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Review Article

Diabetes Mellitus and Regenerative Medicine: New Possibilities for the Regeneration of β Cells and Treatment of Diabetic Foot Ulcer

Abstract

Diabetes mellitus is a very common disease that affects a large number of people in the world and whose treatment is very expensive, also due to its complications. Diabetes is associated with many complications and among them the formation of diabetic foot ulcers is a serious problem. Regenerative medicine, defined as a field that can repair, regenerate or replace cells or tissue, can have a very important role in the treatment of this pathology. Over the years, new protocols to reconstruct β cells to produce insulin have been tried out, solving the problem that characterizes the disease. The diabetic foot ulcer is a very serious complication that is usually localized on pressure points and it can lead to the need to amputate the limb. The diabetic patient is characterized by an impaired ability to heal wounds. Normally, the healing process of a wound follows precise steps, which in the case of a patient suffering from this disease, can be altered. Many studies have shown that regenerative medicine can be a valuable aid in improving wound healing of diabetic patients. Stem cells can be considered an important resource because of their capability of self-renewal and differentiation. Several experiments have shown that transplanting mesenchymal stem cells (MSCs) into the site of the lesion may help in the realization of some steps of the resolution of the wound.

Introduction

Diabetes mellitus is a major disease resulted by hyperglycemia due to a relative or absolute insulin deficiency. This is a health problem that affects a large number of individuals and the number of patients is on the increase [1]. There are two types of diabetes: type 1 diabetes (T1DM) is the first and it is characterized by an absolute insulin deficiency that is due to an immune-mediated destruction of β cells that are the production site of insulin within pancreatic islet of Langerhans. The second type of diabetes is T2DM, and it is characterized by the impairment of insulin signaling and by the insulin resistance followed by the loss of functional β cells [2].

Diabetes is a chronic disease that is related to serious complications such as retinopathy, nephropathy, neuropathy and micro vascular complications. One of the most debilitating complications of diabetes mellitus is the development of chronic foot ulcers. The treatment of this important complication is a clinical, economical and social problem. The healing process of diabetic foot does not follow the classical progression of wound healing and this depends on intrinsic factors, such as neuropathy and vascular abnormalities, and it also depends on extrinsic factors such as wound infection, callus formation and pressure to the site [1]. Diabetic foot may be so complicated as to require amputation and it is a serious consequence.

Regenerative Medicine

Regenerative Medicine is a new field of research and clinical applications aimed at repairing, replacing or regenerating cells, tissues or organs to restore altered functions due to various

causes including congenital abnormalities, diseases and aging [3]. Regenerative Medicine has set itself the goal to restore normal functions, so it can be considered an important opportunity for all the major therapeutic approaches [4]. Regenerative medicine is characterized by an interdisciplinary nature which includes stem cell biology, tissue and organ transplantation, genetics, molecular biology and tissue engineering. So interdisciplinary allows you to open up to new therapeutic approaches to try to solve health problems [5]. Regenerative medicine consists of five key moments, the first step consists in obtaining the original tissue, then it is necessary to isolate the cells and expand them in culture. The next step consists in the assembly of the cells in a delivery or in a maturation system and the application of the product to the clinic. Finally, the last step includes the application of it to the patient. All these stages must take place according to precise procedures and in cleanrooms [6]. (The cleanroom technology consists of activities aimed to control and minimize contaminants in the air. It has three main purposes: protecting the process or product from contaminated air, protecting persons from exposure to contaminants that could harm their health and finally preserving the outside environment from emissions generated by the operations of the facility) [7].

Applications of regenerative medicine in diabetes: β cells

Replacing β - cells has a great potential in the treatment of type 1 diabetes that is characterized by the autoimmune destruction of the insulin-producing pancreatic islet. The β cells are located in the pancreas, an organ composed by an endocrine and an exocrine portion.

The endocrine portion is the region that can produce hormones and it is composed by hormone-producing cells including β cells. It would be useful to use ESC, IPS, “reprogrammed cells” to generate new cells that have their appearance mutated in β cells. This could be possible with stem cells. Normally, adult β cells have a limited capacity to replicate themselves so the potential of stem cells transplantation has to be considered in the treatment of the diabetic patients [8]. The possibility of generating replacement cells and tissues in laboratory was born with the discovery of human pluripotent stem cells. It is possible to use human pluripotent stem cells to form cells in the β cell lineage in vitro with the identification of genes and signals that are important for the pancreatic lineage that are discovered with studies on pancreatic development in model organism [9]. People suffering from type 1 diabetes need insulin replacement therapy, so over the years patients were treated by injections, islet transplants. Because of the shortage of donors for transplantation, there have been different attempts to deal with this disease. Important possibilities have been attributed to stem cells that have attracted the attention of a lot of researchers on how to form the β cells from stem cells. The formation of β cells in vitro can be achieved by several strategies that include: the differentiation from human pluripotent stem cells (hPSCs), the Trans differentiation of other cell types, the differentiation of pancreatic progenitors and finally the expansion of β cells already existing. To get to the formation of β cells, the stem cells lose their pluripotency and progressively move towards differentiation. Many transcription factors expressed during embryogenesis were identified, including Pdx1, Pax4 and Nkx2.2. D’Amour et al. (2005) have realized that to get to the formation of β cells it is necessary to imitate, as much as possible, the conditions of embryonic development. Then, in 2006, D’Amour et al. described some procedures to get to the differentiation of stem cells, in two dimensions, the activation of Pdx1 and the construction of cells containing insulin. D’Amour et al. described the need to expose the stem cells to some soluble factors, and a very important role was recognized to Activin A. One of the most important moments to get the differentiation of stem cells in vitro is the activation of Pdx1. The activation of Pdx1 is followed by the activation of other endocrine markers, including Ngn3, Nkx2.2, Nkx6.1, MafA and MafB. The conclusions of several groups of researchers have outlined the steps to get to the development of pancreatic cells from stem cells: 1) definitive endoderm formation, 2) endoderm patterning, 3) pancreatic epithelium specification and finally 4) endocrine commitment.

To get to the definitive endoderm formation it was necessary to understand embryo development in vitro and this has been possible through studies on stem cells of mice. A combination of Activin A and Wnt is required to achieve the first step of the development. To ensure an efficient and reproducible differentiation it is necessary to identify markers to track and investigate endoderm development. Genes FOXA2 and SOX17 are generally used to demonstrate the presence of definitive endoderm. The flow cytometry of surface markers is another test to study the differentiation and since there are no specific markers for endoderm, is used a combination of markers that allow having information. During the differentiation process some stem cells are lost; some studies are therefore aimed at the resolution of this problem, for example by introducing a bioreactor.

The step of patterning the definitive endoderm is characterized by an important role that is played by the levels and the timing of TGF- β signaling. The third phase of the specification of the pancreatic epithelium is based on the basic knowledge of the fact that in the early stage of pancreatic development, there are distinct regions that follow different regulatory pathways even though they have common progenitors. The last stage has not been fully understood, therefore there is a high variability in the procedures put in place in the various laboratories [10,11].

Applications of regenerative medicine in diabetes: diabetic foot ulcer

One of the consequences of diabetes mellitus, as it has been explained previously, can be the onset of foot ulcers. Diabetes mellitus is characterized by the alteration of wound healing [12]. The normal wound healing process consists of five phases: hemostasis, inflammation, proliferation, epithelialization, and tissue remodeling. Chronic wounds, such as diabetic wounds, are wounds that fail one or more of the wound healing steps [13,14]. Normally, the healing of a wound includes specific steps: coagulation, inflammation, formation of granulation tissue, tissue remodeling [15]. The healing of a wound of a diabetic patient follows a particular course, where many processes are altered and this can lead to the formation of an ulcer. The healing of a wound of a diabetic patient has an altered course about cellular activity, the synthesis of ECM, the release of growth factors, angiogenesis and neovascularization and about inflammation, too. The keratinocytes have the task to migrate from basal membrane and differentiate to form new skin. The keratinocytes of a diabetic patient increase their proliferation but decrease their differentiation and their ability to migrate. The slowness of these keratinocytes can be considered one of the risk factors for the formation of an ulcer. Fibroblasts normally play a very important role in the intervention on wound healing but they are impaired in diabetic patients because of their reduced proliferation and excessive apoptosis. Extracellular matrix components help replenish cells in normal skin but in the diabetic wound they are decreased because of an excessive degradation. The growth factors, which have an important role in cellular signaling during healing of a wound, are reduced in the diabetic wound. Angiogenesis and neovascularization are very important steps to provide oxygen, food, but also to bring together to the site of the lesion inflammatory cells and stem cells circulating. The diabetic patient has a damaged microcirculation.

Inflammation is a fundamental aspect of wound healing but this must be the right time because if the inflammation is prolonged, it can cause harm rather than benefit. Usually in diabetic patients it occurs a state of prolonged inflammation [16]. Diabetic ulcers are usually localized on pressure points, such as metatarsus – phalangeal joints, ankles or heel region. It is assumed that in diabetic wounds the problem of healing is located in the proliferation phase and / or the inflammation phase. After these steps, the epithelialization usually follows without complications [17]. The classic way to treat diabetic foot ulcer consists of three treatments: debridement, antibiotics and revascularization, if it is necessary. Generally, antibiotic therapy is not used for the purpose of healing the wound rather it is useful because of the elimination of any infections from this. The most common

infections of diabetic foot are polymicrobial and they consist of both aerobic and anaerobic bacteria. The use of local therapies may help in healing [15-18]. Studies on regenerative medicine show us how stem cells can be considered an important resource because of their ability to self-renew and to differentiate. Several experiments have shown the usefulness of transplanted mesenchymal stem cells (MSCs) in the diabetic wound site as these cells can promote healing by stimulating various stages of resolution. MSCs have beneficial effects regarding the promotion of cell proliferation, collagen synthesis, the release of growth factors, and neovascularization and cell mobilization into the wound site. These observations were deduced by the examinations of wounds of rats that have benefited in the healing process [16]. Another research showed the use of bone marrow, derived in sterile conditions, which was put in contact with wounds of those patients who had not responded to normal therapies. Another part of bone marrow was put in culture. The bone marrow aspirate was placed in contact with the wound and, after that, the wound was covered. Subsequently, the application was repeated with cells that had been cultured and it was observed that the bone marrow aspirate could promote wound healing because it contains important precursor cells in skin regeneration.

Patient that suffered from type 2 diabetes mellitus and peripheral neuropathy, and that had toe ulcers of digits 1 and 2 secondary to poorly fitted footwear (Figure 1). Marrow aspirate was obtained from her distal tibial metaphysis and placed directly to the wound, covered with non-adherent dressing and left in place for 48 hours. The first dressing change showed granulation tissue (Figure 1). No adjunctive treatments were used, and the wound healed by secondary intention 47 days later with minimal scarring (Figure 1). Although the marrow aspiration from the ipsilateral tibia, there were no marrow site harvest complications in the patient [19,20].

A recent study attempted to use a combination of four modern therapeutic approaches in diabetic wounds of some patients showing resistance to heal. Were combined. The combination of bone marrow MSCs, platelet growth factors, fibrin glue and bone marrow-impregnated collagen matrix has allowed some patients to heal their wounds and allowed others to improve their conditions [21].

Conclusion

Diabetes mellitus is a heterogeneous group of disease that is widespread and that has an impact on the rise. In general this

disease is associated with genetic predispositions that affect the functioning of insulin secretion by pancreatic β cells and altered lifestyle and a wrong diet. Regenerative medicine provides important chance to rebuild the insulin-producing pancreatic β cells. To date, many studies have been conducted and many other will have to be conducted about this very complex yet motivating possibility to try to offer patients other therapies than traditional, with the possibility of offering more advantages. Diabetes mellitus is often associated with complications that can also be very strict and they can compromise the quality of life of the patients. Among the complications, there is the formation of ulcers in the region of the feet, due to impaired wound healing that characterizes the disease. The diabetic foot ulcer is a serious complication as it can incur additional problems that can result in the need for amputation. Over the years different approaches have been developed to treat this complication, but these approaches were ineffective for some patients. Regenerative medicine could offer an alternative, or in some cases it could offer a support to existing therapies. In the last few years, some studies have shown that stem cells applied on the wounds could provide benefits in healing. For the future it would be interesting to study the role they may have induced pluripotent stem (iPS) cells and mesenchymal stem cells in diabetes care. The recent discovery of induced pluripotent stem cells has provided the possibility to revolutionize the field of regenerative medicine. In fact, generating patient-specific pluripotent stem cells with properties similar to those of embryonic stem cell (ESC) has long been a central aim in research on stem cell-based regenerative medicine. In 2006, Takahashi and Yamanaka described the derivation of iPS cells from embryonic and adult mouse fibroblasts through the ectopic co-expression of four genes: Oct4, Sox2, Klf4, and c-Myc. The expression of these four genes was sufficient to reprogram somatic cells to an ESC-like pluripotent state. There are several advantages of using iPS cells for regenerative medicine. In fact, their use can overcome the ethical and political issues associated with the use of embryonic cells. Moreover, they can be used as autologous and patient-specific cells, which eliminate issues related to the immune rejection of grafts, and can thus be expected to become the major tool in the advancement of personalized medicine. Finally, iPS cell production can easily be scaled up, which essentially provides an unlimited source of cells for clinical applications, in contrast with adult stem cells [22-24].

On the other hand, mesenchymal stem cells (MSCs) are multipotent stromal cells that were discovered in bone marrow by Friedenstein et



Figure 1: (1) Dorsal toe ulcers pre-treatment with MDSC. (2) One week after treatment with MDSC, exposed bone and distal interphalangeal joint at the second digit. (3) Wounds completely healed at day 47 with minimal scarring (photo from day 65) [20].

al. and exhibit plasticity i.e. they are capable of differentiating into many cell types in the appropriate microenvironment. Exogenous application of MSCs has been successful in animal and human trials in liver diseases, connective tissue disorders, spinal cord injury, chronic non-healing ulcers, critical leg ischemia and musculoskeletal disorders. In addition, studies have shown that bone-marrow derived mesenchymal stem cells to promote regeneration of infarcted myocardium by promoting neovascularization. Moreover, studies on possibility of utilizing MSCs in regenerative and reparative therapies for neurological disorders particularly Parkinsonism are underway. Furthermore, human mesenchymal stem cells have great potential for tissue engineering but the challenge remains always to generate functional cell types that are useful for transplantation [25,26].

Open toward regenerative medicine, investing in new research and continuing the efforts made so far, it appears to be an important resource for treating a disease in its onset or its complications because diabetes mellitus is often associated with high health costs.

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