case report

Allotransplantation of macroencapsulated parathyroid cells as a treatment of severe postsurgical hypoparathyroidism: case report

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Ann Saudi Med 2016; 36(2): 143-147

DOI: 10.5144/0256-4947.2016.21.3.1130

Persistent hypoparathyroidism, a condition associated with major inconvenience and potential morbidity, is more difficult to treat than other hypofunctional endocrine disorders. Therapeutic alternative in severe postsurgical hypoparathyroidism is allotransplantation of macroencapsulated parathyroid cells. With this technique, it is possible to implant cells or tissues of parathyroid origin to replace them in such patients without immunosuppression. At the present time, durable results have only been reported in parathyroid allotransplantation when immunosuppression to prevent rejection is administered. We report an allotransplant of parathyroid cells in a patient with continuous endovenous requirement of calcium to survive. Macrocapsule containing ~(20 to 30)×10^6 parathyroid cells was constructed with a polyvinylidine difluoride and implanted into the deep femoral artery. The functional activity of the graft, traced for 3 months, allowed to exclude the parenteral administration of calcium and to compensate symptoms of the disease.

SIMILAR CASES PUBLISHED: There have been no more than 3 previous clinical reports of similar parathyroid cell allotransplantation without immunosuppression.

Total thyroidectomy is a standard surgical procedure performed for thyroid cancer and benign thyroid diseases, such as Hashimoto thyroiditis and Graves disease, with low rates of postoperative complications. Nevertheless, after such operations, postoperative hypoparathyroidism occurs in 1.5% to 2.5% of patients.[1] Most cases have a positive clinical effect when treated with oral calcium and vitamin D. However, some patients, due to persistent symptoms of tetany, need frequent parenteral administration of chloride or calcium gluconate.[2] Along with this, long-term drug therapy for hypoparathyroidism has various side effects, such as gastritis, urolithiasis, and nephrocalcinosis, which significantly impair the quality of life of patients. Thus, the treatment of such patients with severe hypoparathyroidism requires an alternative and permanent therapy that would effectively restore parathyroid function, exclude or reduce the doses of substitution drug therapy, and reduce its complications. Parathyroid allotransplantation is reasonable and often the only therapeutic alternative for patients with severe postoperative hypoparathyroidism. Studies by Tolloczko et al[3] demonstrated results, attaining survival and function of grafted parathyroid tissue for at least 18 months, without immunosuppression. However, the main problem associated with this therapy has been rejection by alloimmunization or inflamatory responses with ensuing fibrosis that eventually compromised the survival of the transplanted tissue. Since permanent hypoparathyroidism rarely threats patient's life, the use of immunosuppressive therapy is highly undesirable. To overcome rejection and prolong the viability of parathyroid graft, attempts were made on careful haplotype and ABO group matching. The method of micro- and macroencapsulation of transplanted tissue is based on the principle of creating a mechanical barrier for antibodies and white blood cells, but allowing the nutrition and hormones to diffuse.[4] Our previous researches

on pancreatic islet cells and thyroid cells revealed that grafts into the blood stream may preserve viability and long-term functioning without the use of immunosuppression; therefore, bloodstream is one of the immunologically privileged sites.[5] This case report presents a 3-month clinical follow-up of patients with severe postoperative hypoparathyroidism, in whom parathyroid cell allotransplantation was performed. The patient was symptom free, had no hypocalcemia on lowered doses of oral substitution therapy, and did not need intravenous (IV) calcium after allotransplantation. These preliminary results suggest the possibility of using macroencapsulated parathyroid allograft as an alternative treatment of severe hypoparathyroidism.

CASE

The recipient was a 39-year-old woman, who had undergone total thyroidectomy for Hashimoto thyroiditis at the age of 23. The postoperative period was complicated with severe symptoms of hypocalcemia. The patient suffered from tetany, paresthesia, fatigue, headache, crampy abdominal pain, and constipation for 16 years. She had a number of medical emergencies with seizures. The patient was being treated with oral calcium 1000 mg/d, vitamin D3 400 IU/d, calcitriol 2 μg/d, and IV calcium chloride 4 g 1 to 2 infusions/wk. Despite the treatment, the patient still had symptoms. Her total serum calcium level was up to 1.22 mmol/L, and parathyroid hormone (PTH) level was 6.8 pg/mL. IV calcium chloride administration was repeatedly complicated by the soft tissue necrosis of the upper and lower extremities. Brain computed tomography revealed calcification of the basal ganglia. Considering the severity of hypoparathyroidism and the inefficiency of the substitution therapy, a decision was made to do allotransplantation of macroencapsulated parathyroid. Parathyroid tissue was obtained from a 27-year-old man with parathyroid hyperplasia secondary to renal failure. A fragment of every parathyroid gland was subjected to a histological study (**Figure 1**). The results of serum serology ruled out donor's contamination with HIV, hepatitis B and C, and Treponema pallidum.

The isolation of parathyroid donor tissue and cell culturing were performed in full sterile conditions at all times. Gland samples were delivered to laboratory in a transport medium based on Dulbecco modified eagle medium, containing 10% adult bovine serum and antibiotics (gentamycin, 100 μg/mL; penicillin, 100 U/mL). The storage time of the biomaterial to cell seeding was not more than 5 hours at a temperature of 4°C. The isolation of cell biomass was carried out by mechanical grinding of tissue fragments to the size of 0.1 to 2 mm^3, as well as enzymatic treatment with collagenase type II (1%), trypsin (0.25%), and DNase (0.01%). The time of incubation with enzymes was 18 hours at 4°C, followed by 10 minutes at 37°C, and then cryopreserved. The duration of cryopreservation before transplantation was 30 days. The concentration of PTH in the culture fluid after thawing was 1385 pg/mL. To determine cell phenotype, a immunocytochemical study of culture smears was performed using monoclonal antibodies to human PTH (**Figure 2**). Subsequent culturing confirmed sterility of the graft.

The macrocapsule designed as a cylindrical tube 15 to 20 mm in length and 3 to 4 mm in diameter from microporous, 157-μm thick, polyvinylidine difluoride artificial membrane with a pore diameter of 0.55 to 1.37 μm, and the porosity of 28.2% (**Figure 3**). Then, ~(20 to 30)×10^6 parathyroid cells were injected to the lumen of the capsule.

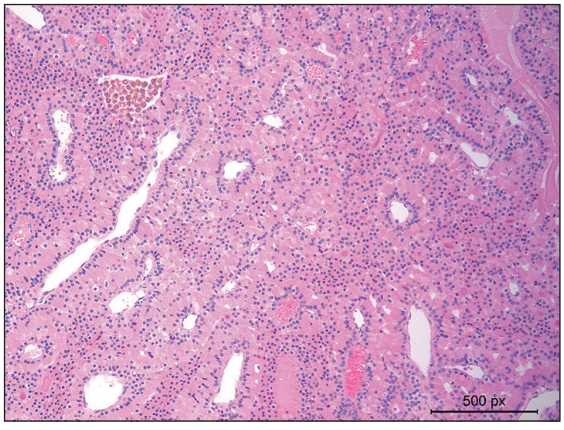

Figure 1. Allogeneic parathyroid gland: diffuse hyperplasia (Hematoxylin–eosin staining; magnification ×10).

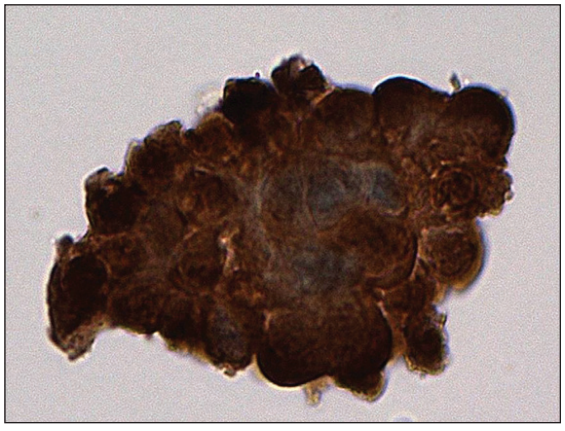

Figure 2. Immunocytochemistry investigation of cell culture; positive cytoplasmic staining of parathyroid cells is observed (magnification ×400).

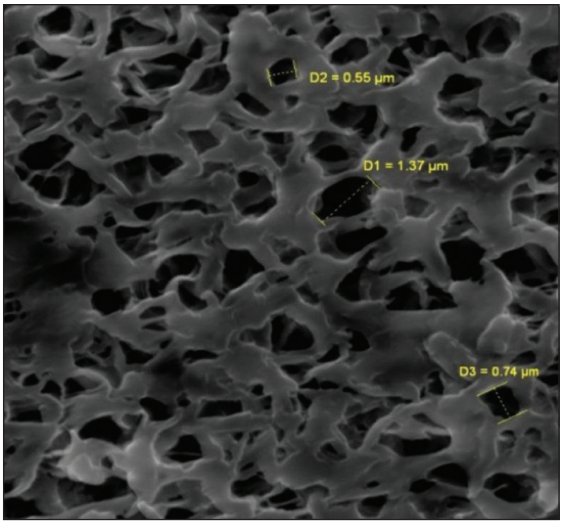

Figure 3. Digital microscopy; polyvinylidine difluoride membrane (magnification ×15 kx).

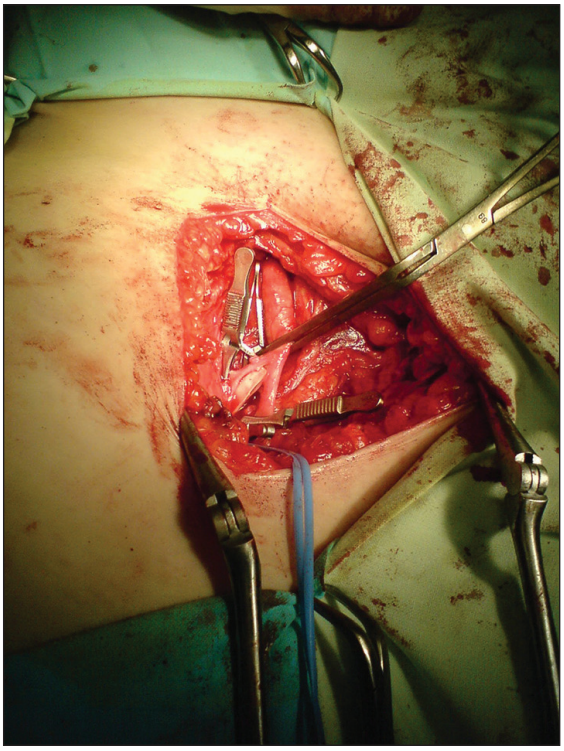

Figure 4A. Macrocapsules with cell transplant implanted into the lumen of the deep femoral artery.

Under spinal anesthesia, an encapsulated graft was implanted into the lumen of the deep femoral artery, followed by plastic repair of the arteriotomy with an autovenous patch (**Figure 4**).

Artery patency was confirmed by palpation at the end of the operation, as well as in the postoperative

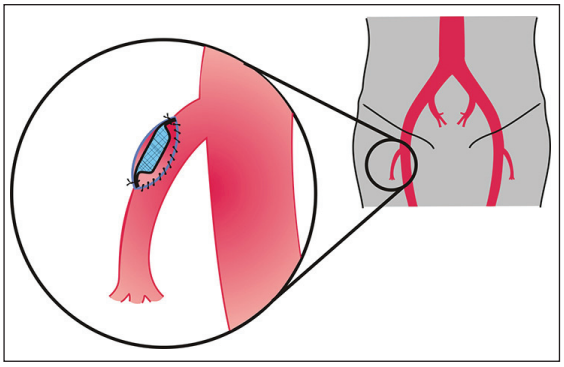

Figure 4B. Scheme of the graft implantation site.

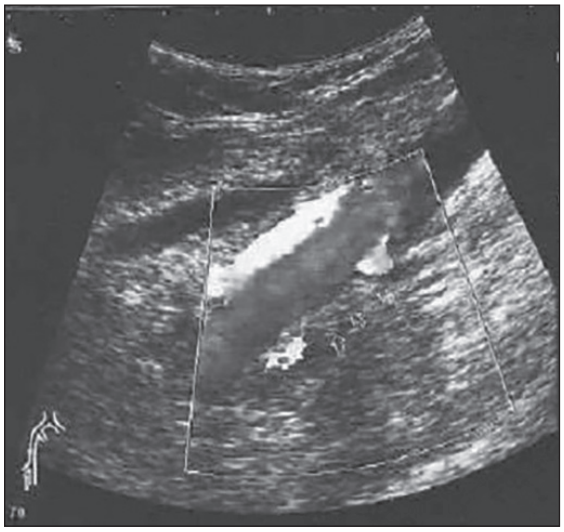

Figure 5. Femoral artery ultrasonography. The arrows indicate deep femoral artery with the graft.

period by Doppler sonography (**Figure 5**). After transplantation, the laboratory control of serum calcium, phosphate, and PTH was performed (Roche, PTH-DRG).

Postoperative period carried no complications. Moderate postoperative pain was controlled with non-steroidal anti-inflammatory drugs. Thrombosis prophylaxis was performed with subcutaneous dalteparin for 5 days. **Figure 6** shows levels of serum calcium and PTH after transplantation. Serum calcium range after transplantation was 1.22 to 1.7 mmol/L during the first 30 days, 1.65 mmol/L at day 39, and 1.9 mmol/L at day 47. Seven days after transplantation, the PTH level was 5.8 pg/mL; however, at day 33 the PTH level increased to 12.3 pg/mL, and at day 90 up to 21.15 pg/mL. The patient received no IV calcium with half the dose of oral replacement therapy. In the post-transplant period, the serum phosphorus level decreased from 1.97

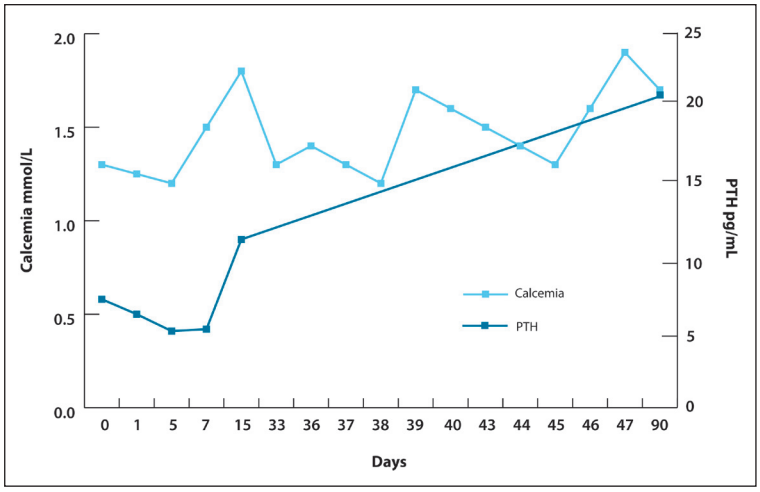

Figure 6. Total serum calcium (light blue line) and PTH (dark blue line) in a patient with severe postoperative hypoparathyroidism after allotransplantation macroencapsulated parathyroid cells.

mmol/L to 1.66 mmol/L, indicating an adequate effect of PTH on renal reabsorption.

In the posttransplantation period, the patient had no symptoms of hypocalcemia except on day 33, which was preceded by dyspepsia. It should be noted that the levels of serum calcium did not correlate with indicators of PTH, which can be attributed to irregular oral administration of calcium due to periodic episodes of gastroduodenitis.

Doppler ultrasound, performed 3 months after the operation, confirmed the patency of the deep femoral artery at the site of the graft without significant hemodynamic disorders. Diameter of the main stem of the deep femoral artery and its branches on the side of surgery was 5.5 mm and 4.3 mm, respectively (linear flow velocity—36 cm/s and 50 cm/s), versus 5.6 mm and 4.4 mm on the opposite side (the linear velocity of the blood flow—82 cm/s and 52 cm/s).

The study was approved by the local ethics committee after obtaining informed consent from the patient to the surgical intervention. The project was financed by the Ministry of Healthcare of the Republic of Belarus.

DISCUSSION

Along with known complications of substitution treatment of hypoparathyroidism, we met another not less serious complication of IV calcium infusion—necrosis of soft tissues of the extremities. It is then desirable to seek a permanent therapeutic alternative that can reinstate normal parathyroid function effectively, replacing or reducing medical supplementation and reducing long-term morbidity. This is especially desirable, as the use of synthetic PTH in such patients has been associated with the appearance of osteosarcomas in animals. Therefore, parathyroid transplantation appears to be an ideal treatment. Nevertheless, there is little experience in this field. The maximum functioning duration of parathyroid allograft (13 years) was recorded in a patient with chronic renal failure, on whom kidney and parathyroid tissue transplantation was performed with subsequent immunosuppression.[6] Tolloczko et al[7] attempted allotransplant therapy using cultured principal parathyroid cells, which were selected according to PTH production, low major histocompatibility complex (MHC) expression, and human leukocyte antigen (HLA matching. In one case, the duration of graft function without immunosuppression reached 18 months; however, the need for replacement therapy remained at baseline. In 1983, the idea about the need to encapsulate parathyroid tissue to prevent rejection and increase transplant vital period arose for the first time.[8] This concept assumed pretransplant immobilization of cells or tissue into microspheres, which creates a mechanical barrier to immunoglobulins, complement components, and immunocompetent cells of the recipient, and, at the same time, does not hinder transport of nutrients and products of cellular secretion. However, most of these microcapsules provoke the development of nonspecific inflammatory response with subsequent per capsular fibrosis and imminent failure of graft functionality.

In this case, we studied the effectiveness of macroencapsulated cryopreserved parathyroid cells obtained from a live, unrelated, and not ABO and HLA compatible donor. We obtained objective evidence that the cell graft still functioned after 3 months. The PTH level was rising, while the patient took half of the dose of the replacement therapy that she had taken before. Doppler ultrasound, performed 3 months after the operation, showed a fixed graft with no stenosis or thrombosis of the deep femoral artery. Based on the results of our earlier researches, it can be argued that the arterial blood stream, as a place for the implantation of encapsulated allograft, has sufficient immunoprotective properties.

In conclusion, we believe that transplantation of encapsulated parathyroid cells may become the method of choice in the treatment of severe forms of hypoparathyroidism. Along with the low traumatism of the surgical intervention and the possibility of its performance in most surgical hospitals, an additional advantage of the method is the possibility of repeated transplantation in the case of a decrease in the parathyroid graft functional activity. Further investigations about the porosity, biocompatibility, and stability of the micropo-

rous membranes are required, as well as there is the need for MHC and ABO donor–recipient matching and for immunosuppression.

Funding sources

Ministry of Healthcare of the Republic of Belarus.

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