SUCCESSFUL IMPLANT OF LONG-TERM CRYOPRESERVED PARATHYROID GLANDS AFTER TOTAL PARATHYROIDECTOMY

Fábio Luiz de Menezes Montenegro, MD, PhD,1 Melani Ribeiro Custódio, MD,2 Sérgio Samir Arap, MD, PhD,1 Luciene Machado dos Reis, MS, PhD,2 Shigueko Sonohara, MS, PhD,3 Inês Vieira Castro, MD,4 Vanda Jorgetti, MD, PhD,2 Anói Castro Cordeiro, MD, PhD,1 Alberto Rosseti Ferraz, MD, PhD1

1 Department of Head and Neck Surgery, University of Sao Paulo Medical School, Sao Paulo, Brazil. E-mail: fabiomonte@uol.com.br
2 Department of Nephrology, University of Sao Paulo Medical School, Sao Paulo, Brazil
3 Department of Radiology, University of Sao Paulo Medical School, Sao Paulo, Brazil
4 Department of Pathology, University of Sao Paulo Medical School, Sao Paulo, Brazil

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Abstract: Background. Parathyroid cryopreservation is essential in some cases of parathyroid surgery. The fate of autografted tissue after long-term cryopreservation is not fully discussed in the literature.

Methods. The successful experience with the use of parathyroid tissues preserved for 21 months and 30 months is reported.

Results. Both patients were women with renal hyperparathyroidism who underwent total parathyroidectomy without autotransplantation. Patient 1 was a 40-year-old woman. At 21 months of follow-up, her parathyroid hormone (PTH) level was undetectable, and despite oral calcium supplements, she was hypocalcemic. Forty-five cryopreserved fragments were thawed and implanted in her forearm. Calcium levels improved, and PTH steadily increased in both arms. PTH levels at 18 months after the autograft were 37.0 pg/mL in the contralateral arm and 1150.0 pg/mL in the implant arm. Patient 2 was a 44-year-old woman. After 30 months, her PTH was undetectable, and she underwent cryopreserved tissue implantation.

Conclusion. These cases show that parathyroid tissue may remain viable even after long-term storage.

Keywords: cryopreservation; hypoparathyroidism; hyperparathyroidism, secondary; parathyroid glands, transplantation; parathyroidectomy

The surgical management of renal hyperparathyroidism is still evolving. Subtotal parathyroidectomy and total parathyroidectomy with autotransplantation have been employed in many patients. Both techniques are associated with different rates of recurrence and permanent hypoparathyroidism. Although total parathyroidectomy without autotransplantation would be theoretically associated with hypoparathyroidism in more cases, this operation has been reevaluated. Unfortunately, permanent hypoparathyroidism after parathyroidectomy is unpredictable. To lessen this risk of hypoparathyroidism, cryopreservation of parathyroid tissue was proposed for delayed autotransplantation.

Some questions arise about the use of cryopreserved parathyroids: How is parathyroid tissue affected by the freezing process? Does the time
elapsed between cryopreservation and implantation affect the function of parathyroid cells? Clinical experience with the use of long-term cryopreserved parathyroid tissue is not fully discussed in the literature. Far from only an academic interest, the understanding of the modifications after cryopreservation may be critical to decide when to implant or how long this tissue can be available. This understanding would be helpful not only to renal-related hyperplasia, but also to other forms of hyperplasia treated by total parathyroidectomy and autotransplantation, as in patients with hyperparathyroidism in multiple endocrine neoplasia.

The experience with parathyroid implantation after long-term cryopreservation is reported in 2 cases.

**CASE REPORTS**

**Case 1.** A 40-year-old woman underwent total parathyroidectomy without autotransplantation because of secondary hyperparathyroidism in 1996. She had a history of renal failure (as a consequence of systemic lupus erythematosus), and she started hemodialysis in 1989. Four years later, she underwent kidney transplantation. After 15 months, she rejected her renal allograft. In 1995, she complained of bone pain and pruritus. Total calcium was 2.88 mmol/L (normal, 2.13–2.63 mmol/L), phosphorus was 2.9 mmol/L (normal, 0.7–1.5 mmol/L), and parathyroid hormone (PTH) was 1178.96 pg/mL (normal, 10–72 pg/mL). Medical treatment failed to improve symptoms, and phosphorus levels were always elevated.

After signing an informed consent, the patient underwent a total parathyroidectomy, and cryopreservation of parathyroid tissue was done, in a study protocol approved by the ethical committee of the Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo. During the operation, 5 enlarged parathyroid glands were identified, 4 in habitual places, close to the thyroid, and 1 supernumerary gland inside the carotid sheath, near the carotid bulb. The time elapsed between harvesting and cryopreservation was about 150 minutes. The fragments were transported in RPMI 1640 (Roswell Park Memorial Institute) supplemented with antibiotics (200 µg/mL streptomycin and ampicillin). After washing, the tissue fragments were placed in cryotubes with RPMI 1640, 50% fetal bovine serum, and 10% dimethylsulphoxide (DMSO) and were kept in a polystyrene foam box in a freezer at −70°C and then stored in liquid nitrogen (−180°C).

There was significant decrease in calcium levels reaching the nadir of 0.78 mmol/mL of ionized fraction (normal, 1.10–1.30 mmol/mL). After a long follow-up, her levels of calcium remained low in spite of oral replacement of both calcium and calcitriol, and the use of cryopreserved material was indicated. At this time, her PTH dosage was zero.

After 21 months of storage, parathyroid tissue was thawed rapidly at 37°C and rinsed with RPMI 1640 on an ice bath, to remove DMSO and serum. Some fragments were sent for evaluation with light and electron microscopy. Forty-five fragments were then rinsed in a sterile saline solution and implanted in her forearm, under local anesthesia. Light microscopy study did not show any degenerative change in the cryopreserved tissue, as lytic necrosis or vacuolar formation (Figure 1). At electron microscopy evaluation, there were no signs of loosening cellular attachments. Mitochondrial membranes were not disrupted, and no dilation of endoplasmic reticulum was observed (Figure 2).

**FIGURE 1.** Photomicrograph aspect of parathyroid tissue at excision of the parathyroid (upper part) and after cryopreservation and thawing for implantation (lower part) (hematoxylin–eosin stain, original magnification x40).
After the implant, the patient’s clinical condition slowly improved. Her levels of PTH have increased since the implant, as shown in Table 1. Later she underwent another kidney transplantation and is doing well, with normal systemic PTH level. She does not need either calcium or calcitriol to be eucalcemic.

**Case 2.** A 44-year-old woman with renal failure due to hypertension had undergone hemodialysis for 6 years. She had complained of bone pain for 3 years. Her calcium, phosphorus, and PTH levels were 2.5 mmol/L, 2.0 mmol/L, and 1245.8 pg/mL respectively. After total parathyroidectomy without autotransplantation, under the same study protocol, she was followed. Cryopreserved parathyroid implantation was advised at 30 months because PTH levels were undetectable. Under the same conditions described above, she received 50 fragments of cryopreserved parathyroid tissue. Light-microscopy examination of cryopreserved tissue showed no signs of cellular damage. One year later, she underwent successful cadaveric renal allograft. Table 2 shows her levels of total calcium, phosphorus, and PTH at different times. She is also off of calcium or calcitriol.

**DISCUSSION**

The first report of successful use of cryopreserved parathyroid tissue in humans was in 1977, in a uremic patient 6 weeks after parathyroidectomy. Brennan et al reported their experience with cryopreserved parathyroid autografts. They performed the autograft in 6 patients who had hypoparathyroidism after surgery for primary hyperparathyroidism. Parathyroid storage varied from 45 days to 18 months. Although no systemic PTH could be demonstrated in the patient with the tissue cryopreserved for 18 months, the authors believed it was successful, as low levels were demonstrated in the grafted arm and the patient improved. As animal studies have suggested that tissue preserved for more than 12 months could not function properly, Brennan et al were the first to report that cryopreserved parathyroid tissue could function even after 12 months of storage. The same group later analyzed a total of 12 patients with deferred autografts, also in the treatment of primary hyperparathyroidism. They observed that the length of cryopreservation and subsequent function of the graft were not related. Recently, Cohen et al in a prospective analysis reported that no functional autograft was observed beyond 22 months of preservation.

In 1980, Wells et al grafted cryopreserved tissue in 6 patients with primary hyperplasia. All cases have been autografted 2 to 6 months following parathyroidectomy. There was clinical evidence of functioning tissue in all but 1 case.

<table>
<thead>
<tr>
<th></th>
<th>Pre-implant</th>
<th>2 mo</th>
<th>6 mo</th>
<th>12 mo</th>
<th>18 mo</th>
<th>35 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mmol/L)</td>
<td>1.8</td>
<td>1.9</td>
<td>2.0</td>
<td>2.0</td>
<td>2.1</td>
<td>2.0</td>
</tr>
<tr>
<td>Phosphorus (mmol/L)</td>
<td>2.9</td>
<td>1.8</td>
<td>2.1</td>
<td>2.3</td>
<td>2.3</td>
<td>1.3</td>
</tr>
<tr>
<td>PTH systemic (pg/mL)</td>
<td>0.0</td>
<td>1.0</td>
<td>10.6</td>
<td>21.4</td>
<td>37.0</td>
<td>37</td>
</tr>
<tr>
<td>PTH graft (pg/mL)</td>
<td>–</td>
<td>2.8</td>
<td>10.8</td>
<td>304.6</td>
<td>1150.0</td>
<td>1433.0</td>
</tr>
</tbody>
</table>
Discussing implantation of autologous cryopreserved parathyroid as 1 modality of reoperation in secondary hyperparathyroidism, Rothmund and Wagner showed a mean necrotic rate of 20% in the fragments analyzed before autograft. They had 4 patients without previous fresh autograft (tissue was cryopreserved between 2 and 10 months) and 6 with a previous fresh autograft (tissue stored from 2 to 42 months). Apparently, adequate function was obtained in all 10 cases, but the success criterion was normocalcemia and there was no reference to PTH levels. In the present cases, there was no clear correlation of calcemia and PTH levels.

Wagner et al reported a very satisfactory response to implantation of cryopreserved parathyroid tissue in 25 patients. In this study, the time of preservation varied from 6 to 52 months. The main criteria for success or failure were calcium or vitamin D requirements, serum calcium levels, and clinical signs of hypocalcemia instead of PTH detection. In this view, there is lack of unequivocal and direct evidence of cryopreserved graft function.

Our cases show the clinical experience with tissue preserved for 21 and 30 months. Before grafting, PTH was undetectable, and in 1 case, calcium levels were low in spite of supplements of both calcium and calcitriol. In this condition parathyroid glands were implanted. After implantation, PTH levels have progressively increased. This experience shows that even after long-term storage, parathyroid tissue can be utilized and detectable levels of PTH may develop. This report is strong evidence of function of long-term preserved parathyroids in humans, which gives clinical support to an in vitro observation that although cryopreservation affects parathyroid tissue, it seems unrelated to the time elapsed from harvesting and possible implantation.

At the time the parathyroid tissue was grafted in the present cases, the study of 1 fragment by light microscopy did not show any particular sign of destruction. The study by electron microscopy in case 1 showed cells without evidence of irreversible injury. Morphological studies may be helpful. In this regard, proper identification of the stored tissue (nodular or diffuse hyperplasia) and frozen section information of degenerative changes would aid decision making during the implant procedure. Smeds et al defined 4 “quality” groups of cryopreserved transplants according to morphology.

Additional studies, such as the Tunnel technique to evaluate apoptosis, seem to give no more information than do conventional hematoxylin-eosin stains.

In both cases described here, the amount of tissue was higher than that usually employed with fresh material. Whether this has affected function is not clear, but no recurrence was observed in both cases. Experimental evidence suggests a correlation between the volume of transplanted tissue and serum PTH concentration.

As the fate of fresh and cryopreserved parathyroid tissue is not clearly understood, additional studies are necessary to define the success rate of grafting fresh and cryopreserved tissue and to assess the factors affect the tissue’s ability to secrete PTH after freezing. There is evidence that the technique of cryopreservation, including both freezing and thawing process, is probably the major factor to optimize survival and secretion of cryopreserved autografts, but hypercalcemia or vitamin D may inhibit graft function, as reported by Sitges-Serra, when discussing the presentation of Cohen et al. Tanaka et al showed that hypocalcemia did affect autografted cryopreserved parathyroid.

In this report, hypocalcemia was observed in spite of satisfactory systemic levels of PTH. The same condition is considered a partially functional graft. Perhaps the condition of patients with low PTH levels, dependent of calcium/vitamin D to avoid symptomatic hypocalcemia, described as nonfunctional by Cohen et al, would be better described as hypofunctional, rather than nonfunctional. More properly, the term nonfunctional would describe no detectable PTH levels. Cacciato et al observed that only some patients are cured of hypocalcemia despite proven graft function. Improper function of cryopreserved graft is

| Table 2. Calcium, phosphate, and parathyroid hormone (PTH) levels before and after implantation in case 2. |
|--------------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| | Pre-implant | 15 mo | 31 mo | 44 mo | 62 mo | 73 mo |
| Calcium (mmol/L) | 2.2 | 2.7 | 2.6 | 2.2 | 1.7 | 2.1 |
| Phosphorus (mmol/L) | 2.9 | 2.4 | 0.9 | 1.3 | 1.6 | 1.4 |
| PTH systemic (pg/mL) | 0.0 | 24 | 53 | 103 | 101 | 95 |
| PTH graft (pg/mL) | – | 565 | ? | 5836 | 4056 | 309 |
still intriguing, and even DNA/RNA nonlethal damage was questioned. Schmitt et al demonstrated that PTH pulsatility, but not calcium sensitivity, is restored in fresh autotransplanted parathyroid, possibly caused by a deficient expression of calcium-sensing receptor. Analysis of calcium-sensing receptor expression in cryopreserved tissue may clarify this question. The absence of a clear relationship of graft and systemic levels of PTH seen in these cases is also remarkable.

Another point to address is the possible interference of PTH fragment 7–84, which may present crossreactivity in many PTH assays. This fragment is nearly as large as intact PTH, but it seems to block the hypercalcemic action of the intact molecule. In patients with normal PTH levels, but still requiring calcium supplements to maintain eucalcemia, one may question if fragment 7–84 is not interfering.

In conclusion, parathyroid tissue cryopreservation may revert the hypoparathyroid state after total parathyroidectomy in renal patients, even after long-term storage. Cryopreservation seems advisable in the surgical management of all cases of parathyroid hyperplasia, should the need to correct postoperative persistent hypoparathyroidism arise.

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REFERENCES