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The Influence of Allogeneic Platelet Gel on the Morphology of Human Long Bones

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A B S T R A C T

The aim of this study is to analyze the morphologic and functional change of human bone defect after its grafting with mixture of platelet gel and autologous cancellous bone. For one year, we have prospectively studied nine consecutive patients, aged 25–73 y, with pseudoarthrosis of long bones, after unsuccessful initial surgeries. We have harvested cancellous bone from patients' iliac crests and mixed with the ABO compatible allogeneic platelet rich plasma (PRP) gel. That mixture has been inserted in the bone defect, and surgically fixated. Radiologically, the defects achieved the bone morphology (the appearance of hazy callus) between 6th and 24th week. The time of functional recovery was varied, between 12 and 40 weeks for partial weight bearing, and between 16 and 48 weeks for free limb mobility and full function of the limb. The overall healing of bone defect was 16 to 36 weeks. Two patients had complications of poor graft ingrowth and one with a reversible postsurgical nerve paresis. On the X-ray scans, solid and fast restoration of bone structure was notable, with excellent bone ingrowth, suitable for full weight bearing. The allogeneic platelet gel had no adverse effects. This method can be used for treating of long bone defects, because of its strong influence on restoration of normal bone morphology. Further investigation is required to establish efficiency relative to other methods.

***Key words**: platelets, bone transplantation, trauma, plasma, bone grafting, morphology*

Introduction

The extensive trauma and subsequent infection can cause large bone defects. Structural properties of diseased bone are presented as a diminished mineralization, lower protein composition, and unnatural distribution of the bone trabecullae. This all leads to brittle bony morphology and inadequate resistance to regular mechanic stress.

The bone defects can be treated by various surgical methods. The most common methods are reconstructions by cancellous bone grafting, and the Ilizarov intercalary bone transport method[1]. The application of these methods results in successful final outcomes. However, un-physiological bone repair, repeated surgical procedures, long hospitalization time, prolonged ambulatory impairment, and high medical costs are still common, with functional and aesthetic con sequences. It is also well known that concomitant diseases, such as diabetes, can to some extent impair the healing of fractures, which has been extensively studied in animals and less well in humans[2].

Recently, growth enhancers such as autologous platelet rich plasma (PRP) in the form of activated platelet gel and recombinant bone morphogenetic proteins (rBMP) have been used with varying results for healing wounds, ulcers, fractures, and in maxillofacial settings[3]. An animal study showed enhanced bone growth when autologous bone was combined with platelet-rich plasma[4]. The healing effects of platelet gel were attributed to the numerous growth factors (GFs) released by the platelets after activation[5]. Some of those identified are: the platelet derived growth factor (PDGF), TGF-α and β (transforming growth factor alpha and beta), EGF (epidermal growth factor), FGF (fibroblast growth factor), IGF (insulin growth factor), PDEGF (platelet derived epidermal

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growth factor), PDAF (platelet derived angiogenesis factor), IL-8 (interleukin-8), TNF-α (tumor necrosis factor alpha), CTGR (connective tissue growth factor), GM-CSF (granulocyte macrophage colony stimulating factor), KGF (keratinocyte growth factor), and Ang-2 (angiopoetin), as reviewed by several authors. The inductive potential of platelet gel in tissue regeneration could also be attributed to its significant antimicrobial activity. Recent studies on patients for the regeneration of long bone and foot and ankle defects have provided promising clinical results when using platelets as a source of GFs. It was demonstrated that with the use of platelet gel as compared to reconstruction with conventional methods, better and stronger bone yield was achieved, and that bone density and growth were enhanced[6].

In the majority of clinical experiments, authors have applied autologous platelets obtained by preoperative apheresis from the peripheral blood of the patient undergoing surgery. However, this may not always be the best solution. In cases of diabetes it has been shown that the release of platelet GFs is decreased in experimental diabetic animals, and that allogeneic PRP as a source of additional GFs can considerably improve diabetic fracture healing[7]. Allogeneic single donor platelet units are easy to obtain, since they are a standard blood bank product; they are highly standardized in terms of platelet content, residual leukocyte and red blood cell content, the centrifugal forces used for their isolation, temperature of centrifugation, techniques of separation and processing and composition of preservative solution. Also, they are available in large quantities and considered safe. Autologous platelet preparations, on the other hand, are subject to enormous variability, which hinders serious studies of their clinical efficacy.

We used the standard blood bank platelet concentrates in the preparation of a graft composed of allogeneic platelet gel mixed with autologous cancellous bone in order to improve the healing conditions in bone defects, which was successfully demonstrated in our pilot clinical case[8]. In the latter case study, we showed that the healing potential of the gel GFs obtained from a high number of allogeneic platelets could be combined with the bone-forming potential of autologous osteogenic and other stem cells from the cancellous bone. We employed the plasticity of the resulting graft mixture for the modeling and all of this contributed to a successful clinical outcome. In the present study, this method was further tested in a series of nine patients.

Patients and Methods

Patients

Here we present the results of a prospective clinical study performed from May 2004 to February 2010 in the University Clinical Centre Ljubljana, Slovenia, in which we have treated defected non-union of long bones using allogeneic platelets as a source of GFs. Nine consecutive patients (3 female and 6 male), age from 21 to 73 years (average 45.9 years), each with a defect of a different long bone (3 femoral, 4 tibial, 1 humeral and 1 ulnar). They had already been unsuccessfully treated with conventional methods in our or other hospitals for at least 9 months (Figure 1). The therapeutic options in these cases had been exhausted. In two of the patients, we have treated osteomyelitis before applying our treatment. We took additional microbiological samples at the time of operation. Two of the patients were insulin independent diabetics.

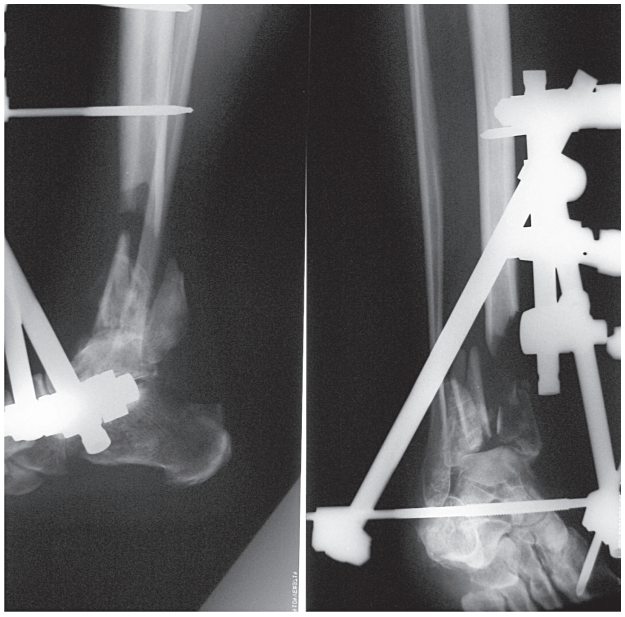

Fig. 1. The bone defect in distal tibia, after open comminuted fracture, reduction, debridement and external fixation.

In our clinical investigation plan, the primary aim was to establish the potency of allogeneic platelet gel added to the transplanted autologous cancellous bone in treatment of post-traumatic bone defect, with a follow-up of one year. The secondary aim was to assess the healing, safety, handling and tolerance of the method, and potential cost benefits.

We have noted all the important parameters in special chart, the clinical and radiological examinations, during the post-operative follow-up of one year. As a survey of the immunological side effects of allogeneic platelets, we performed a screening of HLA antibodies class I, and human platelet antibodies (HPA), before the implant operation and in the third month after the operation.

We prepared the cancellous bone from one or both patients' iliac crests into particles smaller than 5 mm. A standard allogeneic random single donor platelet concentrate (ABO and RhD matched, serologically HIV, HBV, HCV and lues-negative, leukocyte depleted, and irradiated) was added to cancellous bone in equal volume (allogeneic platelet concentrate with approximately 1.4×10^9 mL^{-1} with fibrin glue components for the activation of platelets and polymerization of fibrinogen).

The bone defects were approached through previous surgical incisions after administering a single dose of prophylactic antibiotic. After debridement of the non-un-

ion, we filled the resulting bone defect with a semi-solid, moldable gelatinous graft and stabilized the bone with different types of osteosinthesis (Table 1). We applied suction drainage and closed the wound in layers.

In the follow-up protocol, we assessed the general status after the operation, and the bone configuration with X-ray at 2, 4, 6, and 12 months. We assessed bone remodeling at 6 and 12 months by CT scan. We drew blood samples from each patient at week 14 for the identification of anti-HLA/Class I antibodies and anti-HPA antibodies in order to assess potential immune reactions related to the use of allogeneic platelets. The standard in-house platelet immuno-fluorescence test (PIFT) and antigen capture ELISA test (PAK-12, GTI, Brookfield, USA) were used to screen antibodies[9].

Results

We removed suction drainage on the second or third day after the surgery. The drainage volumes were in average 250±81 mL (200–450 mL). The patients were discharged from the hospitals after 6–8 days, and they have been regularly examined in the outpatients' clinic.

Seven of nine patients have had complete bone ingrowths, with no defect. Different healing times are presented in Table 2.

We have noted major complications during the treatment in three patients (33%); these were consisted of poor incorporation of implant, mental deterioration leading to non-compliance, and radial nerve paresis (one patient respectively). Two of these patients had to undergo further surgery. More detailed data of bone healing are displayed in Table 3. No side effects caused by the implant were observed; no platelet or HLA-class I antibodies were detected in any patient.

The survival of the graft mixture was excellent; most of the volume was preserved and incorporated. The structure of the graft was almost the same as neighboring cancellous bone. The adjacent long bones have presented no structural disintegration. This was critical in the case of distal tibia (patients No 2, 3, 5, and 9), where we have observed diminished incorporation in the distal part, due to the fact that the metaphysis of the tibia is hypovascularized. In the case of femur pseudarthrosis (patient No 6), bone quality was insufficient for the implant to regenerate the whole bone circumference, so healing was prolonged.

Discussion

Long bone defects are a pinnacle of deteriorated morphology of a human bone. It is a culmination of pathological processes that weakened vascular supply. This leads to decreased bone density, lower structural protein content in the bone adjacent to a bone defect.

The treatment of bone defects of long bones after injury is still one of the most difficult tasks in reconstructive bone surgery. The golden standard in bone graft surgery is still the use of autologous bone graft[1]. In certain settings, especially in extensive bone defects, this me-

TABLE 1
FIXATION METHODS USED AND GRAFT VOLUME PER PATIENT

Patient No.	Pseudarthrosis site	Fixation method	Defect size / Graft volume in mL
1	Femur	Internal locking plate	16
2	Distal tibia	External fixator	35
3	Distal tibia	Internal locking plate	45
4	Femur	Tutor brace	15
5	Distal tibia	Internal locking plate	30
6	Proximal femur	Dynamic hip screw with long plate	25
7	Humerus	Internal locking plate	35
8	Ulna	External fixator	30
9	Distal tibia	Internal locking plate	25
		Average graft volume	28.5±9.5

TABLE 2
HEALING TIMES AFTER THE OPERATION FOR SUCCESSFUL CASES

Time in weeks for:	Min	Max	$\bar{X}$	Median
Appearance of hazy callus	6	24	10.0±5.4	8
Partial weight bearing	12	40	22.5±10.4	18
Free mobility and full weight bearing	16	48	31.0±12.2	31
Overall bone healing	16	36	23.0±4.7	24

TABLE 3
DETAILED BONE HEALING DATA

Patient / sex / age (years)	Appearance of hazy callus wks.	Partial weight bearing wks.	Free mobility + full weight bearing wks.	Extent of graft incorporation	Stable bone healing wks.	Complications	Final result 1 y.
1/F/63	8	12	40	Complete	16	None	Healed
2/M/50	8	18	32	Bad prox. ingrowth	24	Left hip fract.	Healed
3/F/49	10	16	–	No distal. ingrowth	24	Reop. pseudart.	Failed
4/M/45	24	32	44	Both ends half	28	None	Healed
5/F/45	8	40	48	Proximally half distally nothing	36, pseudar. reoperat.	Poor compl. + mental disord.	Failed
6/M/73	6	16	24	Complete	24	Short limb	Healed
7/M/33	8	–	16	Complete	16	Radialis paresis	Healed
8/M/21	12	–	16	Complete	16	None	Healed
9/M/34	8	18	30	Complete	24	None	Healed

thod of treatment could be insufficient and could only pose an additional trauma for the patient. Some authors have reported difficulties when treating defected non-unions, such as extremely long healing time and incorporation of the graft, necrosis of the grafts, and reacutisation of infection[10]. Concomitantly, long-term immobilization contributed to the contractures of the joints and soft tissue, and in the long-term perspective, also to the inferior functional and aesthetic results.

Reconstruction by vascularized bone transfer along the Ilizarov intercalary bone transport and cancellous bone grafting has been the most widely used method of treatment for large defected non-unions after injury. There have been several modifications of the Ilizarov method, which retain its versatility, stability and mechanics, but these methods also contribute to a high rate of complications[10]. Long treatment procedures deteriorate also soft tissues, leading to contractures and scars. This influences unnatural position of joints, leading to varus and valgus deviations of adjacent joints. Tissue engineering involves the restoration of tissue structure or function through the use of living cells. The general process consists of cell isolation and proliferation, followed by a re-implantation procedure in which a scaffold material is used. Cell sources can be autologous or allogeneic cells. Autologous cells are usually the better choice, because the allogeneic cells could incite immune rejection by the recipient. Mesenchymal stem cells provide a good alternative to cells from mature tissue and have a number of advantages as a cell source for bone and cartilage tissue regeneration. Some authors report that most tissue engineering applications in the head and neck area would probably involve the use of chondrocytes and osteoblasts along with some type of scaffold material because of the importance of initial support and shaping[11].

Theoretically, the ideal bone graft substitutes should be osteogenic, biocompatible, bioabsorbable, able to provide structural support, easy to use clinically and cost-effective. A composite graft combines an osteoconductive matrix with bioactive agents that provide osteoinductive and osteogenic properties. Bone grafts are used to replace a part of the bony defect or to enhance the healing of a fracture. Because of the inability to procure large quantities of autologous bone and the added morbidity for the patient associated with the autograft donor site, new methods of bone transplant materials have emerged in recent years. The functional properties of bone morphogenetic proteins (BMP) 2 and 7, mesenchymal stem cells (MSC), demineralised bone matrix, and biocompatibile ceramics are presented in many papers describing their use in bone defect treatment[12]. Bone morphogenetic proteins exhibit an extraordinary power to induce new bone formation de novo without the presence of cancellous bone[13]. With their high cost, limited availability and restricted clinical indications, BMPs are a less attractive option for clinical application. Synthetic substitutes that provide a scaffold to support or direct bone formation include calcium sulphate, ceramics, calcium phosphate, cements, collagen, bioactive glass and synthetic polymers. These are available in a variety of formulations, including pellets, cement and injectable paste.

One of the clinical challenges in long bone defects is the induction of appropriate bone formation, especially in patients with diabetes. Several studies have demonstrated the clinical efficacy of various platelet derived GFs. Recent evidence shows that in diabetic patients platelets are handicapped by decreased expression of growth factors and lower potential for healing fracture. The safety and efficacy of allogeneic platelets was also shown in our recent pilot case study. Moreover, the preparation of autologous platelet gel requires pre-operative apheresis and blood draws from the patient, and adds to the complexity, risk and cost of surgery[14]. Although there is some evidence that the GFs are released to some extent in the stored platelet concentrates, the majority of GFs remain intact in the platelet granules if they are appropriately stored for up to 5 days[15]. Based on these facts, we were of the opinion that allogeneic platelets

constitute a superior alternative to autologous preparations obtained by pre-operative apheresis. Therefore, we used a standard platelet concentrate from the blood bank as a component for the activated platelet gel. In theory, allogeneic platelets could have several certain side effects. In order to minimize these, all platelet units in our study were leuko-depleted and irradiated in order to prevent immune and bacteriological side effects, especially alloimmunisation to HLA-Class I and HPA antigens[16]. In fact, there was no evidence of immune reactions or transfusion-transmitted infections following the procedures. There have been no signs of bacterial contamination, which is not strange, based on the recent observation that the platelet gel exhibits significant antimicrobial activity in vitro.

The combined autologous/allogeneic graft showed successful incorporation into the defective pseudarthrosis in 7 out of 9 patients, which was confirmed with the CT scans and plain X-ray film. The problem with the two patients in whom the therapy failed was the poor incorporation of the graft in the distal tibia, where bone healing is compromised through many factors[17]. One of the patients had a deteriorating psychiatric disorder and could not follow instructions later in the study, and one had a poor bone situation arising from previous treatments. The other seven patients with successful outcomes achieved a satisfactory clinical improvement with no side effects related to the procedure. A bacterial infection did not reoccur in cases where an infection was previously treated. Our treatment has concluded a series of numerous operations and rehabilitations for some of our patients. Last, but not least, it is worth noticing that the outdate rate of the platelet units is currently in the range of 8–27% of all prepared platelet units. This leads to the conclusion that the successful use of allogeneic platelets would significantly decrease the amount of wasted platelets, which could consequently favorably change the results of blood banking policies.

We showed that adding a platelet gel to a cancellous bone graft can help achieve a good morphological result of bone defect treatment. It retains grafted bone from resorption and enhances its incorporation into adjacent bone. The standard platelet concentrates from the blood bank did not pose a significant risk for the affected patient. The results indicate good reasons for the application of this method in the treatment of bone defects in long bones. Further investigation is required to demonstrate the comparative efficiency of this treatment. This is the first report of a prospective clinical study monitoring the use of allogeneic platelets mixed with autologous cancellous bone for the treatment of the non-union of long bones after fractures conducted at the University Clinical Centre in Ljubljana. Our new method of tissue engineering seems to have the potential to become a widely approved and accepted method of bone tissue replacement in the treatment of the non-union of long bones.

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UTJECAJ GELA PRIPRAVLJENOG OD ALOGENSKIH TROMBOCITA NA OBLIK DUGIH KOSTIJU ČOVJEKA

S A Ž E T A K

Cilj ovog rada je analizirati morfološke i funkcionalne promjene na defektima dugih kostiju pacijenata nakon graftovanja mješavinom trombocita i autologne spongiozne kosti. Tokom jedne godine prospektivno je analizirano devet pacijenata starosti 25–73 godine sa pseudoartrozom duge kosti, nakon neuspješnog inicijalnog kirurškog tretmana. Mješavinom spongioze uzete sa grebena crijevne kosti i trombocitnog koncentrata ABO kompatibilne alogene plazme (PRP) ispunjen je koštani defekt, i operativno fiksiran. Radiološki, defekti su dobili koštani izgled (pojava tvrdog kalusa) izmedju 6. i 24. tjedna. Vrijeme funkcionalnog oporavka je variralo; 12–40 tjedana bilo je potrebno do mogućnosti parcijalnog oslonca, odnosno 16 do 48 tjedana do pune pokretljivosti i funkcionalnosti uda. Ukupno cijeljenje koštanog defekta iznosilo je 16 do 36 tjedana. Dva pacijenta su imali komplikaciju u smislu slabog koštanog urastanja te prolazne postoperativne pareze živca. Na RTG snimkama bila je vidljiva cjelovita i brza restauracija koštane strukture s odličnim koštanim prerastnjem, potrebnim za puni oslonac. Alogeni trombocitni gel nije imao štetnih efekata. Ova metoda može se koristiti za liječenje defekata dugih kostiju, obzirom na snažnu restauraciju normalnog koštanog oblika. Buduća istraživanja su neophodna kako bi se usporedila učinkovitost ove metode s drugim sličnim metodama.