CASE REPORT

Alleviation of exogenous insulin requirement in type 1 diabetes mellitus after immunoablation and transplantation of autologous hematopoietic stem cells

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KEY WORDS

bone marrow transplantation, type 1 diabetes mellitus

ABSTRACT

An essential component of type 1 diabetes mellitus is autoaggression of the immune system against insulin-secreting pancreatic cells. It is thought that early destruction of the autoaggressive mechanism prior to the complete damage of β cells should halt this process. In a 28-year-old male patient with a 4-week history of type 1 diabetes mellitus, three courses of plasmapheresis had been performed before cyclophosphamide, 2 g/m^2 body surface area, was administered and hematopoietic cells were obtained. Six weeks after the diagnosis, 4 doses of cyclophosphamide 50 mg/kg body weight were again administered together with antithymocyte globulin, and autologous hematopoietic cells were transplanted. The procedure was associated with no significant side effects. Insulin requirement started to drop from the first course of plasmapheresis, and the patient has remained normoglycemic with no need of exogenous insulin or other hypoglycemic agents since the third week after the procedure, which has been 5 months until publication of this report. Independence from exogenous insulin is associated with the implemented therapy (a gradual decrease in insulin requirement has been observed after consecutive stages of the immunosuppressive treatment, with total discontinuation after bone marrow transplantation). The course of the disease and the type of treatment may suggest that such medical procedures could eliminate autoaggressive mechanism in diabetes and prevent further degeneration of insulin-producing cells, thus becoming a new therapeutic option for patients with type 1 diabetes mellitus.

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INTRODUCTION Type 1 diabetes mellitus is a metabolic disease in which autoaggression of the immune system against pancreatic β cells is the key pathomechanism. Most of β cells had already been damaged at the time of diagnosis, but there still existed a pool of approx. 20–40% of active cells which could resume insulin production provided that destruction mechanism was under control.[1] The unaffected pancreatic cells would probably secrete enough insulin to maintain plasma glucose within normal range as long as the process of autoaggression was eliminated. Immunoablation with transplantation of autologous hematopoietic stem cells is a medical procedure which stops autoaggressive immunological mechanisms (and the whole immune system). It has been first tested in diabetics in Brazil.[2] Fifteen patients underwent treatment with high doses of immunosuppressants and the subsequent autologous hematopoietic cell transplantation. Long-standing independence from exogenous insulin with good plasma glucose control has been achieved in 80% of patients enrolled in the study (mean follow-up of 24 months,

TABLE 1 Basic laboratory analyses concerning pre- and posttransplantation period in a patient with type 1 diabetes mellitus after autologous hematopoietic stem cell transplantation

	Diagnosis	Mobilization	Transplantation	Insulin withdrawal (day 24 + after transplantation)	3 months after transplantation
fasting plasma glucose	420	160	140	87	90
exogenous insulin requirement (UI/kg BM)	1.25	0.47	0.36	0	0
HbA_{1c} (normal range: 4.5–5.7%)	13.8%				5.2%
plasma C peptide (fasting/1 h after stimulation; ng/mL)	0.3/0.6		1.15/3.05	1.0/2.0	2.99/4.14

maximum follow-up of 48 months [Voltarelli, unpublished data]). There were no significant adverse effects of the treatment (no death noted, one patient presented with pneumonia). Such positive results together with a low risk associated with autologous hematopoietic cell transplantation have prompted us to undertake a similar therapy. We hereafter present the first Polish case of a successful type 1 diabetes mellitus treatment with autologous hematopoietic stem cell transplantation.

A 28-year-old patient with a short history of type 1 diabetes mellitus was admitted to the Department of Internal Medicine, Endocrinology and Diabetology of the Central Hospital of the Ministry of Interior and Administration in Warsaw. The patient presented typical diabetic symptoms: polydypsia, polyuria, weight loss of about 8 kg within 1 month, and no ketoacidosis. The autoimmune pathomechanism of his diabetes had been confirmed in anti-glutamic acid decarboxylase (anti-GAD) antibody testing, yielding the result of 5.41 U/mL (normal values <1.0 U/mL). On admission, his plasma glucose was 420 mg/dL, and HbA_{1c} of 13.8% (HbA_{1c} normal range, 4.5–5.7%). The patient had been treated with intensive insulin therapy using aspart and neutral protamine hagedorn insulin. Satisfactory glucose control had been achieved gradually. The patient was then transferred to the Department of Hematology, Oncology and Internal Medicine of the Medical University of Warsaw for hematopoietic stem cell transplantation. The patient had no past medical history, his general outcome was 0 on the WHO scale, body mass index (BMI) = 20.7 kg/m^2. Plasma C peptide was confirmed before the procedure (fasting plasma C peptide was 0.3 ng/mL, and 0.6 ng/mL 1 hour after a standard meal; normal range: 1.77–4.68 ng/mL). In HLA testing, DRB1 04, 07; DQB1 0304, 0202 were found, one of which (DRB1 04) is more often observed in type 1 diabetes mellitus.[3] The study was approved by the Bioethics Committee of the Medical University of Warsaw. After giving his written, informed consent, the patient was qualified for immunoablation followed by autologous hematopoietic stem cell transplantation. Before that, on April 9–12, 2008, three courses of plasmapheresis had been performed to immediately decrease the patient's anti-β cell antibody titer.[4]

Hematopoietic stem cell mobilization From April 14, 2008, the patient had received 2 g/m^2 of cyclophosphamide, and from April 15, 2008, 10 μg/kg body mass (BM) of granulocyte colony-stimulating factor (G-CSF) (filgastrim 2 × 48 mln units). On April 22, 2008 leukapheresis was performed and 3.03×10^6 $CD34^+$ cells/kg BM were drawn. No significant adverse effects were noted upon conditioning, and the granulocyte count was $2.9–17\times10^9$/L.

Transplantation On May 1, 2008, a pre-transplantation immunoablative conditioning scheme was started with a daily dose of 50 mg/kg BM of cyclophosphamide on days 5, 4, 3, 2, and 3 mg/kg BM of antithymocyte globulin (ATG Fresenius) on day 5, and in a higher dose of 7 mg/kg BM on days 4, 3, 2, 1. No significant complications were observed during conditioning. On May 6, 2008, the patient was given an autologous preparation of 3.03×10^6 $CD34^+$ hematopoietic stem cells/kg BM. In the peritransplantation period the patient received antiviral (acyclovir), antibacterial (ciprofloxacin), and antifungal (fluconasol) prophylactic treatment, followed by anti-*Pneumocystis jiroveci* prophylactic treatment (cotrimoxazol) after transplantation. On day + 5, a single dose of 5 μg/kg BM of G-CSF (filgastrim 1 × 48 mln units) was added. Neutropenia of $<0.5\times10^9$/L remained for 10 days – the patient became feverish a few times; empiric antibiotic-therapy was administered (cefepime and vancomycin; blood culture tests were negative). No marked abnormalities in laboratory tests were found – a slight, expected worsening occurred during conditioning preceding transplantation (creatinine level – median of 0.6 mg/dL [normal range 0.6–0.8 mg/dL], aspartate transaminase activity – median of 41 U/L [normal range: 18–106 U/L], alanine transaminase activity – median of 65 [normal range: 31–155], plasma bilirubin – median of 1.36 mg/dL [normal range: 0.3–2.4 mg/dL]). On the 13th day after transplantation the granulocyte count exceeded 0.5×10^9/L. The patient was discharged on day 16 after transplantation.

The insulin requirement was 0.47 IU/kg BM at the beginning of the treatment (mobilization), 0.36 IU/kg BM at the day of transplantation, and decreased to 0.17 IU/kg BM on the engraftment day (when the granulocyte count reached $>1\times10^9$/L for 3 consecutive days) (TABLE 1). For a few weeks after engraftment the patient had received 0.11 IU/kg BM of insulin to ensure maximal protection for the rest of β cells. On day

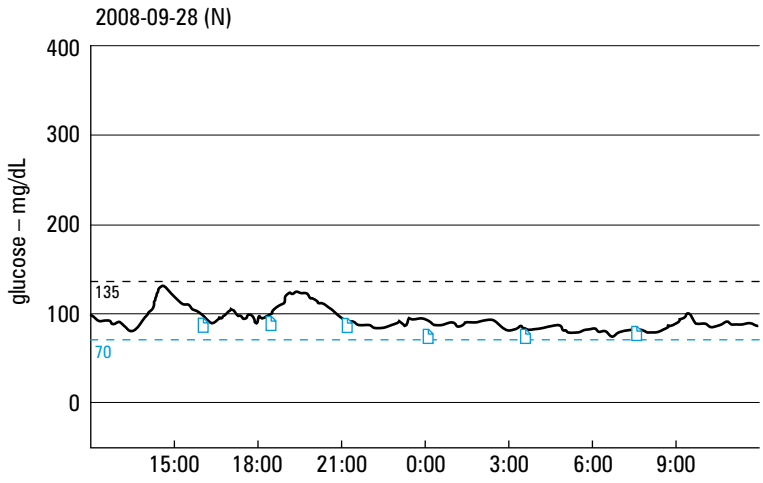

FIGURE 24-hour registration of the patient's plasma glucose

24 after transplantation (June 30, 2008) exogenous insulin treatment was discontinued. During the following days fasting plasma glucose was normal, but a few episodes of hyperglycemia were observed, with maximal plasma glucose of 125 mg/dL (May 25, 2008). Plasma C peptide levels measured in fasting state and 1 hour after a standard meal were 0.3 ng/mL and 0.6 ng/mL, respectively, (normal range: 1.77–4.68 ng/mL) on diagnosis, 1.15 ng/mL and 3.05 ng/mL before transplantation, 1.0 ng/mL and 2.0 ng/mL after insulin treatment withdrawal, and 2.99 ng/mL and 4.14 ng/mL 3 months after bone marrow transplantation (BMT). Three months after transplantation HbA_{1c} was of 5.2% (**TABLE 1**). On discharge, continuation of diabetic diet was recommended due to a possible excretory dysfunction of pancreatic β cells.

Five months has passed since transplantation at the time of submitting the paper for publication, and the patient is in no need of exogenous insulin intake; his plasma glucose remains within normal range. We have not tried any oral antidiabetic drugs stimulating insulin secretion or drugs inhibiting insulin resistance (BMI 20.7 kg/m^2).

Continuous glucose monitoring system On September 26–28, 2008, plasma glucose monitoring using continuous glucose monitoring system (CGMS) was performed. Mean glucose level was 94 mg/dL (minimal 53, maximal 152 mg/dL) in 72-hour monitoring. Minimum and maximum plasma glucose levels were measured immediately after the system was connected. For the remaining monitoring time (99% of the time) glucose level stayed within normal limits and mean plasma glucose was 94 mg/dL (minimum – 76 mg/dL, maximum – 133 mg/dL). The **FIGURE** presents 24-hour glucose level record.

Intravenous glucose tolerance test On September 25, 2008, an intravenous glucose tolerance test (IVGTT) was performed upon i.v. injection of 20 g glucose. The test results are presented in **TABLE 2**.

DISCUSSION The present case has been the first successful Polish attempt to achieve remission in the early phase of type 1 diabetes mellitus following immunosuppressive treatment and the subsequent autologous hematopoietic stem cell transplantation. The method involved destruction of the patient's immune system and also the autoimmune process which is the main pathomechanism in type 1 diabetes mellitus. From the theoretical point of view (it has not been confirmed empirically yet), this method of treatment could only be successful in patients with a short history of the disease, when there are at least some β cells still functioning. As soon as the autoaggressive mechanism is stopped, pancreatic cells might be able to resume secretion of sufficient amounts of insulin to maintain normal glucose level. The patient must fulfill two conditions to be scheduled for transplantation in accordance with our medical protocol:

1 C peptide positive plasma to confirm that endogenous insulin secretion is preserved

2 autoimmune basis of the disease (antibody testing, e.g. anti-GAD).

In our case, the course of the implemented treatment has confirmed what had previously been achieved in Brazil[2]; at the same time it has been the first case described by other authors. At this point in time (several patients have undergone the therapy so far), it is hard to say how long the achieved result will be maintained. Several factors other than efficacy of the procedure may play an important role here, e.g. the number of remaining β cells after treatment (which depends on the time of the diagnosis: the later the diagnosis is made, the fewer the cells will be present), to what degree the patient follows lifestyle recommendations after the procedure (being overweight, risk for type 2 diabetes mellitus) and the chance of recurrent autoimmune disorder. The currently available data (very scarce until now) suggest that the recurrence rate is about 20%, and it is estimated on the basis of the Brazilian data and other studies concerning autologous BMT in different autoimmune diseases.[6]

This results from the fact that constraining stem cells to recreate the immune system does not eliminate the patient's genetic susceptibility to diabetes. Genetic predisposition is responsible only for the risk of developing type 1 diabetes mellitus and the health risk in siblings is 27.3% in monozygotic twins.[5] This means that the remaining 70% of the risk is related to abnormalities in individual development of the immune system after birth.

The risk of death is the most serious complication of transplantation. It is relatively low in autologous BMT. Autotransplantations have routinely been performed in patients aged <70 years with a spectrum of different diseases, and in multiple sclerosis patients, who constitute a group most similar in terms of demographics to type 1 diabetes mellitus, the risk of death on transplantation is close to 0%; since 2000, no cases of death associated with the procedure have been recorded in Europe (n = 63).[6] It will be possible to estimate

TABLE 2 Intravenous glucose tolerance test

time (min)	30	20	10	0	1	3	10	15	20	25	60	90	120
plasma glucose mg/dL (normal range: 70–110)	84	84	89	87	171	170	185	177	175	174	143	122	102
insulin μIU/L (normal range: 2.6–24.9)	2.17	1.72	1.4	2.57	3.99	6.22	6.64	6.49	6.53	5.85	5.16	6.27	4.75

the actual rate of complications following autologous stem cell transplantation in type 1 diabetes mellitus when such procedures are performed in a larger number of patients. To date, no death has been reported (in the data published by the Brazilian group), which however does not exclude such a risk. In each patient the danger of possible post-procedural complications always has to be confronted with the risk of major complications of type 1 diabetes mellitus such as hypo- and hyperglycemia, potentially severe organ and vascular complications, and premature death. Although autologous stem cell transplantation could lead to severe complications, it can be a medical alternative for patients with a short history of type 1 diabetes mellitus.

Our patient is another one (at least the 19th including all those who had transplantations done in Brazil [Voltarelli, unpublished data]) to have achieved remission of type 1 diabetes mellitus after the treatment. We sometimes observe spontaneous remission in patients with type 1 diabetes[7,8], but it happens rather rarely and lasts for a limited period of time. In a group of patients that had been excluded from our experiment, we did not note any case of a spontaneous remission. Furthermore, the current study showed a strong relationship between a decrease in exogenous insulin requirement and the immunosuppressive treatment in our patient. However, the greatest change in insulin requirement occurred after BMT – it dropped from 26 to 12 units within 6 days. Moreover, after upper respiratory tract infections no recurrence of diabetes was observed, which is usually not the case in spontaneous remission (IVGTT and CGMS results after infections). This could further confirm that elimination of the autoaggressive process prolongs the period of remission.

IVGTT demonstrated that the first phase of insulin secretion was not restored after treatment while the second phase was preserved. It is an expected result, because decreased insulin secretion in the first phase is the first metabolic disturbance in natural history of type 1 diabetes mellitus, confirming dysfunction of pancreatic β cells which occurs before clinical symptoms appear. We do not expect our patient to immediately regenerate the lost β cells since at the time of diagnosis as much as 60–80% of his β cells had been destroyed. What we are hoping for instead is to maintain the patient's plasma glucose within normal range due to the activity of the remaining cells. CGMS and self-control results, as well as the documented HbA_{1c}, show that even though the first phase of insulin secretion is impaired, it is possible to achieve satisfactory results as long as the patient follows a diabetic diet.

If the further course of disease in the described patient leads to stable independence from exogenous insulin, and if similar results are obtained in other patients, the procedure will become a new therapeutic method for early diagnosed type 1 diabetes mellitus. It is not unlikely that immunoablative treatment alone (without transplantation) could have similar results, but the methodological scheme described above was based on the data from the Brazilian center; only plasmapheresis was added according to the recommendations of other authors.

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OPIS PRZYPADKU

Uniezależnienie się od egzogennej insuliny w cukrzycy typu 1 po immunoablacji i przeszczepieniu własnych komórek krwiotwórczych

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SŁOWA KLUCZOWE

cukrzyca typu 1, przeszczepienie szpiku

STRESZCZENIE

Cukrzyca typu 1 jest chorobą polegającą na autoagresji układu odpornościowego skierowanej przeciwko komórkom trzustki produkującym insulinę. Tym samym wczesne zniszczenie mechanizmu autoagresyjnego przed całkowitym zniszczeniem komórek β powinno zatrzymać ten proces. U 28-letniego chorego z cukrzycą typu 1 rozpoznaną 4 tygodnie wcześniej wykonano najpierw trzy zabiegi plazmaferezy, a następnie podano cyklofosfamid w dawce 2 g/m^2 i pobrano komórki krwiotwórcze. Sześć tygodni po rozpoznaniu podano ponownie 4 × 50 mg/kg cyklofosfamidu oraz globulinę antytymocytarną i przeszczepiono pobrane wcześniej komórki krwiotwórcze. W trakcie zabiegu nie wystąpiły istotne powikłania. Zapotrzebowanie na insulinę zaczęło się zmniejszać począwszy od pierwszej plazmaferezy, a od trzech tygodni po zabiegu chory utrzymuje normoglikemię bez podawania egzogennej insuliny i innych leków hipoglikemizujących (dotychczas 5 miesięcy). Uniezależnienie od egzogennej insuliny miało związek z zastosowanym leczeniem (stopniowe zmniejszanie się zapotrzebowania po kolejnych etapach leczenia immunosupresyjnego, z ostatecznym odstawieniem po przeszczepieniu szpiku). Taki przebieg choroby i leczenia może sugerować, że wspomniana procedura lecznicza jest w stanie wyeliminować mechanizm autoagresyjny w cukrzycy, zapobiec dalszemu niszczeniu komórek produkujących insulinę i tym samym stanowić nową możliwość leczniczą dla chorych na cukrzycę typu 1.

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