



Replantation of Cryopreserved Human Parathyroid Tissue

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Twenty-five patients with permanent postoperative hypoparathyroidism received cryopreserved parathyroid autografts. Twelve patients had undergone cervical re-operations due to persistent or recurrent hyperparathyroidism and 10 patients had malfunction of a fresh autograft after total parathyroidectomy. Hypoparathyroidism occurred in 2 patients after subtotal parathyroidectomy and in 1 after the resection of a solitary adenoma following previous thyroid resection. The viability of the tissue was examined histologically prior to replantation in 22 patients and the amount of tissue needed for transplantation was determined by the ratio of necrotic cells vs. viable cells in the material. The patients were examined between 6 months and 125 months (median: 40 months) after replantation. Pre-operatively each patient required high doses of calcium and vitamin D metabolites to establish normocalcemia. This medication was reduced postoperatively, with 16 patients requiring no supplemental treatment. Nine patients still needed low doses of calcium and/or vitamin D. At follow-up all patients were free of hypocalcemic symptoms. Our results demonstrate that replantation of autologous cryopreserved parathyroid tissue is safe and effective therapy for permanent postoperative hypoparathyroidism. Thus, we regard it as an essential part of today's parathyroid surgery.

Hypocalcemia occurs frequently following parathyroid surgery, but usually disappears within a few days or weeks. In contrast, permanent hypoparathyroidism is a severe complication after parathyroidectomy. It can not be treated by substituting the deficient hormone like other endocrine organ hypofunctions, e.g., hypothyroidism. Usually the patients must take oral calcium and vitamin D lifelong in order to maintain normocalcemia. This sometimes presents a problem with patient compliance. Despite this medical treatment, bone metabolism is disturbed. Long-term absence of parathyroid hormone (PTH) leads to osteoporosis even in previously normal bone due to decreased function of osteocytes. There is only a minimal activity of these cells because of the lack of PTH [1, 2]. Clinically this results in bone pain, pathological fractures, and reduced bone healing. These effects of permanent hypoparathyroidism are even more severe in a bone altered previously by hyperparathyroidism (HPT).

In 1977 Wells and coworkers [3] introduced replantation of autologous cryopreserved parathyroid tissue as an operative treatment of hypoparathyroidism. The most striking advantage

is the re-establishment of normal calcium metabolism by PTH regulation. Also the patients no longer require periodic blood tests and expensive lifelong medication. Furthermore, there are no signs of increased neuromuscular irritability. Surgical therapy is the method of choice over medical treatment. Despite these advantages, surgical treatment of permanent postoperative hypoparathyroidism has not been as widely accepted as one might expect. The primary reason is the sophisticated and expensive technique of the original method of cryopreservation published by Wells and associates [3].

We developed simpler methods for cryopreservation and storage of the tissue and demonstrated its identical viability compared to the original method [4, 5]. Another reason for the poor utilization of this method might be the poor graft function of cryopreserved tissue, compared to that of transplanted fresh tissue. While fresh autografts will function in >90% of the cases [6, 7] these results could not be achieved using cryopreserved tissue. Four different groups report a total of 27 patients who had received autologous cryopreserved parathyroid tissue. Only 16 (59%) patients developed normal function of the grafts [8-11]. In our opinion the high failure rate is caused by partial necrosis of cryopreserved tissue [12]. However, this problem was not investigated in the 27 patients reported. The patients received the same amount of tissue which would be considered sufficient when grafting fresh parathyroid tissue. According to the results of our earlier investigations, there is evidence that comparable functional results can be achieved when cryopreserved or fresh tissue is autografted if the rate of necrosis in cryopreserved tissue is balanced by transplanting more than 20 cryopreserved tissue particles [12, 13]. In the following we report our updated experience.

Patients and Methods

Twenty-five patients received cryopreserved parathyroid autografts between March, 1980 and March, 1990. During this period a total of 621 patients underwent parathyroid surgery at our institution (417 for primary HPT, 204 for secondary HPT). There were 12 men and 12 women (age 24 to 75 years, median: 44 years) and a male toddler of 18 months who received autografts. Eleven patients had primary HPT and 14 patients had secondary HPT. The most frequent cause of postoperative hypoparathyroidism was cervical re-operations (12 patients) for

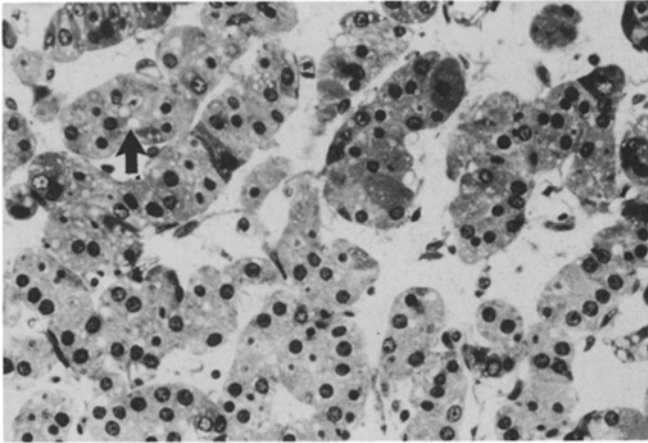


Fig. 1. Histological examination of cryopreserved human parathyroid tissue. Most cells are well preserved with only a slight increase of reversible vacuolar formation (arrow). (Semithin section, methylene blue; freezing rate -1°C per minute).

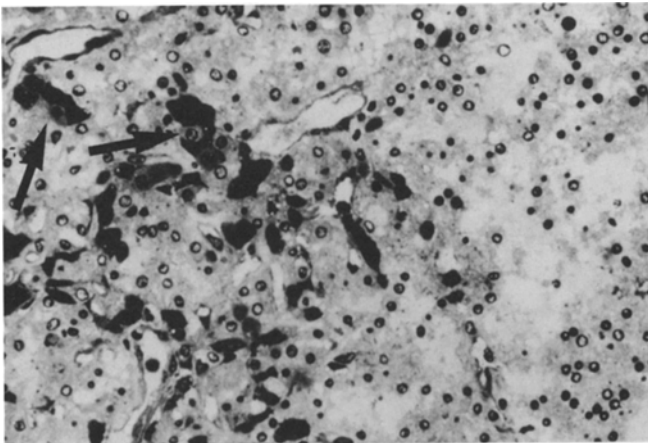


Fig. 2. Cryopreserved parathyroid tissue. Almost all cells show lytic necrosis (light cells), the dark epithelia are condensed and show coagulation necrosis, only a few cells are viable (arrows). (Semithin section, methylene blue; freezing rate -2°C per minute).

recurrent or persistent primary (8 patients) or secondary (4 patients) HPT. Ten patients had to be regrafted due to insufficient function of the previously implanted fresh tissue after total parathyroidectomy for secondary HPT. Two other patients had a subtotal parathyroidectomy for primary HPT and 1 patient had a solitary parathyroid adenoma excised 36 years after bilateral subtotal thyroid resection.

For cryopreservation the parathyroid tumors were cut into cubes of 1 mm to mm in length. These particles were frozen in NUNC® test tubes with 1 ml culture medium (0.8 ml RPMI 1640, 0.1 ml dimethylsulfoxide and 0.1 ml AB blood serum) as described previously [3, 12, 13]. The tissue was stored in liquid nitrogen at -196°C .

Prior to each replantation, except our first 3 patients, 1 vial was thawed for histological examination (Figs. 1 and 2) in a shaking waterbath at 37°C . The particles then were rinsed 5 times in 1 ml RPMI 1640 with 20% AB blood serum to wash out the cryoprotecting DMSO which is cytotoxic above room

temperature. The number of necrotic cells ranged from 0.7% to 52% although the process of freezing and thawing was standardized. Based on the histological data we were able to calculate the number of particles required for successful replantation. Thus we replanted as much functioning tissue as there is found in 20 particles of fresh tissue consisting of viable cells only. To balance the individual number of cell necrosis, 23 to 44 particles (average: 30) were replanted in our patients. In our first 3 patients the histological examination was done retrospectively since we had not known the importance of the histological result for successful replantation at the time they received their cryopreserved tissue. In these patients only 20 particles had been used for autotransplantation. Retrospectively the percentage of cell necrosis in the tissue of these patients ranged from 4% to 20%.

For replantation the tissue was thawed in the same technique as described above. The replantation site was the bending musculature of a forearm. The particles were transplanted into individual muscle pockets. Each pocket was closed by a non-resorbable suture or a metal clip.

In each case the indication for replantation of cryopreserved autologous parathyroid tissue was permanent postoperative hypoparathyroidism. This diagnosis was made in primary HPT when the extirpation of at least 4 parathyroid glands in 1 or more operations in the neck had been documented and when PTH was not detectable in the circulation. The time between parathyroidectomy and replantation of cryopreserved tissue was 5 months in average (range, 0.5 to 15 months). In 10 patients fresh parathyroid autografts after total parathyroidectomy were assumed to be malfunctioning if the patients continually required high doses of calcium and vitamin D metabolites beyond 6 months postoperatively. Cryopreserved tissue was replanted in these cases after an average of 12 months (range, 2 to 84 months). In 4 of these 10 patients early replantation (within 2 to 4 months) was required due to osmotic diarrhea caused by oral calcium medication. One of these 10 patients had a normal functioning autograft for 4 years, but then developed increasing insufficiency of the graft, requiring replantation of cryopreserved tissue which had been stored in liquid nitrogen for 84 months at the time of replantation.

In the 2 patients who had subtotal parathyroidectomy for primary HPT, replantation was performed after 23 months and 36 months. At the time of replantation the required daily calcium intake ranged between 2 g and 3 g, the dose of vitamin D metabolite between 1 mg and 2 mg to achieve a normal serum calcium level. Thus PTH secretion of the cervical remnant would not have been expected. The patient who had thyroid surgery prior to the extirpation of a solitary parathyroid adenoma was replanted after 11 months. At that time he was on 2 g to 3 g calcium gluconate and 50 g ergocalciferole daily, still suffering from paraesthesia.

Follow-up examinations of these 25 patients were performed 6 months to 120 months (median: 40 months) postoperatively. The main criteria for judging success or failure of the cryopreserved autografts were dosage of the required calcium and/or vitamin D, serum calcium levels, as well as clinical signs of hypocalcemia. Selective venous sampling from both arms for PTH detection in patients after total parathyroidectomy and autotransplantation did not prove to be reliable in our hands [14].

Table 1. Total daily calcium and vitamin D intake in 25 patients preoperatively and at the time of follow-up examination, 3 to 120 months (average: 40 months) after parathyroid autograft.^a

Medication	Pre-operative	Follow-up examination
Oral calcium	113 g	8 g
median (range)	5 (2-10) g	1.5 (0-3) g
Calcitriol	62.5 g	3 g
median (range)	2 (0-3) g	0.5 (0-2) g
Calcium gluconate	17.5 g	3 g
median (range)	1 (0-6) g	0.5 (0-1.5) g
With calcium lactate	11.25 g	2.1 g
median (range)	0.7 (0-4.2) g	0.35 (0-1.05) g
And ergocalciferol	4.625 mg	0.375 mg
median (range)	0.25 (0-1.5) mg	0.125 (0-0.375) mg
Dihydroxycholesterol ^b	1 mg	0.5 mg
Hydroxycholecalciferol ^b	0.125 mg	–
Cholecalciferol ^b	0.0125 mg	–

^aSixteen of 25 patients did not require any medication at the time of follow-up examination.

^bOne patient.

Results

Before replantation all 25 patients required high doses of supplemental therapy. The total daily medication of the 25 patients prior to replantation is shown in Table 1. With this medication the serum calcium level ranged from 1.8 mmol/l to 2.5 mmol/l (median: 2.1 mmol/l). Twenty patients considered their life disturbed by frequently recurring paraesthesia; tetanic attacks were rarely reported.

After replantation the medication had to be continued from 1 month to 10 months (median: 6 months) in the 22 patients who received >20 particles of parathyroid tissue (necrosis-rate corrected). With our first 3 patients who received only 20 particles because their tissue had not been examined histologically prior to replantation, the period of continuing medication lasted from 6 months to 21 months. In all 25 patients, the medication could be reduced in increments to lower levels or completely discontinued, depending on the serum calcium concentration. At the time of the follow-up examination, 16 of 25 patients were normocalcemic and free of symptoms of hypoparathyroidism without supplemental therapy. Nine patients still required oral calcium or vitamin D (range, 0.5 mg dihydroxycholesterol only and 2 g calcium combined with 2 mg calcitriol). The doses of supplemental treatment required postoperatively in all patients are shown in Table 1. Calcium levels of these 9 patients were 1.8 mmol/l to 2.3 mmol/l (average 2.1 mmol/l). Replantation in these patients had been performed 6 months to 52 months prior to follow-up examination and all patients were free of symptoms of hypoparathyroidism.

Discussion

Our results show that surgical treatment of permanent postoperative hypoparathyroidism is reliable. In all patients the autografts functioned and most of the patients did not require any medication. In the patients who still required oral calcium and/or vitamin D medication the dose was clearly reduced when compared to the pre-operative dose. All patients felt a signifi-

cant increase in quality of life postoperatively, especially due to the cessation of paraesthesia and tetanic attacks.

The indication for replantation of cryopreserved autologous parathyroid tissue is symptomatic permanent postoperative hypoparathyroidism. In cases of mild or even asymptomatic hypocalcemia which can be sufficiently treated by low dose supplemental treatment, we would not recommend autotransplantation.

The period of time between parathyroidectomy and replantation can not be defined generally. It depends on the type of operation performed and if the patient suffers from renal insufficiency. After subtotal parathyroidectomy, hypocalcemia is seen frequently for several weeks or months. It can be treated conservatively. The severity and the duration of postoperative hypocalcemia correlates with the degree of renal osteopathy. If, however, 6 months postoperatively the patient still requires high dose substitution of calcium and vitamin D, we assume permanent hypocalcemia, requiring replantation. We recommend the same time period after total parathyroidectomy and autotransplantation of fresh tissue, a therapy we prefer in cases of secondary HPT. The postoperative recalcification of the skeleton may last several months and during this time the dose of calcium and vitamin D medication required may not change and would not lead to replantation of autologous tissue [9]. With complications of the conservative treatment (e.g., osmotic diarrhea with adverse effects on calcium resorption) which would require an increase of the medication, we recommend earlier replantation.

Following cervical re-operations the replantation should also be delayed for 6 months since hypocalcemic patients may develop normal parathyroid function. In selected cases earlier replantation may be necessary. This includes patients with a proven aparathyroidism after the extirpation of 4 parathyroid glands, undetectable PTH levels, and symptomatic hypocalcemia despite high dosage substitution therapy. This strategy explains our rather low average waiting time of 5 months after re-operation for primary HPT.

Our results are rather encouraging and are based on the histological examination of the tissue prior to replantation which enabled us to determine the quantity of tissue needed for successful replantation. If the number of necrotic cells was not taken into account, the consequence was a delayed and inadequate function of the replanted tissue, which might be the cause of the results reported from other groups [8–11]. Fresh tissue contains almost no necrotic cells and 20 particles are adequate to achieve a normal parathyroid function [4, 12]. With the use of cryopreserved tissue with a high percentage of cell necrosis, 20 particles may be insufficient. By balancing the rate of necrosis, however, results comparable to those after replantation of fresh tissue can be achieved. If the morphometric procedure of determining the necrotic cell ratio in semi-thin slices [12] can not be performed, the replantation of 30 particles of cryopreserved tissue may be sufficient. In our experience this quantity is enough to achieve normal parathyroid function in most patients.

In general, cryopreservation of parathyroid tissue is required in those patients who are at risk for permanent postoperative hypoparathyroidism after parathyroidectomy (Table 2). According to our experience, we see the indication especially in patients undergoing cervical re-operations. In this group the

Table 2. Indications for cryopreservation of parathyroid tissue.

Re-operation for recurrent or persistent hyperparathyroidism
Subtotal parathyroidectomy
Total parathyroidectomy with autotransplantation of fresh tissue
Parathyroid surgery following thyroid resection

possibility of persistent postoperative hypocalcemia is 20% to 50% [7]. Even after subtotal parathyroidectomy or total parathyroidectomy with autotransplantation of fresh tissue for secondary HPT, permanent postoperative hypoparathyroidism occurs in about 10% of patients [7]. Cryopreservation is also indicated in cases of parathyroid surgery following thyroid resection. The situation is comparable to re-operation for persistent or recurrent HPT unless it is obvious that functioning parathyroid tissue remains in the neck. According to our experience, permanent hypocalcemia is seen in about 10% of these operations. Cryopreservation is not necessary in patients undergoing primary extirpation of a solitary adenoma in the neck, the most common parathyroid operation.

Utilizing these indications implies that parathyroid tissue is much more often cryopreserved than replanted. However, cryopreservation provides an additional and safe option for the patient if permanent postoperative hypoparathyroidism occurs. Therefore, cryopreservation should be regarded as an essential part of today's parathyroid surgery.

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Résumé

Vingt-cinq patients ayant une hypoparathyroïdie postopératoire ont reçu une autogreffe conservée par le froid. Douze de ces patients avaient eu des réopérations en rapport avec une hypoparathyroïdie persistante ou récidivée. Chez 10 patients, l'autogreffe pratiquée pendant la parathyroïdectomie primitive ne fonctionnait pas. Chez deux patients, l'hypoparathyroïdie était en rapport avec une parathyroïdectomie subtotale, et dans un cas, enfin, l'hypoparathyroïdie était le résultat de la résection d'un adénome solitaire chez un patient déjà thyroïdectomisé. La viabilité des tissus a été déterminée histologiquement avant la réimplantation chez 22 patients et la quantité de tissu nécessaire a été calculée selon le rapport cellules nécrotiques/cellules viables dans le transplant. Les patients ont été revus entre 6 et 125 (médiane: 40) mois après la réimplantation. Préopératoirement, tous les patients avaient besoin de hautes doses de calcium et de vitamine D pour garder une normocalcémie. Après l'opération, on a pu réduire ces doses et les supprimer complètement pour 16 d'entre eux. Les neuf autres patients ont toujours besoin de petites doses de calcium et/ou de vitamine D. Tous les patients sont sans signes d'hypocalcémie. Nos résultats montrent que la réimplantation d'autogreffe de tissu parathyroïdien, conservé au froid, est sûre et efficace dans le traitement de l'hypoparathyroïdie post-opératoire permanente. Nous la considérons comme une partie essentielle du traitement chirurgical de l'hypoparathyroïdie.

Resumen

Veinticinco pacientes con hipoparatiroidismo postoperatorio permanente recibieron autoinjertos de paratiroides criopreservada. 12 pacientes habían sido sometidos a reoperaciones cervicales debido a hiperparatiroidismo persistente o recurrente, 10 pacientes presentaron mal función del autoinjerto fresco después de paratiroidectomía total. Se presentó hipoparatiroidismo en dos pacientes después de paratiroidectomía subtotal y en uno después de resección de un adenoma solitario después de resección tiroidea previa. La viabilidad del tejido fue examinada histológicamente antes de la replantación en 22 pacientes y la cantidad de tejido necesaria para trasplante fue determinada por la relación entre las células necróticas y las células viables presentes en el material. Los pacientes fueron examinados entre 6 y 125 (promedio 40) meses después de la replantación. Preoperatoriamente cada paciente requirió altas dosis de calcio y de metabolitos de vitamina D para lograr normocalcemia; esta medicación fue reducida en el postoperatorio, con 16 pacientes libres de tratamiento suplementario. Nueve pacientes todavía requerían bajas dosis de calcio y/o vitamina D. En el seguimiento la totalidad de los pacientes se encontraban libres de síntomas de hipocalcemia. Nuestros resultados demuestran que la replantación de tejido paratiroideo autólogo criopreservado es una forma de terapia segura y eficaz para el hipoparatiroidismo postoperatorio permanente. Por consiguiente, la consideramos como una parte esencial de la cirugía paratiroidea actual.

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