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Commentary Open Access

Is Healing of Fractures Regulated Centrally?

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Commentary

The increased rate of fracture healing and abundant callus formation of long bone fractures in patients with concomitant severe acute traumatic head injury is a well-known orthopaedic phenomenon. Few studies, however, have reported this phenomenon being induced by acute traumatic Spinal Cord Injury (SCI). There is also a well-established clinical relationship between spinal cord injuries and heterotopic ossification. Research is yet to confirm accelerated bone healing in long bone fractures in patients with concomitant SCI and to establish theories about the mechanism causing it.

In a prospective controlled project published in several articles, we compared the radiological bone healing parameters of time to union, thickness of continuous bridging union callus, blood levels of parathyroid hormone, growth hormone, adrenaline, noradrenaline, corticosteroids, leptin hormone, and blood values of growth factors: insulin-like growth factor-II (IGF-II), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), Activin-A a member of transforming growth factor-B super family, and cytokine interleukin-I in 21 patients with acute post-traumatic spinal cord injuries SCI with paraplegia or quadriplegia due to fractures or fracture-dislocation of cervical or dorsal spine and 22 concomitant long bone diaphyseal fractures of humerus, femur, and tibia to patients with spinal cord injuries only without long bone fractures, and to patients with only long bone fractures only. Parathyroid hormone, growth hormone, and corticosteroids were assayed using Immulite, the Automated Immunoassay Analyzer, which is a continuous, random access instrument and performs automated chemiluminiscent immunoassays and the rest of hormones, growth factors, and cytokine were assayed and measured using the Enzyme linked Immunosorbent Assay (ELISA) technique.

Blood samples from different patients' groups were processed by centrifugation and after separation of blood cells; separated sera were preserved at -850 C and used also to count the number of circulating mesenchymal stem cells and monocytes in peripheral blood using the flow cytometry, in each of the above groups of patients.

These sera were used as well, to investigate their effect on the growth of stem cell culture by measuring the cell count of cell line of human bone marrow-derived mesenchymal stem cells MSCs (ATCC-USA) treated with control and patients' sera from different groups after 72 hrs incubation [1,2].

Experimentally, we tested the effect of inflicted dorsal spinal cord injury, producing paraplegia, on the bone healing of hind limb mid-shaft femoral osteotomy fixed by intramedullary K-wire in 12 rabbits, compared to control group of another 12 rabbits with inflicted femoral osteotomy and intra-medullary K-wire fixation only. In a controlled experiments we also, tested the effect of sera from acute post-traumatic spinal cord injury SCI patients with paraplegia and quadriplegia and from rabbits with inflicted dorsal spinal cord injury with paralysis of their both hind limbs on the bone healing of femoral osteotomy of hind limb in another groups of rabbits.

The study results showed that long bone fractures in patients with associated acute traumatic spinal cord injury of quadriplegia or paraplegia heal more expectedly, faster and with exuberant florid union callus ($P>0.001$) and showed statistically significant higher levels of parathyroid hormone and growth hormone ($p<0.005$) and normal corticosteroids levels. Patients with long bone fractures only showed consistent and statistically significant higher level of noradrenaline and adrenaline hormones compared to patients with spinal cord injury alone or associated with long bone fractures ($p<0.001$). Leptin hormone shows statistically significant consistent decrease in patients with spinal cord injury and concomitant long bone fractures compared to its values in healthy subjects ($p<0.001$). The study also, showed statistically significant higher levels of growth factors of PDGF, VEGF, Activin-A, and cytokine I-L-1, along the 3 weeks of follow-up ($P>0.005$). I-IGF-II showed statistically significant subnormal level along the whole follow-up period in the same patients ($P>0.005$) [1,2].

Measuring the cell count of mesenchymal stem cells (MSCs) (CD105+ve/ CD14-ve)and(CD 90+ve/ CD14-ve) in blood of patients from different groups showed high statistically significant cell count in patients with spinal cord injuries with or without long bone fractures, especially in the first 24 hours post-injury which started to decline at the end of the first week, in comparison to the control groups of long bone fractures only and in sera from healthy subjects and although the count of MSCs was declined in the blood of patients of spinal cord injury with or without long bone fractures , it remained elevated statistically significant in comparison to its count in healthy subjects ($P<0.001$).

Measuring the cell count of monocytes (CD45-ECD) and (My4-FITC) in blood of patients from different groups showed high statistically significant cell count in patients of all groups in comparison to the monocytes cell count in blood of healthy subjects ($P<0.005$), with the highest increase was in patients with spinal cord injury only group ($P<0.001$). The cell count in all groups remained elevated statistically significant along the whole three weeks of follow-up ($P<0.005$).

Measuring the cell count of cell line of human bone marrow-derived mesenchymal stem cells MSCs (ATCC-USA) treated with control and patients' sera from different groups after 72 hrs incubation showed high statistically significant cell count and growth and viability rate in patients with spinal cord injury SCI with or without long bone fractures) in comparison to the control group with sera from long bone fracture only patients and sera from healthy subjects. The mean growth rates were 81.46 ± 5.37% and 81.5 ± 6.49% in SCI patients with or without long bone fractures versus 59.77 ± 5.98% in long bone fractures only patients and 52.96 ± 5.11% in the sera from the control healthy subjects ($p<0.0001$). Moreover, we found a positive correlation between fracture union time and thickness of union callus on one side and proliferation of bone marrow-derived mesenchymal stem cells in the tissue cultures on the other side.

Experimentally, in the group of 12 rabbits, we inflicted mid-dorsal

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spinal cord injury and femoral osteotomy. The femoral osteotomies in the animals of this group showed abundant radiological union at 3 weeks in 9 (75%) and after 6 weeks in all the 12 rabbits. Post-mortem dissection of these animals showed solid abundant union on macro-pathological assessment and the Mean ± SD callus area was 2.70 ± 1.1 cm^2. The rabbits of this group, after inflicting the spinal cord injury and the femoral osteotomy, became either weakly ambulatory or non-ambulatory animals, which necessitated a special care in providing adequate drinking, feeding, and housing to those animals and close observation. There were no cases of nonunion in this group as well. Histopathological microscopic examination of fractured femoral specimens showed evidence of mature callus, endochondral ossification, and mineralization. In addition there was remodeling of the callus into lamellar bone formation and bone marrow formation.

In the control group of 12 rabbits, which have been operated upon by femoral osteotomy only, 6 (50%) rabbits showed united femoral osteotomy only after 6 weeks with abundant callus in one, moderate callus in 4, and minimal callus in one. The 6 (50%) femoral osteotomies in the remaining 6 rabbits showed atrophic nonunion with no callus in 4, and hypertrophic nonunion with moderate non- bridging callus in 2. The mean ± SD callus area was 1.82 ± 0.25 cm^2 in united specimens. Microscopically, united specimens showed evidence of mineralization of the cartilage with heavy calcium deposit, bone remodeling and granulation tissue. Ununited specimens showed granulation tissue with non-bridging woven bone and inflammatory phase with early soft callus.

In the experiments to test the effect of sera from spinal cord injury patients on rabbits' femoral osteotomies, and after we operated upon 12 rabbits, they became ill with gross swelling in their operated limbs in 6 of them and died soon within a week after the osteotomy and the insertion of serum allograft on a carrier. The other 6 remained ill till around three weeks when they died subsequently, and their post-mortem dissection revealed severe tissue reaction with variable, but considerable pus collection with definite non-union without a trace of callus in any of them.

In group of 12 rabbits, which have been operated upon by femoral osteotomy and allograft from spinal cord injury rabbit's serum on a carrier, 8(66.7%) femoral osteotomies in 8 rabbits of this group showed abundant radiological union at 6 weeks in radiological assessment and dissection of these animals showed solid abundant union on macro-pathological assessment. The mean ± SD callus area was 1.70 ± 0.10 cm^2 in united specimens. There were 4 (33.3%) nonunion in this group, 3 rabbits showed radiological and in post-mortem dissection atrophic nonunion of their femoral osteotomies without any trace of union callus. In the remaining rabbit, the femoral osteotomy went into hypertrophic nonunion. Microscopic examination of united femoral specimens in this group of rabbits disclosed Woven bone and hyaline cartilage formation with endochondral ossification, while ununited femoral specimens showed soft callus with granulation tissue formation [3].

Afan et al., concluded in their study of the anatomical analysis of the innervation of murine femora and the effects of denervation of these femora on the cellularity of the femoral bone marrow and the mobilization of the osteo-progenitor stem cells into peripheral blood, that the nervous system has an important role in the selective control of bone marrow cellularity and the denervation leads to decrease in the femoral bone marrow cellularity and mobilization of progenitor cells to the peripheral blood. The study also indicates a possible nervous control of cell proliferation within the bone marrow [4].

From the aforementioned results of the study, we concluded that bone healing is not just regulated by autocrine/paracrine and hormonal mechanisms as suggested in the traditional view, but it might be controlled neuronally and centrally. The confirmed accelerated bone healing of long bone fractures in patients with concomitant spinal cord injury SCI in this study could be explained, based on the normal and subnormal level of adrenaline, noradrenaline, and corticosteroids hormones, by denervation of flat or long marrow containing bones at and below the level of spinal cord injury, in particular the sympathetic nervous pathways and whether this denervation is due to the pathology of spinal cord damage at the site of injury or patho-physiologically, by inhibitory feedback to the accurate nucleus of the sympathetic nervous system in the hypothalamus. This in turn, according to Afan et al., could lead to mobilization of undifferentiated mesenchymal stem cells MSCs and osteoprogenitor cells to peripheral circulation in abundance, which has been confirmed by flow cytometry, with considerable statistical significance compared to the control group, to induce accelerated healing of long bone fractures with abundant, exuberant, and florid union callus, The statistically significant higher levels of growth factors and cytokines in patients with or without long bone fractures indicate either its release from the damaged segment of the spinal cord crossing the cord-blood barrier or its secretion by autocrine mode from the MSCs itself to guide the MSCs to home to the fracture site and to induce its multiplication and proliferation as confirmed in the vitro part of the study by revealing the statistically significant mitogenic effect of sera from SCI patients with or without long bone on the growth rate and cell count of cell line of human bone marrow-derived mesenchymal stem cells MSCs. In the vivo experimental part on rabbits, the osteogenic effect of sera from rabbits with inflicted dorsal SCI on the healing of femoral osteotomy on another 12 rabbits, was not substantiated , as the healing of the femoral osteotomies in these rabbits were very similar to the control groups, may be due to the low concentration of the humeral or growth factors in the serum allograft or due to the use of the inappropriate carrier of calcium sulphate bone substitute granules due to the lack of collagen sponge carrier.

What lacking in this extensive study was the use of osteocalcin, bone sialoprotein, alkaline phosphatase, or other indicators kits of differentiation of MSCs into osteoblasts in the MSCs cell line culture to ensure the osteogenic effect of the sera from SCI patients, but anyhow this does not affect our proposed theory of acceleration of healing of long bone fractures in SCI patients as, we think that the mitogenic and osteogenic effect of these sera are due to abundance of growth factors and cytokines, which are the results and by-products and not the cause or the basic mechanism of the osteogenesis which we have already explained.

The therapeutic implications of the aforementioned proposed theory may indicate the use of sympatholytic drugs as B-blockers and others to enhance bone healing especially, in the situations where the fracture environment is deprived of its normal vascularization or its supply of MSCs and prone to end up by delayed or non-union as in high grades of open fractures. On the other hand sympathomimetic drugs such as α-adrenergic agonists, β-adrenergic agonists, and dopaminergic agonists, can be used to prevent or reduce the incidence of heterotopic ossification in SCI patients.

References

1. Khallaf FG, Kehinde EO, Mostafa A (2016) Growth factors and cytokines in patients with long bone fractures and associated spinal cord injury. J Orthop 13: 69-75.

2. Khallaf FG, Kehinde EO, Mostafa A (2015) Bone healing and hormonal bioassay in patients with long bone fractures and concomitant spinal cord injury. SM J Orthop 1: 1009.

3. Khallaf FG, Kehinde EO, Hussein S, Al Shinawy SI (2016) The effect of induced brain injury and spinal cord injury on the union of femoral osteotomy in rabbits. Int J Biol Med Res 7: 5394-5401.

4. Afan AM, Broome CS, Nicholls SE, Whetton AD, Miyan JA (1997) Bone marrow innervation regulates cellular retention in the murine hemopoietic system. Br J Haematol 98: 569-577.