers have been investigating the possibility that human embryonic stem cells could be developed as a therapy for treating diabetes. Some studies in mice show that embryonic stem cells can be coaxed into differentiating into insulin-producing beta cells, and new reports indicate that this strategy may be possible using human embryonic cells as well. Researchers in Spain reported using mouse embryonic stem cells that were engineered to allow researchers to select for cells that were differentiating into insulin-producing cells. Bernat Soria and his colleagues at the Universidad Miguel Hernandez in San Juan, Alicante, Spain, added DNA containing part of the insulin gene to embryonic cells from mice. The insulin gene was linked to another gene that rendered the mice resistant to an antibiotic drug. By growing the cells in the presence of an antibiotic, only those cells that were activating the insulin promoter were able to survive. The cells were cloned and then cultured under varying conditions. Cells cultured in the presence of low concentrations of glucose differentiated and were able to respond to changes in glucose concentration by increasing insulin secretion nearly sevenfold. The researchers then implanted the cells into the spleens of diabetic mice and found that symptoms of diabetes were reversed **(Soria et al., 2000)**.

Lumelsky and his colleagues (2001) described a series of experiments in which they induced mouse embryonic cells to differentiate into insulin-secreting structures that resembled pancreatic islets. They started with embryonic stem cells and let them form embryoid bodies—an aggregate of cells containing all three embryonic germ layers. They then selected a population of cells from the embryoid bodies that expressed the neural marker nestin. Using a sophisticated five-stage culturing technique, the researchers were able to induce the cells to form islet-like clusters that resembled those found in native pancreatic islets. The cells responded to normal glucose concentrations by secreting insulin, although insulin amounts were lower than those secreted by normal islet cells. When the cells were injected into diabetic mice, they survived, although they did not reverse the symptoms of diabetes. According to Lumelsky, this system is

unique in that the embryonic cells form a functioning pancreatic islet, complete with all the major cell types. The cells assemble into islet-like structures that contain another layer, which contains neurons and is similar to intact islets from the pancreas.

Some researches had also provided more evidence that human embryonic cells can develop into cells that can and do produce insulin. **Schuldiner et al., (2000)** reported that human embryonic stem cells could be manipulated in culture to express the PDX-1 gene, a gene that controls insulin transcription. In these experiments, researchers cultured human embryonic stem cells and allowed them to spontaneously form embryoid bodies (clumps of embryonic stem cells composed of many types of cells from all three germ layers). The embryoid bodies were then treated with various growth factors, including nerve growth factor. The researchers found that both untreated embryoid bodies and those treated with nerve growth factor expressed PDX-1. Embryonic stem cells prior to formation of the aggregated embryoid bodies did not express PDX-1. Because expression of the PDX-1 gene is associated with the formation of beta islet cells, these results suggest that beta islet cells may be one of the cell types that spontaneously differentiate in the embryoid bodies. The researchers now think that nerve growth factor may be one of the key signals for inducing the differentiation of beta islet cells and can be exploited to direct differentiation in the laboratory. Complementing these findings is work done by **Jiang et al., (2007) & Kernieli et al., (2007)** using human embryonic cells of the same source. In preliminary findings, he has shown that human embryonic stem cells can differentiate and express the insulin gene.

Assady et al., (2001) further characterized insulin-producing cells in embryoid bodies. The researchers found that embryonic stem cells that were allowed to spontaneously form embryoid bodies contained a significant percentage of cells that express insulin. Based on the binding of antibodies to the insulin protein, the researchers" estimates that 1 to 3 percent of the cells in embryoid bodies are insulin-producing beta-islet cells. The researchers also found that cells in the embryoid bodies express glut-2 and islet-specific glucokinase, genes important for beta cell function and insulin secretion. Although the researchers did not measure a time-dependent response to glucose, they did find that cells cultured in the presence of glucose secrete insulin into the culture medium. The researchers concluded that embryoid bodies contain a subset of cells that appear to function as beta cells and that the refining of culture conditions may soon yield a viable method for inducing the differentiation of beta cells and, possibly, pancreatic islets. Taken together, these results indicate that the development of a human embryonic stem cell system that can be coaxed into differentiating into functioning insulin-producing islets may soon be possible.

Several research groups are trying to apply Lumelsky "s results with mice to induce human embryonic stem cells to differentiate into insulin-producing islets **(Jiang et al., 2007 & Kernieli et al., 2007)**.

Ultimately, type 1 diabetes may prove to be especially difficult to cure, because the cells are destroyed when the body's own immune system attacks and destroys them. This autoimmunity must be overcome if researchers hope to use transplanted cells to replace the damaged ones.

Many researchers believe that at least initially, immunosuppressive therapy similar to that used in the Edmonton protocol will be beneficial. A potential advantage of embryonic cells is that, in theory, they could be engineered to express the appropriate genes that

would allow them to escape or reduce detection by the immune system. Others have suggested that a technology should be developed to encapsulate or embed islet cells derived from islet stem or between the islet cells and cells of the immune system. Such encapsulated cells could secrete insulin into the blood stream, but remain inaccessible to the immune system **(Abdi et al., 2008)**.

Before any cell-based therapy to treat diabetes makes it to the clinic, many safety issues must be addressed. A major consideration is whether any precursor or stem-like cells transplanted into the body might revert to a more pluripotent state and induce the formation of tumors. These risks would seemingly be lessened if fully differentiated cells are used in transplantation. But before any kind of human islet-precursor cells can be used therapeutically, a renewable source of human stem cells must be developed. Although many progenitor cells have been identified in adult tissue, few of these cells can be cultured for multiple generations. Embryonic stem cells show the greatest promise for generating cell lines that will be free of contaminants and that can self renew. However, most researchers agree that until a therapeutically useful source of human islet cells is developed, all avenues of research should be exhaustively investigated, including both adult and embryonic sources of tissue **(Aguayo and Bonner, 2010)**.

progenitor cells in a material that would allow small molecules such as insulin to pass through freely, but would not allow interactions

Cord blood-derived stem cells

In type 1 diabetes, autoimmune destruction of pancreatic islet β cells reduces an individual's ability to regulate blood glucose, ultimately resulting in poor blood circulation, heart disease, stroke, infection, kidney failure, and often premature death. Each day, millions of patients with type I diabetes receive insulin injections to survive, but these injections do nothing to address the underlying T cell-mediated autoimmune dysfunction. For many years, attempts to address the underlying autoimmunity have been unsuccessful **(Couzin-Frankel, 2011)** due to the polyclonal nature of the autoimmune response and the global challenges of immune regulation in type I diabetes patients **(Mathieu and Gillard, 2011).** Combinations of individual approaches have been proposed to address these challenges, but adherence to these approaches will be complicated and costly. Alternative approaches are needed. Stem cells have been touted as a means of replacing lost pancreatic islet β cells and curing type I diabetes, but this approach is doomed in the absence of a treatment for the underlying autoimmune response **(Bluestone et al., 2010 & Vane Belle et al., 2011)**.

While traditional stem cell therapy is not likely to be effective for long-term treatment of type I diabetes patients, recent studies suggest that alternative approaches using stem cells may overcome the autoimmune component of the disease. Human cord blood-derived stem cells and mesenchymal stem cells have been shown to modulate immune activity *in vitro* **(Zahao and Mazzone, 2010)**. Subsequent studies have demonstrated that cord blood – derived stem cells can be used to alter immune function and improve markers of type I diabetes in non-obese diabetic mice **(Zhao et al., 2009)**. Cord blood – derived stem cells have been shown to modulate the immune function of type I diabetes patient-derived islet β cell-specific pathogenic T cell clones in co-culture **(Zhao and Mazzone, 2010)**. Studies in animal models also suggest that cord blood – derived stem cells treatment may allow the patient to regenerate the native population of islet β cells without stem cell transplantation

(Zhao et al., 2010). To translate these findings into a clinically feasible therapy, lymphocytes through co-culture with cord blood – derived stem cells. If shown to be safe and effective, immune modulation by cord blood – derived stem cells has the potential to address type I diabetes and other autoimmune diseases while reducing risk to the donor, minimizing ethical concerns, and avoiding graft-versus-host disease **(Young et al., 2012)**.

Transplantation therapies for type 1 DM include whole organ transplantation **(Larsen, 2004)**, transplantation of isolated islets **(Shapiro et al., 2000)** and regeneration therapy **(Yamaoka, 2002)**. Although the transplantation of both a whole organ and isolated islets has been successfully used in the clinical treatment of type 1 DM, a shortage of donors limits the widespread use of this treatment modality. Additionally, the quality of a donor's pancreas is an important criterion for islet isolation. On the other hand, regeneration therapy, in which stem cells are stimulated to differentiate into insulin-producing cells that can be used to replace lost β cells, is free of such supply limitations **(Kobayashi et al., 2009)**.

Mesenchymal stem cells (MSCs) were first isolated from bone marrow **(Friedenstein et al., 1966)** and have the potential to differentiate in culture into muscle cells, adipocytes, osteocytes, chondrocytes **(Pittenger et al., 1999)**, cardiomyocytes **(Fuduka, 2001)** and pancreatic β cells **(Jiang et al., 2007)**. Moreover, following systemic injection, MSCs have been shown to be incorporated into a variety of tissues, including bone **(Pereira et al., 1998)**, muscle **(Ferrar, 1998)**, lung **(Perreira et al., 1995)** and epithelium **(Spees et al., 2003)**. Although insulin-producing cells can be developed from bone marrow MSCs **(Karnieli et al., 2007)**, adipose tissue-derived stem cells **(Chandra et al., 2009)** and human umbilical cord blood-derived we developed novel process to re-educate a patient's mononuclear cells **(Parekh et al., 2009)**, the number of MSCs that can be cost-effectively isolated and differentiated remains a major limitation. We found that fibroblast-like cells from Wharton's jelly of the human umbilical cord were similar to MSCs in the bone marrow and could be induced to differentiate into adipogenic cells, osteogenic cells, cardiomyogenic cells, and insulin-producing cells **(Wang et al., 2004)**. Because MSCs from the umbilical cord can be easily isolated and expanded in culture, they may provide a novel source of cells for cellular type 1 DM therapies **(Wang et al., 2011)**.

Peri-Jiun et al., (2012) found that Wharton's jelly from the human umbilical cord contains fibroblast-like cells, which are similar to MSCs. They investigated the ability of these cells to differentiate into insulin-producing cells, as well as the potential curative effects of transplanting the insulin-producing cells into the livers of diabetic rats. Simultaneously, we tested the usefulness of the modified Port-A catheter in transplantation. **Jiang et al., (2007)** illustrated that human umbilical cord MSCs could be differentiated into insulin-producing cells following incubation under specific conditions. Based on current references of pancreas endocrine cell development, a combination of various factors, including activin A, sodium butyrate, growth factors in serum free media supplements were used in this study due to the controversy surrounding insulin uptake by cells from media supplements **(Rajapopal et al., 2007)**. **Peri-Jiun et al., (2012)** used human C-peptide to characterize insulin production by human umbilical cord MSCs. Proinsulin, the precursor of insulin, is composed of 3 segments, the A-chain, B-chain, and C-peptide. Although C-peptide is released from proinsulin, unlike the A- and B-chains, it

is not taken up by the cells. Thus, levels peptide can be used as a marker of insulin secretion. After exposure of MSCs to differentiation conditions, immunocytochemical staining revealed that the cells expressed both insulin and C-peptide.

In this study, streptozotocin (STZ) was used to induce type 1 DM because there of C-

is extensive evidence that hyperglycemia induced by STZ can be lowered by stem cell therapy. STZ is a naturally occurring chemical that is toxic to pancreatic β-cells in mammals and can produce an animal model of type 1 DM **(Hussain and Theise, 2004).**

EQUIPMENTS

1. Centrifugation.
2. Incubator.
3. Fluorescence microscope.
4. Microtome cryostat HM 500 OM (Micorm International Gm 6 H).

MATERIALS

1. Hank's balanced salt solution.
2. Serum free Dulbecco's modified Eugle's medium (DMEM, Gibco, Grand Island, NY).
3. Trypsin 25%.
4. 10% fetal bovine serum (FBS, Sigma St. Louis, MO USA).
5. HyQTase diluted with SFM-A.
6. Tissue culture plates (Corning, Fisher scientific international, Hampton).
7. SFM-A contained DMEM\ F12 Gibco, Grand Island, NY).
8. SFM-B contained DMEM\ F12 Gibco, Grand Island, NY).
9. SFM-C contained DMEM\ F12 Gibco, Grand Island, NY).
10. Glucagon – like peptide (Sigma Aldrich).
11. Paraformaldehyde 4% (Dako Cytomation Inc, CA).
12. Phosphate-buffered saline (Dako Cytomation Inc, CA).
13. Mouse anti-human C-peptide antibody (chemicon, Billerica, MA).
14. Dual endogenous enzyme blocks solution.
15. Chromogen substrate.
16. Hematoxylin and eosin (Sigma Aldrich, St. Louis).
17. RNeasy Purification Reagent (Qiagen, Valencia, CA).
18. Minly reverse transcriptase (Amersham, Uppsale, Sweden).
19. Primer sequences (Mission biotech, Taiwan).
20. GA garose gel stain 1%.
21. Master SYBR Green (Roche, Basal, Switzerland).
22. A light Cyclar 480 (Roche, Indianopolis, IN).
23. SMEM-LG (5.5 mM glucose) , (Gibeo, NY).
24. SMEM-HG (25 mM glucose) , (Gibeo, NY).
25. C-peptide ELISA kit (Mercodia, Uppsula, Sweden).
26. Streptozotocin Ampoules.
27. Port – A catheter.
28. Blood glucose meter (Roche, Indianapolis, IN).
29. Formaldehyde 4% (Ferak, Berlin, Germany).
30. OCT in liquid nitrogen (Sakura Fintek, USA).
31. Rabbit antihuman C- peptide antibodies 91 – 100), (Santa Cruz, Santa Cruz, CA).
32. Cy3 –labelled goat anti-human IgG antibodies (1:200), (Chamicon).
33. Rhodamine conjugated goat anti rabbit IgG antibodies (1;500), (Chamicon).
34. Ascensia ELITE glucometer (Bayer corporation, Elkhart IN).

35. Petri dishes (Becton Dickinson Labware Franklin Lakes, NJ).
36. Fetal bovine serum (Invitrogen, Carlsbad, CA).
37. Rat anti-mouse monoclonal antibodies (e Bioscience).
38. Rat anti-mouse IgG antibodies (e Bioscience).
39. CyAn ADP (Dakocytomation).
40. BD Cytofix \ cytoperm fixation\ permeability kit (Bs Biosciences, San jose, CA).
41. Pacific blue – conjugated IFN-y (eBioscience).
42. Biotinylated anti-TGF-B1 Ab (Catalog number BAF 240 R & D system, Minneopolis, MN).
43. Strepavidin-conjugated FITC (Vector Lab.).
44. Mo Flo (DakoCytomation).
45. Qiagen kit (Valencia, CA).
46. Quantitect Reverse Transcription kit (Qiangen, Valencia, CA).
47. ABI Prism 7900 HT Fast – Time PCR System (Applied Biosystems, CA).
48. Validated gene-specific RT2 PCR Primer sets (Super Array, Frederick, MD).
49. Amouse Th1 – Th2 – Th3 PCR array kit .
50. PCR array data analysis soft ware (Super Array).
51. UV mouse insulin enzyme-linked immnuosorbent assay (EIA) kit (Alpco Diagnostics, NH).
52. Immunostaining (Vector Lab)>
53. Guina pig polyclonal anti-insulin Ab. (Dako Cytomation, Carpinteria, CA).
54. Mouse anti- glucagon m Ab. (sigma).
55. Mouse anti-TGF- B1, m Ab. (Sigma).
56. Mouse anti-SMAD4 m Ab . (Santa Cruz Biotechnology, santa Cruz, CA).
57. Rabbit anti- Ki 67 m Ab .
58. Rat anti-macrophage marker F4 \ 80 m Ab (Novus Biological Littleton Co).
59. Hamster anti-mouse dendritic cell marker CD IIc (BS Pharmingen).
60. Cy5 –conjugated affini Pure Donkey anti – rat IgG (Jackson Immuno-Reseearch Lab , CA).
61. In situ cell death detection kit (Fluorescent) (Roche Applied Science , Indianopolis, IN).
62. Mouse IFN-y ELISA kit (Biolegend Inc, San Diego, CA).
63. Mouse IL – 4 and IL – 11 ELISA kit (Ann Arbor , MI).
64. TGF – B1 ELISA kits (Promega, Medison, WI).
65. Petri dishes.
66. Serum-free culture medium (Lonza, Walkervilla, MD).
67. 250 albino rats (6 – 10 weeks in age) .

OBJECTIVES

Millions of type I diabetes patients worldwide must have daily insulin injections to survive. However, it is not a cure; it does not halt the persistent autoimmune response. Nor can it reliably prevent devastating complications such as neuronal and cardiovascular diseases, blindness, and kidney failure. This compelling need brings a sense of urgency to find a cure for type I diabetes that can not only overcome the shortage of insulin-producing β-cells, but also halt the progression of autoimmunity. Here we demonstrate that treatment with transplantation of insulin – producing cells by using cord blood – derived multipotant stem cells can reverse established type I diabetes, not only by controlling the autoimmunity but also by promoting β-cell regeneration leading to the restoration of euglycemia in a diabetic rat model. Thus, these findings provide a new approach for the treatment of type I diabetes

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DESIGN

In the present study for reversal of type I diabetes, mesenchymal stem cells from human umbilical cord will induced in three stages to differentiate into insulin-producing cells and evaluated by immunocytochemistry, reverse transcriptase, and real-time PCR, and ELISA. Differentiated cells will transplant into the liver of diabetic rats using a Port-A catheter via the portal vein. Blood glucose levels were monitored weekly

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ANTICIPATED OUTCOME

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Transplantation of differentiated mesenchymal stem cells from cord blood – derived multipotant stem cells will reversed type I diabetes in the drug – induced diabetic rats, and consequently blood sugar level will decreased.

* The method will provide novel strategy for the treatment of type I diabetes.

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