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Cord blood (CB) stem cells for wound repair Preliminary report of 2 cases

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Abstract

In 2 patients, to promote skin wound/lesion repair we used fibrin-platelet glue combined with HLA compatible (2 mismatches accepted) buffy coats containing CD 34+ cord blood cells.

The fibrin platelet glue was prepared with autologous apheresis platelets and cryoprecipitate. The original product was divided into 3 and 4 aliquots respectively for a correspondent number of applications. At each application, the margins of the lesion were infiltrated with 3 ml of cord blood buffy coat, containing 30×10^3 CD 34+ cells. No graft versus tissue reaction was seen in our patients in a follow-up of 3–7 months.

The level of improvement, scored arbitrarily from 0 to 4, was 3 and 4, respectively.

Our conclusion is that the use of cord blood cells along with fibrin platelet glue is of clinical interest.

Keywords: Cord blood; Progenitors stem cells; Wound repair; Platelet glue; Fibrin glue

1. Introduction

The clinical use of CB dates back to 1989 when the first CB transplant was successfully carried out in a patient with Fanconi anemia [1]. Since then CB has also been used with some success for unrelated transplantation using both HLA identical but also mismatched units [2]. Consequently, cord blood bank systems have been set up both in Europe and in the States [3,4] and CB cells are employed for transplantation both because of their ready availability for patients lacking related and unrelated HLA identical donor and because of the reduced risk of severe acute or chronic GVHD even in the case of 2–3 loci incompatibility. At our immunohematology department, a CB bank is active and close to 1000 typed CB are banked. In our department, a section for the preparation and clinical application of cryo-platelet glue is active as well [5] and, because of the quality of the results obtained over time, the number of requests for application in different disease condition is increasing. The success rate is exceeding 85% with wound healing in patients refractory to conventional

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treatment, surgery included. For the uncommon patients who fail our cryo-platelet glue it is felt that unresponsiveness is due to the lack of specific tissue stem cells or their unresponsiveness to normal appropriate stimuli [6–8] or to the inability of circulating multipotent uncommitted stem cells to reach the place where they have to differentiate [9]. In this line is the report by Badiavas and Falanga who successfully treated chronic wound with bone marrow-derived cells [10]. To face the problem of chronic wounds unresponsive to therapy, cryo-platelet glue included, it was felt by our group that local injection of HLA compatible (2 mismatches accepted) cord blood cells was an appropriate therapeutic attempt.

2. Material and methods

Two patients were enrolled after appropriate written consent after failure of adequate multiple courses of medical and/or surgical treatment. The demographic of these patients is summarized in Table 1.

The autologous fibrin-platelet glue (FPG) was obtained and used as previously described [5]. From each donation 2 aliquots of cryoprecipitate and 4 of dry platelets were obtained. After preparation, 1 aliquot of cryoprecipitate and 3 of platelets were frozen and kept at −80 °C until used. For the preparation of the gel, one aliquot of cryoprecipitate and one of platelets are mixed and 0.5 g of calcium gluconate dissolved in 3–4 vials of Botropase are added. Botropase (Ravizza Farmaceutici S.p.A, Milano, Italia) is a thrombin-like drug commercially available that contains Botroxobin, a Russell's viper venom component, that promotes coagulation through factor X activation and platelet activation. The fibrin-platelet glue is prepared at the bedside in Petri dishes, cut, moulded and used as needed. Cord blood cells collected and stored according to FACHT standards [6] were typed employing SSP/SSO reagents.

For class I the ABC and for class II the DR and DQ loci were typed. When needed, sequencing was carried out using ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). CD 34+ cells in the cord blood units were measured using a Facscan apparatus (Becton–Dickinson, S. Jose, Canada) and reagents. WBC counts were obtained by a cell Coulter (ADVIA™ 120 Hematology system, Bayer, Tarrytown, NY, USA). Sterility was evaluated using BACTEC™ BD Peds Plus/F (Becton–Dickinson, S. Jose, Canada).

Cord blood cells stored in liquid Nitrogen were thawed a +37 °C, centrifuged and the buffy-coat divided into 3 and 4 aliquots respectively for successive weekly applications. Each aliquot containing a minimum of 26×10^3 CD 34+ cells and of 12×10^6 WBC after washing in saline was injected in the margins of the skin lesion, followed by the application of the FPG.

3. Results

Two consecutive patients were included in our preliminary study, after they failed standard conventional therapies for longer then 1 year. In Table 2 the clinical results of treatment are reported along with the treatment modalities. Patient number 2 had also failed with repeat cryo-platelet gel application. When included in the study, the skin lesion was clearly infected and no granulation tissue was seen. After the second application of CB followed by FPG the lesion was cleared with no

Table 1
Characteristics of the 2 patients submitted to CB

Patients	Age/Gender	Diagnosis	Previous therapies	Size (cm) of the skin lesion	Number of HLA mismatches
1	82/M	Trauma	Debridement	11×7	1 in locus C
2	76/F	Radiation injury	Local cryo-platelet-gel	8×3	1 in locus B and 1 in locus C

Cryo-platelet glue treatment.

Table 2
Treatment modality and clinical results

Patient	CB Applications	No of CD $34+\times10^3$/appl	No of WBC$\times$ 10^6/appl	FPG application	CR (scored 0–4)
1	3	30	14	3	4
2	4	26	12	3	3

CB: cord blood; FPG: cryo-platelet gel; CR: clinical result: 0 no improvement, 1 clear but modest, 2 partial, 3 almost complete, 4 complete resolution of the skin lesion.

sign of infection. In this as in the other case improvement was anticipated by increased vascularity of the perilesional skin and formation of granulation tissue.

No sign of graft versus tissue reactions was seen in a follow-up period of 3–7 months.

4. Comment

Cell-based therapies will benefit from a source of pluripotent stem cells alternative to the autologous ones, whose collection has potential limitations such as the age of the patient, his cardiac condition and the need for general anaesthesia for bone marrow aspiration. Cord blood cells are known to contain pluripotent cells which can differentiate into different cell lines in the presence of lineage specific induction factors. Cord blood cells are collected easily and are ready for clinical use when an appropriate request is made. Their responsibility in determining graft versus tissue reaction is modest and, given the reports of their plasticity, it is conceivable that they can produce new skin cells or rejuvenate senescent fibroblasts unresponsive to locally produced or external administration of transforming growth factors or cytokines. In keeping with this is the very recent report of the successful use of bone marrow-derived cells in the treatment of non healing chronic wounds in 3 patients with wounds of more than 1 year duration in whom there was histologic evidence suggesting engraftment of applied autologous cells [10]. The 2 patients in our case list got resolution of their wounds even though patient no. 2 had been included in a report from our group as the sole negative result with FPG [5]. Of course, there are limitations in our very experimental study. We have not been able, presently, to demonstrate that there was a change from CD 34+ cells injected into skin progenitor cells. We have only seen a dramatic increase in cellularity and in immature cells in the site of the cord blood injections. We cannot state if a rejuvenation of senescent local fibroblasts took place instead of a colonization by cord stem cells secondary to their plasticity. We have only shown what the feasibility of this treatment should be if it is not complicated by graft versus tissue reactions when up to 2 HLA mismatches are accepted between the cord blood cells and the recipient. We have also shown that, from a clinical point of view, optimal results are met even in patients unresponsive to conventional therapies and to the local application of cryo-platelet gels. On the basis of these results we are planning a larger study after an appropriate consent by our local ethical committee.

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