Radial Shock Waves and Autologous Growth Factors-Combined Therapy for Bone Delayed Unions
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Tissues in the musculoskeletal system have a wide variety of healing properties, that give our specialty a particular need of understanding each organ in fields as different but yet important as molecular biology or biomechanics. Muscle and ligament tend to heal completely most of the time, while nerve and cartilage seem to heal only as functionally sufficient scar tissues. Most medullary lesions result in irreversible paralysis and cartilage loss in joint replacements. Bone is somehow in the middle of these two scenarios, having a good rate of healing in most of the cases, but with many biological and biomechanical variables that can change the final outcome in terms of healing and functional recovery. Nature has designed a nearly perfect system of bone healing, that involves bleeding, cell migration, differentiation and activation, and finally the delicate process of bone remodeling, matrix maturation and biomechanical stabilization[1-3]. If one of these steps is disturbed, bone healing is in jeopardy. It is well known that poorly vascularized bone heals slowly and has more chances of delayed unions. High energy trauma, open fractures, infection or unstable fixations may also result in difficult healing environments[4-6].

The best opportunity an orthopaedic surgeon has to treat a difficult fracture is his first approach and intervention. Science and technology have provided the tools to give biomechanical stability in most of the cases, and the knowledge of cellular and molecular biology has also changed the vision of our interventions in the past two decades. However, even the best possible treatments sometimes fail, and the biological consequences of our interventions can result in non-healing.
This is a real problem that opened research lines for many groups in the world, trying to prevent and treat delayed unions and pseudoarthrosis[7].

Successful bone healing in delayed unions and nonunions is a challenge for the orthopaedic surgeon. There are many treatment options ranging from simple immobilizations to open fixation and grafting. Treatment is based in the biological and biomechanical “re-stabilization” of the fracture. It requires a particular approach in every case, analyzing the causes of the nonunion, the type of fracture, the anatomical and biomechanical considerations of the lesion, and the best treatment option available for each situation[6].

Surgical interventions in delayed fracture healing and nonunions involve large operations that significantly increase pre and postoperative risk factors, and also represent a longer, more difficult recovery period and rehabilitation process. Bone grafts are commonly used as osteoconductors in fracture defects[8,9]. The use of non biological osteoconductive elements such as hydroxyapatite has also served the purpose of defect filling[10]. However, the osteoinductive properties of grafts are fully dependent on the host’s capability to create a proper healing environment allowing bone graft substitute incorporation[9]. The vascularity, the amount of undifferentiated cells and the third messengers that activate the healing processes are probably as or more important than the physical filling of a tissue gap. The modulation of bone remodeling is a primary function of hormones and growth factors that could be used potentially as osteoinductors in cases where the natural process of bone healing is endangered[11,12]. Surgical procedures are necessary when the biomechanical stabilization is required. Surgery is also useful when tissue stimulation is needed to complete bone healing. It creates a new traumatic environment with bleeding, growth factor liberation, cell migration and differentiation that stimulates new bone formation. Partial decortication procedures, as the ones described by Judet [13], have been used widely in many situations. Grafting and tissue healing augmentation with Autologous Growth Factors (AGF) are currently used in surgery to provide adequate osteoinduction and osteoconduction in order to solve the clinical situation[14-18].

In cases where the biomechanical stability of a fracture has been reached, but the biological status of bone healing is delayed, many non-invasive techniques have been described in order to obtain bone callus[6]. These techniques apply biophysical enhancement elements to increase bone healing in difficult circumstances. Electrical stimulation and magnetic fields have been used successfully in some cases [19-24], but the most documented and widespread system currently
used is the stimulation of endogenous growth factors by focalized high energy ultrasound single pulses, or shockwaves[25,26].

**ESWT and Bone Healing**

Extracorporeal Shock Wave Therapy (ESWT) has been studied in both experimental [27-39] and clinical [40-49] fields, showing good and excellent results in bone healing for delayed unions and pseudoarthrosis. The use of shockwaves focalized on the fracture site increases bone formation by means of vascular stimulation, periosteal reaction and osteoblast activation, probably due to the endogenous liberation of Nitrous Oxide, growth factors and free radicals[25]. These have been proven experimentally by Wang et al. [50,51], showing significant differences in animal models. Clinical trials of Shaden, Rompe, and Wang [45-47] have shown bone healing in delayed unions and pseudoarthrosis using high energy ESWT devices in single sessions with excellent results. There are several recent clinical studies on the effect of ESWT for nonunion and delayed union. Schaden et al. [45] reported their experience with ESWT for the treatment of delayed osseous union and nonunion in 115 patients with a success rate of 75.7%. In a prospective study, Rompe et al. [47] evaluated the effect of high-energy extracorporeal shock waves in the treatment of 43 patients with tibial or femoral nonunions. They showed 72% of bony consolidation after an average of 4 months with no adverse events besides transient local hematoma. Wang et al. [46] reported a success rate of 80% at 12 months follow-up in the treatment of 72 nonunions of long bones with ESWT. These studies, with a success rate range of 70% to 80% in the achievement of bony union, showed moreover an adequate relief of symptoms, a functional recovery and no systemic complications. Therefore, the use of ESWT in the treatment of nonunion is highly recommended.

We have used both techniques in delayed unions, having as a primary indication for surgery the biomechanical instability of the fracture, and for ESWT the biological instability of the delayed union. We have used high energy ESWT for five years and our results show a 79% rate of healing without surgery, a number that reproduces the data from our colleagues in Europe and Asia. According to literature data and the recommendations of the ISMST, the energy required for bone healing treatments using ESWT must exceed 0.3mj/mm², and must be done in one single session of at least 2000 shockwaves. This requires a high energy ESWT electromagnetic, electrohydraulic or piezoelectric generator that can
deliver this amount of energy. These high energy shockwaves are painful and the procedure must be done under sedation or anesthesia. We have used shockwaves in a protocol with 1000 impulses of progressive energy that create an anesthetic area on the field of treatment, and then we can apply the 2000 high energy shockwaves we consider necessary on therapeutic levels. We believe using lower flux densities in a larger number of impulses does not increase bone stimulation, because reaching the target areas is difficult with devices that deliver these energies. One may apply >10,000 low energy shockwaves to a bone and never be able to reach cell stimulation in the fracture site. Rompe, Wang and Schaden have shown excellent results with high energy in one session, and there are no reports of similar results with lower energy protocols.

Radial shock waves have been ruled out for bone treatments because of the low energy they deliver and the depth of the tissues that need to be treated[52]. Radial shock waves spread from the contact point in the skin and lose power as they go deeper. Furthermore, due to its physical properties, this type of shock wave is not able to be focused on a deeper point[25,26]. However, the vascular and cellular effects of radial shockwaves on insertional tendinosis have proven superficial and periosteal revascularization in bone, and probably the good results of this technology in soft tissues is also caused by the stimulation of endogenous growth factors[50,51]. Using radial shockwaves should also create proper environments for healing stimulation in certain areas of bones close to the application site, but probably not on the whole fracture, especially in large bones like the femur or tibia.

AGF and Bone Healing

One of the turning points in orthopaedic surgery has been the growing interest in biological enhancement and orthobiologic procedures to improve bone healing. Ever since Marshal Urist described Bone Morphogenetic Protein in 1965 [53, 54], orthopaedists have been aiming for the “holy grail” of a substance that could easily and rapidly consolidate a fracture. Research in PTH, PTHrp, OP1 and AGF is growing, and an important number of papers and presentations today deal with this particular line of research[5, 55, 56]. Many forms of bone healing enhancers are commercially available, and the experience is getting stronger in evidence worldwide. The use of stem cells and haematopoietic concentrates are also very popular in many other tissues, and slowly growing into bone healing as a therapeutic tool[6]. The traditional enhancement factor used in the treatment of nonunions has been the autogenous bone graft in spite of its limited availability
and the high rate of morbidity in the donor site (10 to 30%) [5,16]. Now, bone tissue engineering based therapies are emerging and increasing the current treatment choices [11].

AGF is a preparation of autologous plasma with a very high platelet concentration. The alpha granules in these platelets contain several biologically active growth and differentiation factors known by their important effect on bone tissue regeneration and neovascularization [57,58,5]. Moreover, the local hematoma produced with AGF activation serves as an osteoconductive scaffold to the repair process [59]. The uses of native growth factors offer various advantages to recombinant ones. Even if AGF contains a higher concentration of native growth factors, their biological proportion remains unchanged as well as their possible interactions [57,16]. Because of its autologous origin, AGF is safe from transmissible diseases and is free from immunological reactions [57]. The only possible adverse event with the use of AGF is the development of antibodies against bovine thrombin [16]. Therefore, we prefer the activation of human thrombin to avoid this undesirable complication.

We obtain our AGF from the own patient’s blood, prepare it in a cell saver in order to obtain the PRP (platelet rich plasma) and the puffy coat, and then concentrate platelets rich in alpha granules to at least 1,000,000 platelets/µL [57]. This concentrate of PRP/AGF is then ready to prepare in the operating room in the consistency that the surgeon requires for each particular procedure [57]. In mixtures with powder grafts or with bone chips it is used in a liquid form, and in arthroscopic procedures it is concentrated to a thick gel that can be managed easily inside the joint. In bone reconstructive surgery like total hip revisions it is also gelled with grafts so the handling of the AGF is easy and delivered into the proper site the surgeon desires. The gellification process is aided with calcium gluconate that reacts with the patient’s blood and thrombin to create the gel in the form we need. This concentration varies according to the surgical needs and is a simple procedure.

Using AGF in percutaneous applications should be able to enhance the healing process in bone. However, the vascular stimulation and the local bleeding is absent in percutaneous procedures, and is probably a key factor in the stimulation of bone formation. ESWT has proven a vascular stimulation and the generation of endogenous growth factors, and could be used to create the proper environment for AGF to act as osteoinductors in percutaneous procedures.
Our experience with autologous growth factors (AGF) in surgical procedures for delayed unions and pseudarthrosis is very encouraging. Using the osteoinductive power of AGF with bone grafts has reduced bone healing time and resulted in stable consolidations. On the other hand, our experience with ESWT in delayed unions has reproduced the data from the literature, and our protocols for each treatment are well defined and followed. However, we would like an easier scenario for certain fractures that are stable, in superficial bones and show signs of delayed union. We came up with the idea of using a combined therapy of low energy shockwaves and the percutaneous application of AGF and wanted to determine if this approach would have similar results to those obtained from open surgery or high energy ESWT. With solid scientific background, it seemed feasible to obtain good results with this protocol, reducing treatment time and costs.

**ESWT & AGF Combined Therapy**

For stable delayed unions in superficial bones, we have used a combined therapy of radial shockwaves followed by the injection of percutaneous AGF. If delayed unions are seen as unfertile soil, shock waves would act as a plow and AGF as a seed. We believe the combination of these therapies can result in the stimulation of bone healing in the same way high energy ESWT or surgical procedures do. The potential advantages are a short minimally invasive procedure with low cost. Having this in mind we started a pilot trial in voluntary patients booked for surgery for delayed stable non unions in 2004. The patients selected to receive the treatment with radial shock waves and injection of AGF were skeletally mature with nonunions or delayed unions of long bones not deeper than 1.5 inches, like tibia, ulna, fibula, distal femur and humerus. The contraindications to undergo this treatment were basically the same of high-energy shock wave treatments but since anesthesia is not required, criteria are less demanding. A patient was not treated with radial shock waves and AGF if he met one of the following criteria: inadequate stabilization, local bone infection, pathologic fracture, tumor within the shock wave field, coagulopathy, use of anticoagulants or pregnancy.

We have treated 16 delayed unions since February 2004. They were 5 females and 11 males with an average age of 39 years (range, 17 to 61 years). One patient received treatment for two delayed unions: a supracondylar fracture of the humerus and an olecranon osteotomy from the original surgical approach. Five
fractures had delayed healing (3 to 6 months from the initial injury or the last operation), and 11 fractures had an established nonunion with an evolution of more than 6 months (range, 6 to 13 months). We treated 6 tibias, 4 humeri, 5 ulnas, and 1 metatarsal. All nonunions were atrophic and did not show signs of established pseudoarthrosis or hyperthropic radiological changes, and had defects under 3 mm. (Table 1)

We have developed a protocol for the application of radial shock waves supplemented with AGF. This single ambulatory procedure is done in an operating room under aseptic conditions and takes between 30 and 40 minutes.

Our process to prepare AGF is the standardized autologous procedure. With a double centrifugation technique, a platelet rich plasma with at least 1,000,000 platelets/µL in a 5ml volume is obtained[57]. This procedure requires a sterile management and pyrogen free disposable materials. Since the technique lasts approximately 1 hour, the patient blood sample (25 cm³) has to be taken at least one hour before the shock wave application.

We do not use local anesthesia in these cases, and we prefer to use our progressive shockwave protocol to generate a reasonable anesthetic level to both apply therapeutic shockwaves and inject the AGF. With the patient placed on a standard operating table, the fracture site is localized with an X-Ray image intensifier.

With no anesthesia, we applied the following protocol: 4000 continuous impulses with 1 to 4 Bar, equivalent to 0.03 to 0.18 mJ/mm² energy flux density at a frequency of 6 to 10 Hz. In all cases we have used the Swiss Dolor Clast® radial shockwave generator produced by EMS in Switzerland. Once the fracture is localized in position and depth, a lubricating gel is applied over the skin. The shock waves are delivered with a 15mm hand applicator in direct contact with the skin, with circular movements in order to achieve stimulation in all the fracture area. The initial pressure of wave application is 1 Bar (<0.03mJ/mm²) at 10 Hz, and it is gradually increased. Then, at 500 impulses the pressure is raised to 2 Bar (0.06 mJ/mm²) and at 1000 impulses to 3.5 Bar (0.16mJ/mm²). Simultaneously, the frequency is decreased initially to 8 Hz and then to 6 Hz. After the first 2000 shock waves that are used for analgesia, we start the treatment with 2000 continuous impulses with 3.5 to 4 Bar (0.16 to 0.18 mJ/mm²) at 6 Hz. During the procedure the patient can experience some pain that is used to check the positioning of the device. Major vascular and neural structures and areas with metallic internal fixation are avoided.
Table 1. The epidemiological data of our patients. Only one did not show signs of radiological healing after 12 weeks of radiological follow up. Three of them were complications of osteotomies in previously healthy bones.

When the application of shock waves finished, 10 ml of the previously prepared AGF is activated with calcium gluconate and injected percutaneously under fluoroscopy in different points of the fracture soft callus. In the fractures with a gap larger than 3 mm, the AGF is mixed with lyophilized cryopreserved 300 micron bone allografts.
The rehabilitation process starts from the first day after treatment. All patients were immobilized for at least two weeks, and weight bearing was allowed in tibial fractures using leg braces. Patients clinical follow-up was made weekly during the first month and monthly during the first year. The intensity of pain before and after the treatment was assessed with a visual analogue scale from 0 to 10, having 0 for no pain and 10 for worst pain. Radiologic images were taken to assess callus formation, fracture defects and the presence of bone union at 6 weeks and 3, 6, and 12 months after treatment.

Bone union after delayed fracture healing or established nonunion was assessed by clinical and radiological evaluations. Clinical assessment included pain intensity, fracture stability and range of motion. Radiologic evolution was determined by defect size, callus formation and stability. We considered an excellent result when both clinical and radiological stability has been achieved at 12 weeks after the treatment. We considered a good result when radiological stability is incomplete at 12 weeks, but the clinical and radiological evolution shows improvement during the follow-up. Bad results are patients with clinical or radiological instability or those who required surgical treatment.

Patients were evaluated blindly by a group of radiologists to determine the evolution of the healing process. The clinical follow-up was not blinded due to the nature of the procedure and the patient’s knowledge of the type of treatment he received. We had excellent results in 14 cases (87.5%). This group had both clinical and radiological stability of the fracture at 12 weeks, even after periods as long as 17 months with no response to previous treatments. Most of the patients had stable delayed unions or nonunions that were on the limit for surgical revision. (Figure 1) We performed this treatment even in patients with serious conditions around the fracture, like vascular grafts and skin flaps. (Figure 2)

We had no healing in one tibia, originally an open segmentary high energy fracture treated with double plates and grafts. One of the fractures healed and the other one did not. We treated the nonunion, but the patient showed no signs of bone callus after six months. A revision surgery was performed and the stabilization was done with an IM nail. The other bone that did not heal was an olecranon osteotomy that had a 3 mm gap. Even though the elbow was immobilized, the K wires broke, a sign interpreted as fracture instability. The patient required surgical revision and grafting. (Figure 3)
Figure 1. Patient #8, 20 year old male, sports related accident with single diaphyseal mid-third ulnar fracture. Initially treated with closed reduction and immobilization. Four months after injury there is pain in pronosupination and X-Rays show no signs of healing (Upper Left). An open procedure is proposed with open reduction and internal fixation with a 3.5 DCP plate. Instead, we performed our protocol with rESWT (Upper Right) and AGF (Lower Left). Twelve weeks after the procedure there is a stable callus and the patient is pain free (Lower right).

Our preliminary results were so encouraging that we have stopped the treatments of delayed unions with high energy ESWT on these particular indications: stable delayed unions or nonunions in superficial bones. However, we must be sure if the effect of one of our treatments is enough by itself, or if the combination of rESWT and AGF is the one that is giving us these promising results. We have already started an experimental study in an animal model of nonunions in dogs, to determine the differences between groups treated only with rESWT, only with AGF, a third group treated with a combined therapy, and a control group. This study will tell us the role of each therapeutic variable in order to go further in its use.

The development of effective and safe methods to enhance the complex process of bone healing is one of the most important research questions in present orthopaedics. Biophysical stimulation of bone by means of extracorporeal shock waves induces regenerating responses in bone tissue[25]. The use of recombinant or autologous growth factors in bone is also well documented and widely used today.
Figure 2. Patient # 7, 52 year old male, motor vehicle accident with massive skin loss that required grafting. Open GIII C fracture with loss of humeral artery that required Dacron graft. Six months after stabilization with an IM nail shows no signs of healing and the patient has permanent pain (Left). Infection is ruled out by labs and bone scans. rESWT&AGF is performed. Six weeks after treatment there is no pain and X Rays show signs of healing (Center). Twelve weeks after treatment there is a stable mature callus and the patient is painless (Right).

Figure 3. Patient # 9, 61 year old female, motor vehicle accident. The initial diagnosis was an inter-supracondylar fracture treated with double plates. The surgical approach required an olecranon osteotomy that did not heal. The Supracondylar fracture did not heal as well. rESWT&AGF was done in both Supracondylar and olecranon nonunions (Left). The humerus healed properly after 12 weeks post treatment, but the olecranon did not show any signs of bone callus. There is probably a mechanical instability left because the K wires were broken in the 12 week X Ray control (Right).
We propose the use of a combined therapy of low energy radial shockwaves and autologous platelet derived growth factors in the treatment of biomechanically stable delayed unions and nonunions in superficial bones, as another minimally invasive tool that represents lower costs and in our experience is giving similar results as of those obtained with open grafting procedures or high energy ESWT. Further research is absolutely necessary, not only in the animal model we are currently working on, but also in multicentric clinical trials. Not everything new is always good, but one must remember that everything good was new once.

Literature

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