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Treatment of Delayed Union of Long Bones by Percutaneous Injection of Autologous Stem Cells

Leczenie zrostu opóźnionego kości długich przezskórnym podaniem autogenego szpiku kostnego

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Abstract

Background. Disturbances in the union of bone fractures is a complication which prolongs treatment time, having a measurable influence on the costs and also connected with serious hardship and even invalidism of the patient.

Objectives. The purpose of this study was to assess the results of percutaneous bone marrow grafting for the treatment of delayed union of the long bone.

Material and Methods. Thirty-seven patients were treated in 2001 to 2005 at the Clinic of Traumatology and Hand Surgery of the Silesian Piasts University of Medicine in Wrocław, Poland by percutaneous bone marrow grafting because of delayed union of the long bone. Injections were three times every two weeks. Ten milliliters of marrow were aspirated and injected percutaneously immediately into and around the delayed union under radiological control.

Results. Bone union following percutaneous bone marrow injection was achieved in 28 of the 37 patients. The average time to union following the first injection was 16 weeks.

Conclusions. Stromal stem cells possess osteogenic properties which can be useful in the treatment of delayed union. Percutaneous injection with autologous stem cells is an effective treatment to induce bone healing and prevent nonunion (*Adv Clin Exp Med* 2007, 16, 1, 43–48).

Key words: delayed union, stem cells, bone marrow, long bone.

Streszczenie

Wprowadzenie. Zaburzenia zrostu kostnego są powikłaniem przedłużającym czas leczenia, co ma wymierny wpływ na koszty leczenia i łączy się z dużymi uciążliwościami dla pacjenta, a w niektórych przypadkach nawet inwalidztwem.

Cel pracy. Ocena wyników leczenia zrostu opóźnionego kości długich przezskórnymi iniekcjami szpiku kostnego.

Materiał i metody. W latach 2001–2005 w Klinice Chirurgii Urazowej i Chirurgii Ręki AM we Wrocławiu leczono 37 pacjentów z powodu zrostu opóźnionego przezskórnymi iniekcjami szpiku kostnego. Iniekcje wykonywano 3-krotnie w odstępach 2-tygodniowych. Podawano 10 ml pobranego szpiku w okolicę stawu rzekomego pod kontrolą radiologiczną.

Wyniki. Zrost kostny uzyskano u 28 chorych na 37 leczonych. Średni czas uzyskania zrostu kostnego wynosił 16 tygodni.

Wnioski. Komórki szpiku kostnego mają osteogenne właściwości, które można wykorzystać w procesie zrostu kostnego. Leczenie za pomocą przezskórnych iniekcji szpiku kostnego pozwala uzyskać zrost kostny, zapobiegając wytworzeniu się stawu rzekomego (*Adv Clin Exp Med* 2007, 16, 1, 43–48).

Słowa kluczowe: zrost opóźniony, szpik kostny, mezenchymalne wielopotencjalne komórki szpiku (MWKS), kości długie.

Bone is a tissue that has the ability to heal itself when fractured. Occasionally, critical defects can form when part of the bone is lost or excised; in this case, the bone fails to heal and requires bone reconstruction to prevent a nonunion defect [1]. Disturbances in the union of bone fractures is a complication that prolongs treatment time and has a measurable effect on costs and is also connected with serious hardship and even invalidism of the patient. A fracture is determined to have a delayed union when it has not healed after three months and a nonunion when it has not healed after nine months or more since the fracture [1, 4–6]. Between three months and nine months after the fracture there is time to prevent nonunion.

The diagnosis of disturbances is based mainly on subjective and radiographic examination. Ultrasonic and isotope examinations can provide valuable information. It is worth stressing the necessity of their simultaneous application to achieve a complete quantitative and qualitative assessment of the healing process of the bone tissue [3]. Table 1 presents the most important symptoms differentiating pathological states of bone healing.

In preparing the treatment plan, it is necessary to determine the extent of the surgery and to choose the kind of stabilization. In addition, it is necessary to remember pharmacological treatment and noninvasive methods helping bone union. Among the latter are the possibilities of using

magnetic fields, low-energy laser therapy, and ultrasound [1, 2, 5, 6].

One of the methods of treating disturbances of bone union is the use of the mesenchymal properties of pluripotent marrow cells [7]. The structure of bone tissue, its function, and metabolism connected with the processes of osteogenesis, resorption, mineral homeostasis, and healing of damages are based on the presence of highly specialized types of bone tissue cells [8]. These arise from two lines of stem cells: mesenchymal and hematopoietic. The undifferentiated endosteal cells and osteocytes belong to the mesenchymal line. Circulating monocytes, pre-osteoclasts, and osteoclasts belong to the hematopoietic line [9]. Undifferentiated mesenchymal cells appearing inside bone canals, in the periosteum, endosteum, and in marrow, can undergo differentiation into chondrocytes, adipocytes, myoblasts, and osteoblasts, depending on the application of the proper conditions [10]. Mesenchymal stem cells cultured *in vitro* synthesize extracellular matrix containing, among other factors, collagen types I and IV, fibronectin, and laminin. The cells also produce a range of cytokines, including interleukin 7, 8, and 11 [11]. The cells have an irregular shape, are mononuclear, contain a small amount of cytoplasm, and remain in an undifferentiated state until stimulation [11]. The idea of using mesenchymal stem cells in treating bone damage is based on “tipping” the balance between osteoblasts and

Table 1. Differentiation of the states of bone healing

Tabela 1. Różnicowanie stanów gojenia się kości

	Clinical examination (Badanie przedmiotowe)			Radiological features (Badania radiologiczne)		Scintiscanning (Scyntygrafia)
	pain	skin warmth	fractures mobility	fragments contours	marrow cavity	
Diagnosis (Rozpoznanie)						capture of marker
Immature bone scar (Niedojrzała blizna kostna)	lack	intensified	springing	blurred	closed	much intensified
Delayed union (Zrost opóźniony)	marked	intensified	springing	blurred	open	intensified
Hypertrophic nonunion (Staw rzekomy hipertroficzny)	lack, small	intensified	slight	thickened with periosteal growth	closed	intensified
Oligotrophic nonunion (Staw rzekomy oligotroficzny)	small	intensified	marked	rounded	closed	intensified
Dysplastic nonunion (Staw rzekomy dysplastyczny)	lack	correct, decreased	large	thinned	closed	intensified
Aplastic nonunion (Staw rzekomy aplastyczny)	lack	correct	large	sometimes blurred, often unchanged	open	much decreased

Table 2. Evaluation of union according to Hammer's division**Tabela 2.** Ocena zrostu wg podziału Hammera

Degree (Stopień)	Picture of callus (Obraz kostniny)	Pseudoarthrosis fissure (Szpara stawu rzekomego)	Union compactness (Zwartość zrostu)
1	homogenous bone structure	blurred	achieved
2	massive bone trabeculation	hardly observable	achieved
3	bone bridge	observable	uncertain
4	trace. Lack of bone bridge	clear	not achieved
5	lack of callus	clear	not achieved

osteoclasts to the advantage of the former [12]. This is possible by supplying precursors of such cells to the damaged site and creating special conditions for their proper differentiation.

An assessment of the results of percutaneous bone marrow grafting for the treatment of delayed union of long bones was the purpose of this study.

Material and Methods

Between 2001 and 2005, 37 patients were treated by percutaneous bone marrow grafting at the Clinic of Traumatology and Hand Surgery of Silesian Piasts University of Medicine in Wrocław, Poland because of delayed union of long bones. In the tested group, men constituted the majority (66%) and the age of the operated patients ranged from 18 to 52 (average: 31 years). The bones involved were the tibia (11 cases), femur (6), radius (10), and the humerus (10). The time from fracture to injection was from three to nine months. Examination of the patients consisted of subjective examination and additional examinations, i.e. radiological and scintiscanning. All patients were followed up retrospectively during control examinations and observation for at least six months after the last injection. Non-achievement of bone union or no improvement in radiological results after three months was classified as a bad result and the patient was qualified for further treatment. The division of Hammer was used for assessing union from the control X-rays (Table 2) [12].

In the tested group of patients, 27 of the fractures were treated surgically and 10 had had conservative treatment (reposition and cast). Injections were three times every two weeks. The injection of bone marrow was performed on an outpatient basis under local anesthesia. With the patient supine, the iliac crest and the delayed union site were prepared and draped in a sterile manner. One percent lignocaine was infiltrated into the skin down to the periosteum. Bone marrow was aspirated from the iliac crest 1 cm posterior to the

anterior superior iliac spine. Ten milliliters of marrow were aspirated and injected percutaneously immediately into and around the delayed union [16] under radiological control. Radiographs were repeated at four-week intervals.

Results

Bone union following percutaneous bone marrow injection was achieved in 28 of the 37 patients. The average time to union following the first injection was 16 weeks (range: 10–26 weeks) (Figures 1–2). Progress into nonunion was observed in 9 cases. In this group, 6 cases were classified as atrophic nonunion and 3 as vascular nonunion. There were better results in the group in which the fracture had been treated conservatively than in the group in which it had been surgically treated. The particular reasons for the formation of nonunion were lack of mechanical conditions to achieve union (5 patients: non-anatomical position of fragments, lack of or too short a period of immobilization after surgical treatment despite unstable osteosynthesis, use of the wrong plate, stabilization without contact of fragments) and disturbances in blood and nerve supply (4 patients). There were no cases of infection following the percutaneous injections and no complications at the donor site.

Discussion

Fracture healing requires the co-operation of many cellular populations regulated by nervous and molecular factors to ensure the proper biological and mechanical environment and to restore the blood supply damaged by injury. Non-fulfillment of any of these factors leads to union disturbances which delay it and, in some situations, they make it completely impossible.

In some cases it is very difficult to define whether pseudoarthrosis or delayed union is being treated. Nonunion is defined when a minimum of

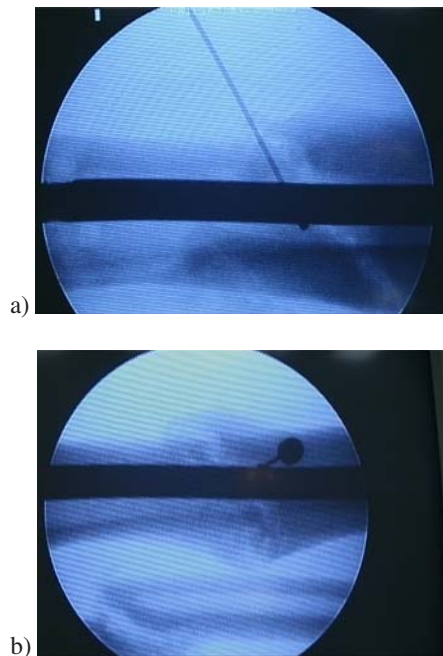


Fig. 1. Patient T.Z., age 30, disease history no. 7647/05, tibial bone. Percutaneous bone marrow grafting: a) injection into delayed union, b) injection around delayed union

Ryc. 1. Chory T.Z., lat 30, nr historii choroby 7647/05, kość piszczelowa. Przeszkórna iniekcja komórek szpiku kostnego: a) iniekcja w miejsce zrostu opóźnionego, b) iniekcja wokół zrostu opóźnionego

nine months has elapsed since injury and the fracture shows no visible progressive signs of healing for three months, and delayed union when it has not healed after three months. These criteria have been modified, as it is difficult to put a fixed time period on the definition. Taylor [4] stated that the criteria of nine months cannot be applied to every fracture and suggested that fracture nonunion of a long bone could be considered when there is a minimum of six months with no progression towards union. Our criteria for defining delayed union were six months and no evidence of union or three months without progression towards union. In our opinion it is very important to treat delayed union, before nonunion, despite the fact that some authors have written about good results also with nonunion [19, 20].

The next essential problem is to define exactly which cells work and what amount gives the best effects. The next problem is that there is no agreement on the name to be given to these cells which are injected. Names such as “mesenchymal stem cells” and “bone marrow stromal stem cells” (SSCs) are frequently used. Difficulties in giving a single name to these cells is also a consequence of their nature. They have been shown to be precursors of many specialized cell types, not of only



Fig. 2. Patient T.Z., age 30, disease history no. 7647/05. Tibial delayed union treated with percutaneous bone marrow grafting: a) 12 months delayed union, b) progression to union after 3 months

Ryc. 2. Chory T.Z., lat 30, nr historii choroby 7647/05. Kość piszczelowa leczona przezskórnymi iniekcjami szpiku kostnego: a) zrost opóźniony po 12 miesiącach leczenia, b) zrost kostny po 3 miesiącach od zastosowania iniekcji szpiku kostnego

mesenchymal lineage, such as bone, cartilage, and fat, but they have also been shown to become hepatic and neuronal cells, making the name “mesenchymal stem cells” inaccurate. Bone marrow is a good source of stem cells, but not unlimited. It is estimated that there are 32 million nucleated cells per milliliter, and only 1 in 18,000 of nucleated cells is a stem cell. Because only a few milliliters of bone marrow is aspirated, there are only a few thousand SSCs that we can obtain from the bone marrow. Muschler [21] wrote that increasing the amount of bone marrow aspirated is not a solution, because each single aspiration does not coincide with a significant increase in the number of SSCs, suggesting that after a certain amount, what is aspirated is blood and not bone marrow, and aspiration of 10 ml gives the best result [22].

Treatment by percutaneous injection with autologous stem cells is one of the methods which can be used in the treatment of delayed union of

long bones which give good results [14, 15]. Goel [16] reported achieving union in 75% of patients in 14 weeks, Niedźwiecki [14, 15] achieved union in every case of delayed union in 3.2 months and in every case of nonunion in 3.4 months following the first injection, Connolly et al. [17] used autologous bone marrow injection and reported healing the fractures of 18 of 20 patients in an average time of seven months. In the present study, bone union was achieved in 70% and the average time to union was 16 weeks. The differences in the results are most likely due to the definition of causes of bone union disturbances. This should be remembered. Treatment with mesenchymal pluripotent marrow cells is a complementary therapy applied after ensuring a correct reduction of fractures, immobilization, and a blood supply adequate to needs [18]. In the present study, cases of incorrect stabilization of fractures and accompanying damage to vessels and nerves were the main cause of treatment failure. Diagonal drilling of a fracture fissure overcoming the cartilage appearing on the fragments' endings and enabling the

migration of cells and restoring common circulation between both fragments seems essential [19]. Introducing bone marrow into those fissures completes the new method of Beck's bone drilling with the new osteogenic material [20].

Percutaneous injection with autologous stem cells is an easy and safe procedure. This method is inexpensive, does not require extra instruments, has regulatory reasons, and can be preformed as a "minimally invasive procedure". In comparison with an "open" technique, the risk of devascularization and infection at the fracture site, where healing is already impaired, is minimized.

Stromal stem cells possess osteogenic properties which can be useful in the treatment of delayed union. Percutaneous injection with autologous stem cells is an effective treatment in inducing bone healing and preventing nonunion. Percutaneous injection with autologous stem cells is a safe, time-saving, and economical "minimally invasive" alternative to "open" techniques in treating delayed union.

References

- [1] **Tylman D, Dziak A:** Traumatologia narządu ruchu. PZWL 1996.
- [2] **Górecki A:** Czynniki wzrostu i tkanka kostna. Oficyna Wydawnicza ASPRA-JR; Warszawa 2004.
- [3] **Zgliczyński SzL:** Radiologia. PZWL, Warszawa 1989.
- [4] **Taylor CJ:** Delayed union and nonunion of fractures. In: Campbell's Operative Orthopaedics. 1992, 28 1287–1345.
- [5] **Russell AT, Taylor CJ, Lavelle DG:** Fractures of tibia and fibula. In: Fractures in Adults, Rockwood and Green, 1991, 3, 1915–1982.
- [6] **Saleh M:** Non-union surgery, part 1. Basic principles of management. Int J Orthop Trauma 1992, 2, 4–18.
- [7] **Bruder S et al.:** Growth kinetics, self-renewal, and the osteogenic potential of purified human mesenchymal stem cells during extensive subcultivation and following cryopreservation. J Cell Biochem, 1997, 64, 278–294.
- [8] **Bruder S et al.:** The effect of implants loaded with autologous mesenchymal stem cells on the healing of canine segmental bone defects, J Bone Joint Surg Am 80A, 985–996.
- [9] **Ohgushi H, et al.:** Stem cell technology and bioceramics: from cell to gene engineering, J Biomed Mater Res 1999, 48, 913–927.
- [10] **Pittenger M, et al.:** Multilineage potential of adult human mesenchymal stem cells, Science 1999, 284 (5411), 143–147.
- [11] **Richards M, et al.:** Marrow-derived progenitor cell injections enhance new bone formation during distraction. J Orth Res 1999, 17, 6, 900–908.
- [12] **Słynarski K:** Osteoindukcyjne właściwości wielopotencjalnych komórek szpiku. Ortop Trauma Rehab 2000, 3, 8–10.
- [13] **Hammer R, Hammerby S, Lindholm B:** Accuracy of radiologic assessment of tibial shaft fracture union in humans. Clin Orthop 1985, 199, 233–238.
- [14] **Niedźwiecki T:** Wpływ szpiku kostnego na gojenie złamań, zrostów opóźnionych i stawów rzekomych kości długich. Chir Narz Ruchu Ortop Pol 1993, LVIII, 3, 194–203.
- [15] **Niedźwiecki T, Niedźwiecki Ł:** Wspomaganie gojenia stawów rzekomych kości piszczelowej przeszczepianiem autogenego szpiku kostnego. Nowiny Lekarskie 2001, 70, 4, 322–328.
- [16] **Goel A, Sangwan S, Siwach R, Ali A:** Percutaneous bone marrow grafting for the treatment of tibial non-union. Injury, Int J Care Injured 2005, 36, 203–206.
- [17] **Connolly J, et al.:** Autologous Marrow Injection as a Substitute for Operative Grafting of Tibial Nonunions. Clin Orthop 1991, 266, 259–270.
- [18] **Lucarelli E, Donati D, Cenacchi A, Fornasari P:** Bone reconstruction of large defects using bone marrow derived autologous stem cells. Transfusion and Apheresis Science 2004, 30, 169–174.
- [19] **Kettunen J, Makela E, Turunen V, Suomalainen O, Partanen K:** Percutaneous bone grafting in the treatment of the delayed union and non-union of tibial fractures. Injury Int J Care Injured 2002, 33, 239–245.
- [20] **Garg N, Gaur S, Sharma S:** Percutaneous autogenous bone marrow grafting in 20 cases of ununited fracture. Acta Orthop Scand 1993, 64, 671–672.

- [21] **Muschler G, Nitto H:** Age- and gender-related changes in the cellularity of human bone marrow and the prevalence of osteoblastic progenitors. *J Orthop Res* 2001, 19 (1), 117–125.
- [22] **Buewell R:** The Function of Bone Marrow in the Incorporation of a Bone Graft *Clin Ortop* 1985, 200, 125–141.

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