

Parathyroid transplant: from auto transplant to stem cells differentiation

Abstract

To deal with hypo parathyroidism, surgeons been used the auto transplantation of parathyroid tissue for decades. In the present time it is a very useful technique to avoid permanent hypoparathyroidism during surgery. Has a successful rate of 99% of graft survival. Nevertheless, not always it is possible to identify or prevent the damage of parathyroid glands during surgery and proceed to perform an auto graft.

With this evidence it was developed the cryopreservation of parathyroid tissue to have an alternative for a probable postsurgical hypoparathyroidism. The indications of cryopreservation of parathyroid glands are for patients with elevated risk of hypoparathyroidism (reoperation, total para thyroidectomy, etc). Instead of the excellent results of auto transplantation of fresh tissue, the cryopreserved parathyroid transplant have <70% of success. This put in controversy the utility of cryopreserved parathyroid banks.

Exist a limited medical therapy for hypoparathyroidism with supplementation of Calcium and Vitamin D. And the administration of recombinant PTH (1-84) in adults with hypoparathyroidism is expensive and still has no clinical standardization. All these make necessary the search of other therapies. In this way, it is been tried the allotransplantation of parathyroid glands with poor results. But recently, the research in Stem Cells (SC) in stromal tissues, like parathyroid glands or thymic gland, tonsils or even the adipose tissue have obtained promising results in differentiating cells in parathormone secreting cells (PT-like cells) opening a new possible cell therapy for hypoparathyroidism.

Keywords: hypoparathyroidism, parathyroid transplant, autotransplant, allotransplant, cryopreservation, stem cells differentiation, rhPTH

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Introduction

The most frequent complication in thyroid surgery is hypocalcemia. This could be permanent because a failure of parathyroid glands (in adverted excision or compromise of vascularization).^{1,2}

Autotransplant

Several decades ago, surgeons tried to solve the problem of hypoparathyroidism with auto transplant of parathyroid tissue during surgery. The transplant of autologous parathyroid glands was first described, a more than a 100 of years ago in animal models by Halsted, with 60% of success when implant parathyroids of dogs in rectus abdominis.³ In thyroid surgery was first described in 1926 by Lahey & Murray.^{4,5} Briefly, the method consists of mechanical disruption and graft of the parathyroid glands in muscle tissue (sternocleidomastoid or forearm). Until now it is described several methods of auto transplant, grafting the tissue into small pieces of 1 x 1 mm in pockets muscle.⁶ or disintegrated tissue is injected intramuscularly and subcutaneously with a blunt needle.^{7,8} The effectiveness of this procedure has been demonstrated in a high percentage of cases. A prospective study of 104 consecutive autografting in patients undergoing thyroid surgery (total or subtotal resection), show a 99% of successful cases (103 cases).⁹

The auto transplant is also used in patients undergoing parathyroid surgery (total parathyroidectomy) with primary hyperparathyroidism associated to multiple endocrine neoplasia and secondary hyperparathyroidism for chronic kidney disease. Fresh autologous

parathyroid tissue (approximately 60 mg) in forearm is autografted to prevent postoperative hypoparathyroidism. This technique has a higher percentage of persistence or recurrence in secondary hyperparathyroidism. In reoperations increases the risk of permanent postsurgical hypoparathyroidism.¹⁰ For this reason, many centers prefer the subtotal or partial parathyroidectomy in renal patients, for the lowest risk of recurrence.¹¹

Some authors tried to reduce the risk of recurrence by intraoperative graft selection. This is done with a parathyroid stereo magnifier identifying normal tissue (best functionality and less proliferative potential- type A).¹² Thus, 37 patients with total parathyroidectomy and immediate autotransplantation identifying tissue type A (low proliferative potential), only one had recurrence at 32 months of surgery, and was easily cured by resecting some grafts from his forearm.¹³ This technique has not been reported again, it is a difficult and poorly reproducible method.

It has been proposed that the effectiveness of autotransplantation (99%) is probably due to the rapid vascularization and re-innervation observed in animal models, which occurs at 4 days and a week, respectively.^{14,15} *In vitro* studies have shown that induces angiogenesis parathyroid tissue independently of the presence of calcium or PTH secretion.¹⁶ It has also been reported an increase of 12 times in VEGF mRNA expression (vascular endothelial growth factor), a potent angiogenic factor produced by various tumors and hypoxic tissue. Likewise, inhibition of this factor with its soluble receptor, determines an inhibition of angiogenesis.¹⁷

The autotransplant is commonly used but demand the identification of parathyroid tissue during surgery, that could be compromised (intrathyroid parathyroid gland, excised parathyroid, avascularized parathyroid). The strong recommendation to avoid hypoparathyroidism is an adequate surgical technique and a high volume surgeon.¹⁸

Cryopreservation

Considering the success of autotransplant, it has been used the cryopreservation to preserve parathyroid tissue for a future autologous transplant if would be needed, because of postsurgical hypoparathyroidism in patient with reoperation or in total parathyroidectomy.¹⁹ Different techniques of cryopreservation have been used considering the cryopreservation media (mainly avoiding animal derived supplements). And the time of cryopreservation it has been a critical feature that must be considered in management of tissue banks and in clinical indication of cryopreserved parathyroid tissue autotransplant.²⁰

Clinical reports of autotransplant of cryopreserved parathyroids show since 1979 to 2014 between 25 -100% of success approximately. It is recognized a 70% of success in average. Those reports use a classification of the clinical response in different categories (total response, partial response, no response) depending on the need of supplementation of calcium and vitamin D.²¹ Other authors mention that a cryopreservation of more than 24 months alters the function of cryopreserved tissue that compromises the success of a future parathyroid autotransplant.²²

Indeed, this evidence are take into account to eliminate parathyroid tissue reserved for more than a year in some European tissue Banks. The recommendations are that cryopreservation was made in cases of parathyroid hyperplasia and in center with a high volume of thyroid and parathyroid surgeries.²³ In many cases it is not possible the cryopreservation of parathyroid tissue, for institutional capacity or surgeon recognition of damaged parathyroids. And if the patient evolves with postsurgical hypoparathyroidism have no chance of autologous transplant. For this reason, many researchers (principally surgeons) has been developed other alternatives like a allotransplant of parathyroid tissue or cells.

Allotransplant

The Allotransplant of parathyroid tissue implies the use of immunosuppressant drugs. So, several researches focus the effort in the decrease of allogeneic reaction of the graft by culturing cell and decreasing the expression of HLA antigens. Or creating a mechanical barrier between the host and the grafted tissue by microencapsulation. Tolozcko et al.,²⁴ reports the culture and selection of parathyroid cells that produce PTH and the graft of at least 3x10⁶ cells in patients with hypoparathyroidism as an alternative treatment.²⁴ The same group in 2010 report a series of patients allotransplanted with these technique, with the selection of PTH producing cells and matching HLA antigens between donor and host. They report a partial clinical response and a failure of grafted parathyroids cells in more than a year of outcome (18-20 months).²⁵ In our experience with allotransplant, we report a graft of microencapsulated cryopreserved parathyroid tissue. It was observed a partial but significant response in a patient with a severe hypoparathyroidism. This patient lived with a continuous intravenous administration of calcium by central venous catheter. With the microencapsulated allotransplant it was possible just keep an oral supplementation of calcium and vitamin d and it was effective approximately for 20 months.²⁶⁻²⁸

Medical therapy

In the present, there is no medical treatment as an alternative for these patients; recently FDA approved a recombinant PTH for the treatment of hypoparathyroidism. But is not an alternative by now because the cost and the lack of long term clinical evidence. There are several clinical trials (REPLACE, RELAY, REPLACE, REPEAT) studying the safety and effects of doses of 25 and 50 mcg of rhPTH (1-84) in adults with hypoparathyroidism.²⁹⁻³¹ In this way, several groups are trying to obtain a new source of cells that produce and secrete PTH for cell therapy.

Cell therapy

In our experience, we develop a method to culture and immortalize a cell line of human parathyroids, called RCPH, this cell line was in mortal with more than 200 passages keeping the production and secretion of PTH. Do not show tumorigenicity in both models, *in vitro* or *in vivo* (NOD/SCID rats grafted). Nevertheless, with time and cryopreservation periods between studies the line decline the production of PTH.^{32,33}

For this reason, many groups have focused their efforts on optimizing the culture of parathyroid to obtain a cell source in sufficient quantity to maintain PTH secretion and thus allows to control calcium homeostasis. Some authors report isolation of stem cells (SC) from parathyroid glands. Others have been able to stimulate the differentiation type PT PTH-secreting cells from embryonic SC or thymic epithelial cells.³⁴ Shih et al.,³⁵ describe the isolation of human parathyroid SC that expresses specific parathyroid markers by FACS, RT-PCR and calcium uptake in real time. They also checked the osteogenic and adipogenic potential of these cultures (schPT).³⁵ Fang et al.,³⁶ reported an expansion of a population of resident stem cells in the parathyroid glands (CD44 + / CD24-) in cases of benign parathyroid (as well as recently demonstrated in parathyroid cancer) isolated by cell sorting (FACS).³⁶

From these and other cell types has been tried to stimulate cell differentiation into parathyroid-like cells (PT-like) with the ability to secrete PTH as regulated by extracellular calcium. Bingham et al.,³⁷ proposes the use of a differentiation protocol where cultures are exposed to Activin A and Shh (Sonic soluble hedgehog). They obtain expression of differentiation markers (CXCR4) and adult parathyroid markers (GCM2, PTH and CaSR), and PTH production in embryonic SC (hES) cells and thymic epithelial cultures exposed to these media (transdifferentiation).^{37,38}

With these results, other authors try to differentiate other sources of Stem Cells into PT-like cell. Park YS et al.,³⁹ harvest Stem Cells for tonsils, and with the described protocol of parathyroid differentiation, they obtain promise results with PT-like cells that produced PTH. This author then propose a 3D culture to create an organoid in order to maintain the structure and the paracrine and autocrine relation between cells to get a better graft survival.³⁹ There is some hypothesis of differentiation of Adipose derived SC into PT-like cells that could be a promise source of cells for treat patients with hypoparathyroidism.⁴⁰

In our laboratory, we have protocols of harvesting, expansion and cryopreservation of adipose derived stem cells, and there is a Project of differentiation of adipose SC into PT-Like cells in order to get a new cell therapy for hypoparathyroidism.⁴¹

Conclusion

Hypoparathyroidism is a relevant clinical problem, with few

medical alternatives. An autologous parathyroid transplant is a very successful technique to avoid a permanent postsurgical hypoparathyroidism, nevertheless the recognition of damaged parathyroid tissue or even the recognition of parathyroid tissue itself is difficult and is not always possible to perform an autograft. In the last 10 years there is a little advance in the allotransplant of parathyroids. But recently becomes a promise new medical and cell therapies. The rhPTH (1-84) and the technique to differentiate PT-like cells. We think that differentiation of PT-like cells or even differentiation of stromal SC of cryopreserved parathyroids could be the best technique to treat patients with hypoparathyroidism with an auto regulated source of cells and without risk of rejection or immunosuppressant therapy.

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Conflicts of interest

The author declares there is no conflict of interest.

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