The effect of induced brain injury and spinal cord injury on the union of femoral osteotomy in rabbits

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ABSTRACT

Objective: The aim of this study is to assess healing potentialities in experimentally induced fractured femur of NZW rabbits subjected as well to induced brain or spinal cord injuries. Materials and Methods: we tested the effect of inflicted traumatic brain injury (TBI) and inflicted spinal cord injury on healing of femoral fractures in animals. 12 rabbits with inflicted brain injury and femoral osteotomy fixed with intra-medullary k-wire group (A) and 12 rabbits with inflicted spinal cord injury with femoral osteotomy and fixation group (B) were followed-up radiologically at 3 and 6 weeks and compared to control group of 12 rabbits with femoral osteotomy and fixation only group (H). 24 rabbits with femoral osteotomy and fixation and 1 ml serum allograft on bone substitute carrier from traumatic brain injury rabbits in 12, group (C) and from spinal cord injury in 12, group (D) were compared to control groups of 12 rabbits with femoral osteotomy and serum allograft from rabbits with femoral osteotomy only, group (E), 12 rabbits with femoral osteotomy and serum allograft from healthy rabbits group (F), and 12 rabbits with femoral osteotomy with carrier without allograft group (G). Results: Group (A) and (B) rabbits showed early union callus in 3 weeks in 20 rabbits (83.3%) and all osteotomies united in 6 weeks with abundant callus. Groups C, D, E, F, G and H animals had nonunion rates of 58.3%, 66.7%, 50%, 58.3%, 41.7% and 50% respectively. The mean ± SD of callus formed was 2.71± 1.31cm2for groups A and B animals compared to 1.85± 0.25cm2 for groups C, D, E, F, G animals (p<0.001) and 1.82± 0.25cm2 for group H animals (p<0.001). Conclusion: We concluded that central nervous tissue damage enhances bone healing and serum from rabbits with traumatic brain injury or spinal cord injury has no osteogenic effect.

1. Introduction

The possible association between traumatic brain injury and accelerated fracture healing has long been recognized. Patients with central nervous tissue damage have been noticed to have increased incidence of heterotopic ossification and this phenomenon has been extensively described. Evidence supporting accelerated osteogenesis in patients with traumatic brain or spinal cord injuries is still controversial. The investigations of this relation in the literature have been divided between studies and authors who denied its presence and others who proved it. 1-16 Garland et al.1-6 found no increase in fracture callus and no shorter healing time in patients with traumatic brain injury and femoral or tibial diaphyseal fractures. Newman et al.14, Giannoudis et al.15, and Yang et al.16 on the other hand demonstrated accelerated long bone fractures healing mainly in femoral diaphyseal fractures with significant shorter union time and enhanced abundant union callus in patients with concomitant severe head injuries 1-18

The primary objective of this experimental study was to test the effect of inflicted traumatic brain injury (TBI) and inflicted spinal cord injury on the healing of femoral diaphyseal fractures in rabbits. The second objective was to test the hypothesis that serum from traumatic brain injured and spinal cord injured animals has osteogenic effect.

Material and methods

In the following experiments, New Zealand white male rabbits weighing between 3 and 4 kg and aged about 12 months were used. The rabbits were anaesthetized using Ketamine and Xylazine mixture. There were 12 animals in the following experimental groups;

Group A: Traumatic brain injury (TBI) using the lateral fluid percussion injury (LFPI) technique was inflicted on rabbits in this group by exposure of the left parietal bone and trephine the skull mid-way between bregma and lambda using 2.5 mm drill bit and injecting Bulkamid Hydro-gel below the dura. The left femur was exposed and osteomatized in the mid shaft using a saw. There after the induced fracture was fixed internally by inserting a 2.5 mm K-wire in tra-medullary in a retrograde manner.

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Group B: Spine of the mid-dorsal region in the rabbits of this group was exposed and spinal cord injury in this region was inflicted to produce paraplegia or paraparesis in the rabbits together with left hind limb mid-shaft femoral osteotomy and intramedullary K-wire fixation.

Group C: The femoral osteotomy and intra-medullary K-wire fixation were inflicted in the rabbits of this group with allograft of 1ml of the sera from rabbits with severe head injury on calcium sulphate tiny bone substitute cube as a carrier, was added.

Group D: In the rabbits of this group, the femoral osteotomy and intra-medullary K-wire fixation were inflicted with allograft from spinal cord injured rabbits' sera on a carrier was added.

Group E: In the rabbits of this first control group, femoral osteotomy and retrograde intra-medullary K-wire fixation were inflicted with allograft from rabbits with only long bones fracture's sera on a carrier was added.

Group F: In the rabbits of this second control group, femoral osteotomy and intra-medullary K-wire fixation with adding of allograft, from the healthy rabbits' sera on a carrier were added.

Group G: In the rabbits of this third control group, femoral osteotomy and retrograde intra-medullary K-wire fixation were inflicted without any allograft sera, but tiny bone substitute cube of calcium sulphate was added at the femoral osteotomy site.

Group H: This was the fourth control group where rabbits' left femur was osteomatised in the mid-shaft with retrograde intra-medullary fixation by 2.5mm K-wire without any additives.

After we operated upon 4 rabbits by inflicting spinal cord injury in the mid-dorsal spine to produce paraplegia or paraparesis, together with right humerus and left femur osteotomies and fixation of both osteotomies by intra-medullary K wire, the 4 rabbits died very early in the follow-up, despite the utmost care to provide them with adequate drinking, feeding, and healthy shelter. We were obliged to cancel this group of experimental animals. It has, also been planned in these experiments to test the effect of sera from head injury and spinal cord injury patients on rabbits' femoral osteotomies, but after we operated upon 13 rabbits belonged to 4 groups, they became ill with gross swelling in their operated limbs and 7 of them died soon within a week after the osteotomy. The other 6 remained ill until 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 and we obliged to cancel these groups, as well and replaced them by groups with transplantation of sera allograft from rabbits with inflicted head injuries and from rabbits with inflicted spinal cord injuries with controlled groups of addition of sera from rabbits with only femoral osteotomy and from healthy rabbits.

The fracture union and the amount of union callus formed were determined radiologically at the end of 3 and 6 weeks of experimental observation and compared to control groups. Only plain x-ray of the fractured femur was used for all assessments. At the end of the 6 weeks the animals were euthanized and the femoral bone subjected to osteotomy was excised, examined macroscopically, and sent for Microscopic Histopathological assessment by one pathologist (SH). Specimens were sent fresh to the Pathology Lab. On arrival in the Path Lab, specimens were immediately immersed in 10% buffered formalin for 30 minutes followed by treatment in decal solution.

Statistical Analysis: Results were analysed with SPSS for Windows (Version 16). Means and standard deviations were determined. Mean scores between groups of experimental animals were compared using chi square and the Student t-test. p value < 0.05 was considered statistically significant.

Material and methods

In the following experiments, New Zealand white male rabbits weighing between 3 and 4 kg and aged about 12 months were used. The rabbits were anaesthetized using Ketamine and Xylazine mixture. There were 12 animals in the following experimental groups:

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Group B: Spine of the mid-dorsal region in the rabbits of this group was exposed and spinal cord injury in this region was inflicted to produce paraplegia or paraparesis in the rabbits together with left hind limb mid-shaft femoral osteotomy and intramedullary K-wire fixation.

Group C: This was the fourth control group where rabbits' left femur was osteomatised in the mid-shaft with retrograde intra-medullary fixation by 2.5 mm K-wire without any additives.

Group D: In the rabbits of this group, the femoral osteotomy and intra-medullary K-wire fixation were inflicted with allograft from rabbits with severe head injury on calcium sulphate tiny bone substitute cube as a carrier, was added.

Group E: In the rabbits of this first control group, femoral osteotomy and retrograde intra-medullary K-wire fixation were inflicted with allograft from spinal cord injured rabbits' sera on a carrier was added.

Group F: In the rabbits of this second control group, femoral osteotomy and intra-medullary K-wire fixation with adding of allograft, from the healthy rabbits' sera on a carrier were added.

Group G: In the rabbits of this third control group, femoral osteotomy and retrograde intra-medullary K-wire fixation were inflicted without any allograft sera, but tiny bone substitute cube of calcium sulphate was added at the femoral osteotomy site.

Group H: This was the fourth control group where rabbits' left femur was osteomatised in the mid-shaft with retrograde intra-medullary fixation by 2.5mm K-wire without any additives.

We were obliged to cancel this group of experimental animals. It has, also been planned in these experiments to test the effect of sera on a carrier was added.

Group I: In the rabbits of this group, the femoral osteotomy and intra-medullary K-wire fixation were inflicted with allograft from spinal cord injured rabbits' sera on a carrier was added.

Group J: In the rabbits of this group, the femoral osteotomy and intra-medullary K-wire fixation were inflicted with allograft from rabbits with only long bones fracture's sera on a carrier was added.

Group K: In the rabbits of this group, the femoral osteotomy and intra-medullary K-wire fixation were inflicted without any allograft sera, but tiny bone substitute cube of calcium sulphate was added at the femoral osteotomy site.

Group L: This was the fourth control group where rabbits' left femur was osteomatised in the mid-shaft with retrograde intra-medullary fixation by 2.5mm K-wire without any additives.
operated limbs and 7 of them died soon within a week after the osteotomy. 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 and we obliged to cancel these groups, as well and replaced them by groups with transplantation of sera allograft from rabbits with inflicted head injuries and from rabbits with inflicted spinal cord injuries with controlled groups of addition of sera from rabbits with only femoral osteotomy and from healthy rabbits. The fracture union and the amount of ununited callus formed were determined radiologically at the end of 3 and 6 weeks of experimental observation and compared to control groups. Only plain x-ray of the fractured femur was used for all assessments. At the end of the 6 weeks the animals were euthanized and the femoral bone subjected to osteotomy was excised, examined macroscopically, and sent for Microscopic Histopathological assessment by one pathologist (SH). Specimens were sent fresh to the Pathology Lab. On arrival in the Path Lab, specimens were immediately immersed in 10% buffered formalin for 30 minutes followed by treatment in decal solution.

Statistical Analysis: Results were analysed with SPSS for Windows (Version 16). Means and standard deviations were determined. Mean scores between groups of experimental animals were compared using chi square and the Student t-test. p value < 0.05 was considered statistically significant.

Results

96 rabbits were operated and completed the planned 6 weeks follow-up, and have been subjected to the post mortem dissection for macro-pathological and micro-histo-pathological assessment.

In group (A) 12 rabbits were operated upon by inflicted brain injury and femoral osteotomy. The follow-up X-rays of the 12 animals showed radiological union after 3/52 in 11 (91.7%) and after 6/52 in all the 12 with an abundant callus formation and Macro-pathological assessment, after euthanizing the rabbits showed solid abundant union at the osteotomy site and the mean + SD callus area was 2.72±1.15 cm². There were no cases of nonunion in this group. X-rays of the results of two rabbits' femoral osteotomies in this group are shown in figures (1) and (2). The Histopathological result showed evidence of endochondral ossification and heavy mineralization in mature callus. In addition there was remodeling of the bridging callus into lamellar bone formation. A4 showed mixture of woven and lamellar bone whereas A5, A6 & A7-2 showed lamellar bone and bone marrow formation as shown in figure 7.

In group (B) 12 rabbits were operated upon by mid-dorsal inflicted 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. 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 cm². 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. X-rays of the results of two rabbits' femoral osteotomies in this group are shown in figures (3) and (4). Histopathological microscopic examination fractured femora 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 group (C) 12 rabbits have been operated upon by femoral osteotomy and allograft of head injury rabbit’s serum on a carrier was added. 7 (58.3%) of the femoral osteotomies in 7 rabbits of this group showed abundant radiological union at 6 weeks radiological assessment and dissection of these animals showed solid abundant union on macro-pathological assessment with the mean + SD callus area was 1.91±0.21 cm². There were three atrophic nonunion and two hypertrophic nonunion in the remaining 5 (41.7%) rabbits' femoral osteotomies of this group. X-ray of the result of one rabbit’s femoral osteotomy in this group is shown in figure (5). Microscopic examination of united femoral specimens in this group of rabbits revealed predominant bridging of woven bone with beginning of lamellar bone formation and residual granulation tissue was noted and ununited specimens showed scanty of woven bone with gross soft callus of granulation tissue and hyaline cartilage.

In group (D) 12 rabbits have been operated upon by femoral osteotomy and allograft from spinal cord injury rabbit’s serum on a carrier was added. 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 cm² 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.

In group (E) (control group), 12 rabbits have been operated upon by femoral osteotomy and allograft of long bone osteotomy rabbit’s serum on a carrier was added. The follow-up of these rabbits both radiologically, on post-mortem dissection, and macro-pathological assessment showed union in 6 rabbits (50%) with abundant callus in one, moderate callus in 5 and nonunion in 6 rabbits (50%). The nonunion was atrophic in 4 and hypertrophic in 2. The mean + SD callus area was 1.89±0.23 cm² in united specimens. Microscopic examination of united femoral specimens in this group of rabbits revealed mature callus, while the ununited specimens showed non-bridging woven bone with heavy inflammation.

In group (F) (control group), 12 rabbits have been operated upon by femoral osteotomy and allograft of healthy rabbit’s serum on a carrier. The 12 rabbits have finished their 6 weeks follow-up. In 7 rabbits (58.3%) the femoral osteotomy appeared radiologically united with abundant callus in one, and moderate callus in 6, which was confirmed in post-mortem dissection and macro-pathological assessment. The mean + SD callus area was 1.82±0.17 cm² in united specimens. The femoral osteotomies in the remaining 5 rabbits (41.7%) showed nonunion, 1 hypertrophic nonunion and 4 atrophic nonunion. Histopathological examination in united specimens showed woven bone and hyaline cartilage formation with endochondral ossification. Ununited specimens showed reparative phase of soft callus and granulation tissue.

In group (G) (control group), 12 rabbits have been operated upon by femoral osteotomy and addition of a carrier, without any allograft serum on it. The 6 weeks radiological assessment of these rabbits and the post-mortem dissection and macro-pathological assessment showed union with moderate callus formation in 5 rabbits' osteotomies (41.7%). The femoral osteotomies in the...
remaining 7 rabbits (58.3.7%) showed nonunion, hypertrophic nonunion in 2 and atrophic nonunion in the remaining 5. The mean ± SD callus area was 1.85±0.29 cm² in united specimens. Microscopically, united specimens showed woven bone and hyaline cartilage formation with endochondral ossification. Ununited specimens showed reparative phase of soft callus.

In group (H) (control group), 12 rabbits have been operated upon by femoral osteotomy only without any additives. In 6 (50%) rabbits, the femoral osteotomy united 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. X-ray of the result of one rabbit’s femoral osteotomy in this group is shown in figure (6). The mean ± SD callus area was 1.82±0.25 cm² 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. The Histopathological result of another osteotomy in this group is shown in figure (8).

All data of femoral osteotomy union in the 8 groups are shown in table (1) and some Histopathological reports of rabbits operated in group (A) and (B) are shown in table (2).

### Table 1: Results of healing of femoral fractures in the different groups of experimental animals

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>No of rabbits &amp; femoral osteotomies</th>
<th>No of united cases</th>
<th>No of non-united cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>12</td>
<td>12 (100%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Group B</td>
<td>12</td>
<td>12 (100%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Group C</td>
<td>12</td>
<td>7 (58.3%)</td>
<td>5 (41.7%)</td>
</tr>
<tr>
<td>Group D</td>
<td>12</td>
<td>8 (66.7%)</td>
<td>4 (33.3%)</td>
</tr>
<tr>
<td>Group E©</td>
<td>12</td>
<td>6 (50%)</td>
<td>6 (50%)</td>
</tr>
<tr>
<td>Group F©</td>
<td>12</td>
<td>7 (58.3%)</td>
<td>5 (41.7%)</td>
</tr>
<tr>
<td>Group G©</td>
<td>12</td>
<td>5 (41.1%)</td>
<td>7 (58.3%)</td>
</tr>
<tr>
<td>Group H©</td>
<td>12</td>
<td>6 (50%)</td>
<td>6 (50%)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>96</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 2: Histopathological examination of post-mortem femoral bone specimens of some of head and spinal cord inflicted injuries with femoral osteotomy and K-wire fixation

<table>
<thead>
<tr>
<th>Name</th>
<th>Procedure</th>
<th>Gender</th>
<th>Union</th>
<th>Callus</th>
<th>Microscopy</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>MLOF osteotomy and fixation by K wire. In addition, Head Injury</td>
<td>0.0±0.0</td>
<td>United</td>
<td>Callus present</td>
<td>Callus mature and remodel into lamellar bone</td>
<td>Final remodeling stage</td>
</tr>
<tr>
<td>A2</td>
<td>MLOF osteotomy and fixation by K wire. In addition, Head Injury</td>
<td>0.0±0.0</td>
<td>United</td>
<td>Callus present</td>
<td>Endochondral ossification and mineralization. In addition there is remodeling of the callus with new bone formation</td>
<td>Early remodeling stage</td>
</tr>
<tr>
<td>A3</td>
<td>MLOF osteotomy and fixation by K wire. In addition, Head Injury</td>
<td>0.0±0.0</td>
<td>United</td>
<td>Callus present</td>
<td>Callus mature and remodel into lamellar bone</td>
<td>Healed fracture</td>
</tr>
<tr>
<td>B4</td>
<td>Femoral osteotomy + K wire + dorsal spinal cord injury</td>
<td>0.0±0.0</td>
<td>United</td>
<td>Callus present</td>
<td>Callus mature and remodel into lamellar bone</td>
<td>Healed fracture</td>
</tr>
<tr>
<td>B5</td>
<td>Femoral osteotomy + K wire + dorsal spinal cord injury</td>
<td>0.0±0.0</td>
<td>United</td>
<td>Callus present</td>
<td>Callus mature and remodel into lamellar bone</td>
<td>Healed fracture</td>
</tr>
<tr>
<td>B6</td>
<td>Femoral osteotomy + K wire + dorsal spinal cord injury</td>
<td>0.0±0.0</td>
<td>United</td>
<td>Callus present</td>
<td>Callus mature and remodel into lamellar bone</td>
<td>Final Remodeling stage</td>
</tr>
</tbody>
</table>

1-Figure 1: X-ray of femoral union with abundant callus after 6 weeks of femoral osteotomy and inflicted traumatic brain injury rabbit group (A)

Figure 2: X-ray of post-mortem dissected left femur with femoral union with abundant callus after 6 weeks of femoral osteotomy and inflicted traumatic brain injury rabbit group (A)
Figure 3: X-ray of femoral union with abundant bridging callus in 3 weeks after femoral osteotomy and inflicted spinal cord injury in rabbit group (B)

Figure 6: X-ray of femoral nonunion 6 weeks after femoral osteotomy only in rabbit group (H)

Figure 4: X-ray of post-mortem dissected left femur with femoral union with abundant callus after 6 weeks of femoral osteotomy and inflicted spinal cord injury rabbit group (B)

Figure 7: Histopathology showing abundant callus formation in femur 6 weeks after femoral osteotomy and inflicted traumatic brain injury rabbit group (A)

Figure 5: X-ray of femoral nonunion in femoral osteotomy with serum allograft from inflicted traumatic brain injury rabbit group (C)

Figure 8: Histopathology showing evidence of mineralization of cartilage with heavy calcium deposit, bone modeling and granulation tissue in rabbit in control Group (H) with poor callus formation and evident non-union
Discussion

Early clinical reports in researching the correlation between accelerated bone healing and nervous tissue damage in head and spinal cord injuries were inconclusive and demonstrated no evidence of accelerated union or increased callus formation.1-8

Several studies later, reported a possible association between traumatic brain injury and accelerated bone healing and have addressed the issue of released growth factors in addition to bone morphogenetic protein (BMP), in brain-injured patients. They suggested that trauma to the central nervous system (CNS) may increase the release of, or decrease uptake of, bone formation mediators that can enter the systemic circulation. Other chemicals may be released from the brain, which act to stimulate local production of BMP or other growth factors or mediators.19-28

Spencer11, Perkins and Skirving17 have compared groups of patients with and without head injuries, with their fractures of the femoral shaft have been fixed by intra-medullary nailing. They concluded that callus was significantly greater in the group with head injury with accelerated time of radiological union.

Renfree et al20 inferred that the release of circulating osteogenic factor from the site of head injury is a possible explanation for the connection between CNS injury and the formation of new bone at a distant location.

Our results of 24 femoral unions with moderate to abundant callus formation, 20 (83.3%) of them showed early abundant union callus in 3 weeks, after femoral osteotomy and inflicted head injury in 12 rabbits group (A) and femoral osteotomy and spinal cord injury in another 12 rabbits group (B) and the results of the control group (H), where 12 rabbits have been operated with only femoral osteotomy with 6 (50%) out of these 12 rabbits ended by non-union and the other united 6 showed this union only after 6 weeks, indicate possible more expected, fast and accelerated union with florid callus formation of femoral osteotomy in rabbits with associated inflicted head or spinal cord injuries and these results were statistically significant (p<0.001).

In the other two groups (C) and (D), 12 rabbits in each group finished the 6 weeks follow-up after surgery of femoral osteotomy, fixation and serum allograft from rabbits with head injury group (C), and from rabbits with spinal cord injury group (D), 5 (41.7%) out of 12 ended up by nonunion in group (C), while 4 (33.3%) out of 12 went into nonunion in group (D). The numbers of femoral osteotomies union and nonunion are very similar to the results of the control groups from (E) to (H). The differences have no statistical significance. This may indicate that serum from rabbits with head injury and spinal cord injury contain no specific protein or humoral factor to stimulate bone healing and accordingly, they have no effect on bone healing. This may again suggest a possible mechanism which could be responsible for acceleration of fractures healing in head or spinal cord injuries of altered nerve signaling pathway in which a possible release in the damaged brain or spinal cord of factors like the classical neurotransmitters(mainly the principal inhibitory neurotransmitter GABA gamma-amino butyric acid), neuropeptides (vasoactive intestinal polypeptide VIP, calcitonin gene-related peptide CGRP), neurotrophines (nerve growth factor NGF, brain derived neurotrophic factor BDNF) to cause sympathetic relative inhibition. This in turn, as proved in other previous studies, leads to mobilization of undifferentiated mesenchymal stem cells to peripheral circulation and the fracture site. Afan et al.29 believed that the CNS has a role in the selective control of bone marrow cellularity that the denervation leads to decrease in the femoral bone marrow cellularity of undifferentiated mesenchymal stem cells and egress of abundance of these cells to the peripheral circulation.29-39

In the histo-pathological assessment of operated rabbits from all groups, after they have finished the 6 weeks follow-up and have been euthanized and their operated femora have been dissected and sent for histo-pathological assessment, we noticed that nearly all fractured femoral specimens from groups A and B rabbits of nervous tissue damage and femoral osteotomy showed mature bridging hard callus with early remodeling of the callus woven bone into lamellar bone and reformation of bone marrow, indicating fast fracture union and accelerated phases of bone healing.

Our results support the clinical and animal experimental previous studies that have suggested that traumatic brain injury or traumatic spinal cord injury may accelerate fracture healing time, increase union callus, and reduce the occurrence of fracture nonunion. Our results also, document that in experimental animals, there is no osteogenic effect of the serum taken from animals with inflicted brain or spinal cord injury as allograft to induce faster or abundant union callus at the femoral osteotomy site.

The results of in-vitro studies of the effect of serum from patients with a traumatic brain injury on cultured cells have not been uniformly conclusive. Although two studies documented activation of osteoblasts on exposure to serum from patients with a brain or spinal cord injury, that finding was not substantiated in other subsequent studies and no substance or protein has been identified as the causative agent.19,39

Despite the current lack of conclusive results, a study by Binder et al.19 found strong evidence that patients with a traumatic brain injury possess a humoral mechanism for accelerated fracture healing by demonstrating a mitogenic effect of the serum taken from those patients on cultured osteoblasts. Kureret al.39 reported similar effect in patients with spinal cord injury and heterotopic osseous. However, there is no evidence that the in-vitro culture changes, which the serum of patients produce can lead to clinically significant changes in fracture healing or fracture methods of management.38

In this context, other recent studies have shown that neurotrophins and their receptors are both expressed during fracture healing and are found in bone and cartilage. An association between nerve growth factor NGF and wound healing has also noted. Moreover, in recent transcriptional profiling studies of murine fracture healing, neurotrophic factors were shown to be expressed early after fracture.40-42

Our results of the presence of early union callus after three weeks from femoral osteotomy in 83.3% of the rabbits operated with associated inflicted brain injury or spinal cord injury are on the contrary to what Boes et al. recently, noticed of a smaller callus at three weeks at the femoral fracture site in the brain injured forty-three Sprague-Dawley rats. In a trial to explain this phenomenon they mentioned that reduced callus size contradicts the concept that traumatic brain injury increases endochondral ossification and may represent a shift from the recruitment of cells into the endochondral progression to one more direct bone formation or alternatively, the fractures have already progressed into remodeling phase of fracture repair where the size of union callus will be reduced.
Conclusion

From the results of the current study, we can conclude that fractures in animals with inflicted brain or spinal cord injury heal more expectedly, rapidly, and with formation of exuberant abundant callus which progress early into remodeling, suggesting a central neuronal control and a neural or combined neuro- hormonal or neuro-humoral mechanism to explain this accelerated osteogenesis. We can also, conclude that serum from animals with traumatic central nervous tissue damage has no osteogenic effect in vivo. To confirm this basically neural theory, a further experimental work on animals is needed, especially, to disclose the effect of sympatholytic drugs on fracture healing.

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