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THE EFFECT OF AUTOTRANSPLANTATION OF BONE MARROW WITH LASER IRRADIATION ON THE HEALING OF NON-UNION FRACTURES IN THE FEMORAL BONE OF DOGS

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ABSTRACT

In this study, the effects of autotransplantation and laser irradiation on the healing of experimentally induced non-union fractures in the femoral bone were investigated clinically and radiographically in 12 dogs. The fractures were left without fixation for 40 days and over, and following the occurrence of non-union state the fractures were treated at 3 different schedules in the experimental animals, which were divided into three groups, each containing 4 dogs:

Group 1 (Control): The non-union fractured femoral bones were treated by the routine surgical procedures using intramedullary fixation achieved with Steinmann intramedullary pins.

Group 2 (Intramedullary fixation with autotransplantation of bone marrow): Following the surgical treatment of non-union fractured femoral bone and their fixation by the Steinmann intramedullary pins (by the same procedure applied in group 1), 4-6 ml of fresh bone marrow was implanted at the fracture site. The bone marrow was aspirated freshly and aseptically from marrow cavity of the opposite femur from the same dog.

Group 3 (Autotransplantation of bone marrow with He: Ne I.R. Laser irradiation): In addition to the procedures applied in group 2, the surgical site was exposed to laser irradiation type He : Ne I.R. with a total dose of 2.7 J/cm2 for 12 irradiation sessions. Each daily irradiation session was for a period of 5 min for 3 successive days, followed by one-day rest.

The concluded results were:

1- Autotransplantation of fresh bone marrow at the site of the treated non-union fractures has a role in accelerating or improving the healing process, by promoting excessive callus formation, whereby the fracture line disappeared at the end of the 4^{th} post-treatment week. In comparison, the callus in group 1 was characterized by its poor quality and the fracture line disappeared at the end of the 5^{th} post-treatment week.

2- The use of laser irradiation with autotransplantation of fresh bone marrow, greatly improved the healing process by increasing the periosteal reaction and callus formation, and lead to disappearance of the fracture line at the end of the 3^{rd} post-treatment week. Furthermore, this technique improved the functional use of the affected limb in comparisons with groups 1 and 2.

3- The laser irradiation made the remodeling stage shorter (finished within about 3 months) in comparison with groups 1 and 2, in which it continued for more than 3 months.

الخلاصة

تأثير الزرع الذاتي لنخاع العظم والتشعيع بالليزر على التئام الكسور غير الملتئمة لعظم الفخذ في الكلاب

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في هذه الدراسة، تم استقصاء تأثير الزرع الذاتي لنخاع العظم والتشعيع بالليزر، سريرياً وشعاعياً، على درجة الالتئام للكسور غير الملتئمة، والمستحدثة تجريبياً لعمد عظم الفخذ في أثني عشرة كلباً.
بعد إجراء الكسر المستعرض في عمد عظم الفخذ ، تم تركها دون تثبيت ولمدة أربعين يوماً (أو أكثر)، لغرض حصول حالة عدم الألتئام والتي تم التأكد منها من خلال المتابعة السريرية والشعاعية لتلك الكسور.
تم علاج تلك الكسور غير الملتئمة بثلاث طرق مختلفة، من خلال تقسيم الحيوانات ، عشوائياً إلى ثلاث مجاميع، ضمت كل منها أربعة كلاب، وكما يلي:
المجموعة الاولى (السيطرة)؛ تم تثبيت الكسور غير الملتئمة لعمد عظم الفخذ فيها باستخدام سفود داخل العظم نوع ستينمان، وبأتباع الطرق الجراحية التقليدية المستخدمة لهذا الغرض.
المجموعة الثانية (الزرع الذاتي لنخاع العظم)؛ بعد تثبيت الكسور غير الملتئمة لعمد عظم الفخذ بواسطة سفود داخل العظم نوع ستينمان، تم زرع منطقة الكسر بمقدار ٤-٦ ملم من نخاع العظم الطازج والذي تم شفطه طازجاً وبطريقة عقيمة من منطقة المدور الكبير لعظم الفخذ للطرف الأخر من نفس الحيوان.
المجموعة الثالثة؛ تم علاج الكسور فيها بنفس الطرق المتبعة في المجموعتين الأولى والثانية، بالإضافة إلى تعريض منطقة إصلاح الكسر بالتداخل الجراحي، إلى جلسات من التشعيع بالليزر نوع هيليوم نيون – تحت الحمراء (He:Ne, IR)، وبجرعة كلية بلغت ٢،٧ جول/سم٢، وبواقع جلسة واحدة يومياً مدتها ٥ دقائق ولمدة ثلاث أيام على التوالي ، يعقبها يوم استراحة وبواقع أثني عشر جلسة تشعيع. تم متابعة كافة الحيوانات وللمجاميع الثلاثة سريرياً و شعاعياً ، حيث كانت المتابعة السريرية يومياً والشعاعية أسبوعياً ولمدة ثلاثة أشهر متتالية. وأثبتت نتائج الدراسة ما يأتي:

١- أدى الزرع الذاتي لنخاع العظم إلى تحسين وتسريع عملية التئام الكسور غير الملتحمة.
٢- كان لاستخدام التشعيع بالليزر مع الزرع الذاتي لنخاع العظم أثراً إضافياً في تسريع عملية التئام الكسر وتحسن مضطرد في الأداء الوظيفي للطرف المصاب.
٣- عجل التشعيع بالليزر من عملية ترميم العظم.

INTRODUCTION

A fracture could be defined as disruption of the continuity of the osseous tissue accompanied by injury of neighboring soft tissues and some times of nerves and blood vessels (1). Delayed union or non-union is the lack of union between the fractured ends, and several techniques have been followed for its treatment. These techniques include; implantation of compact bone (2 & 3), transplantation of bone marrow (4), the use of electromagnetic field (5), and invasive low intensity pulsed ultrasound (6). Low power laser irradiation has been also introduced for treatment of this type of fractures (7, 8 & 9).

The purpose of the present study was to investigate the effect of autotransplantation of fresh bone marrow on the healing of non-union fracture of the femur of the dog after intramedullary fixation, and to compare the results with those obtained after exposing the fracture area to laser irradiation type He:Ne I.R.

MATERIALS AND METHODS

Twelve adult apparently healthy dogs of both sexes and of a local breed were used. The dogs were housed under the same circumstances and given food and water ad libtum. A transverse fracture was induced experimentally in the mid shaft of the femoral bone of each dog. The fractured bone was left without fixation for more than 40 days, and a clinical and radiological follow up was done for all animals to ensure the occurrence of non-union state. The animals were then divided randomly into 3 groups, and treated as follows: Group (1); the ununited fracture was fixed by intramedullary pin, and served as control. Group (2); following intramedullary fixation of the ununited fracture, bone marrow (4-6 ml) was transplanted in the fracture site. The bone marrow was aspirated freshly and aseptically from the marrow cavity of the opposite femur, from the same dog. Group (3); the same surgical procedures followed as in groups 1 and 2, and additionally the involved limb was exposed to laser irradiation type He : Ne I.R., (He:Ne Infrared; Space Laser, Italy), with a total dose of 2.7 J/cm2 for 12 irradiation sessions. Each daily irradiation session was for a period of 5 minutes for 3 successive days, followed by one day rest. A distance of about 30 cm was left between the source of irradiation and the affected site. The dogs in all groups were followed up clinically and radiologically (daily clinical examination and weekly radiological examination), for 3 months.

RESULTS

Clinical findings:

In the first group, the fracture site was warm, reddened, swollen, painful, with subcutaneous hemorrhage, and there was loss of function of the affected limb. These signs and symptoms continued till the 5^{th} post-operative (P.O.) day and began to disappear after the 6^{th} and 7^{th} P.O. days. Similar signs were seen in group (2), but they were more sever, and disappeared within 7-10 days following surgery. The same signs were seen in group (3), but they were more severing and hematoma appeared at the fracture site. These signs and the hematoma disappeared 10-14 days following surgery and irradiation. The functional limb usage was regained at the end of the 8^{th} P.O. week in group (1), whereas it was regained at the 7^{th} P.O. week in groups 2 and 3.

Radiological findings:

In group (1), the fracture line was obvious at the first two P.O. weeks (Fig. 1). At the end of the 3^{rd} P.O. week, the reaction of the external callus was less than in the previous week and the fracture line was still evident. During the 4^{th} P.O. week, the fracture line began to disappear gradually, with little reaction of the external callus, and the bone began assuming its normal shape. The fracture line disappeared completely during the 5th P.O. week and a little reaction of the external callus and normal shape of the bone were seen (Fig. 2). During the 6th, 7th, and 8th P.O. weeks, an increased resorption of the external callus was seen and the bone assumed a greater similarity to the shape of a normal bone (Fig. 3 & 4). At the 12th P.O. week, the external edges of the compact bone above and down the fracture line were in good alignment indicating active remodeling and the bone was almost of a normal shape (Fig. 5).

In the second group, the fracture line remain clear during the 1st and 2nd P.O. weeks with a mild reaction of the external callus was seen. During the 3rd P.O. week (Fig. 6), the fracture line was hard to visualized and disappeared completely during the 4th P.O. week (Fig. 7). An increase in the reaction of the external callus was seen starting from the 1st up to the 5th P.O. weeks. At the 6th P.O. weeks, a decrease in the size of the external callus began and continued up to the 12th P.O. weeks (Fig. 8 & 9).

In the third group, the fracture line was clear during the 1st P.O. week and began to disappear gradually during the 2nd P.O. week. During the 3rd P.O. week, the fractured line disappeared completely, and the reaction of the external callus which started at the 1st P.O. week was still severing (Fig. 10). During the 4th, 5th, and 6th P.O. weeks, the size of the external callus began to decrease gradually, and disappeared almost completely at the 7th P.O. week (Fig. 11). Bone remodeling became more evident at the 8th P.O. week with complete disappearance of the external callus and the bone appeared almost normal (Fig. 12).

Figure 1: Radiograph of the limb of a dog from group (1), at the end of the first P.O. week. Note that the fracture line is visible and there is a reaction of the external callus.

Figure 2: Radiograph of the limb of a dog from group (1) at the end of the 5th P.O. week. The fracture line disappeared completely and there is little reaction of the external callus.

Figure 3: Radiograph of a limb of a dog from group (1) at the end of the 7th P.O. week. Note resorption of the external callus and that the bone assumed a normal shape.

Figure 4: Radiograph of the limb of a dog from group (1) at the end of the 8th P.O. week. Note increased resorption of the external callus.

Figure 5: Radiograph of the limb of a dog from group (1) at the end of the 12th P.O. week. Bone remodeling is continuous at the fracture site.

Figure 6: Radiograph of the limb of a dog from group (2) at the end of the 3rd P.O. week. The fracture line is almost absent and there is continuous reaction of the external periosteum.

Figure 7: Radiograph of the limb of a dog from group (2) at the end of the 4th P.O. week. The fracture line disappeared completely and there is mild increase in the size of the external callus.

Figure 8: Radiograph of the limb of a dog from group (2) at the end of the 7th P.O. week. Note resorption of the external callus.

Figure 9: Radiograph of the limb of a dog from group (2) at the end of the 12th P.O. week. Note the continued bone remodeling and the decreased size of the external callus.

Figure 10: Radiograph of the limb of a dog from group (3) at the end of the 3rd P.O. week. Note complete disappearance of the fracture line and the continuous reaction of the external periosteum.

Figure 11: Radiograph of the limb of a dog from group (3) at the end of the 6th P.O. week. Note the small size of the external callus as a result of resorption.

Figure 12: Radiograph of the limb of a dog from group (3) at the end of the 12th P.O. week. Note that the external callus is almost disappeared completely and that the bone assumed almost normal shape.

DISCUSSION

The clinical findings in the first group, reported in the present study were expectable. The redness, swelling, warmth, sensation to pain, loss of function, and hematoma are well known to occur after fracture of bone. They were all could be attributed to trauma and inflammation and the release of growth factors from macrophages and platelets in the blood clot as well as from the dead bone by lysis and acidification of the matrix (10). Inability of the animal with a fractured femur, treated with intramedullary fixation, to use the affected limb is attributed to pain sensation caused by pressure of exudates on peripheral nerve endings. Additionally, it could be due to the incomplete callus formation between the broken ends of the bone (11). Animals in this group used the limb with broken bone at the end of the 5th P.O. week. This finding is in accordance with that of other, who stated that broken limb bones fixed with intramedullary pin regain its functional usage within 9 weeks after surgery. Radiographically, external callus formation was seen at the first and second P.O. weeks, a time at which the fracture line was still visible. This finding is in agreement with that found by (12), who stated that the fracture line remains till the 10th P.O. week. During the 3rd and 4th P.O.

weeks, the external callus began to increase gradually and the fracture line began to disappear gradually at the end of the 4th P.O. week, and disappeared completely by the end of the 5th P.O. week. Cases of delayed union have been found to involve lowered osteogenic activity as a result of reduced blood supply or improper fixation (13). This could explain the small size of the external callus seen in the present study. At the 6th, 7th, and 8th P.O. weeks, bone remodeling and resorption of the external callus were seen. These processes extended till the 12th P.O. weeks. This finding is in accordance with the statement made by (14), who stated that the final stages of healing may last for approximately 12-18 months.

In animals of the second group, the inflammatory signs seen at the fracture site were more sever than those seen in the first group and could be attributed to the greater trauma induced through bone marrow implantation. These animals began using their affected limbs with caution after the 1st P.O. week and during the 2nd P.O. weeks, and this could be attributed to the ability of the transplanted bone marrow to induce new bone growth. This is supported by the finding through palpation of the fracture site of good attachment between the broken ends of the bone. This finding is in agreement with that of (15). Animals within this group regained the functional use of the affected limb at the end of the 7th P.O. week. Radiographically, there was mild external callus formation during the first two P.O. weeks and this process was more intense than in the first group, and lead to unclear of the fracture line at the 2nd P.O. week. This could be due to the transplanted bone marrow. Bone marrow is the principal source of osteogenitor cells that play an important role in bone formation (16). Furthermore , the bone marrow has the ability to stimulate the differentiation of chondroplast to osteoplasts after calcification of the matrix, thus forms a strong bridge between the broken ends of the bone (17). During the 3rd and 4th P.O. weeks, the fracture line was completely disappeared with little increase in the external callus. This is in accordance with the findings of others (15), were they found that marrow transplantation lead to an increase in callus formation during the 2nd and 3rd P.O. weeks and enhance the healing during the 4th P.O. week. Disappearance of the fracture line may indicate filling of the gap between the two ends of the broken bone by bony material. Others (4 & 18), have stated that marrow cells are capable of stimulating the formation of new bony growth and enhance the healing process. During the 5th P.O. weeks, the callus was still large and this could be due to the effect of the marrow on the periosteum and the continued formation of osseous tissue. Other (19), has found that the direct use of marrow as an autograft on the broken site or as inoculam through the skin leads to stimulation and bone formation and enhance the healing process in many cases including cases of delayed union or non-union.

In the third group, the inflammatory signs were similar to those seen in the second group. However, more extensive hematoma was seen. This could be due to the increased blood supply induced by laser irradiation (20). Dogs in this group re-used their broken limb starting on the 2nd P.O. weeks. This could be attributed to the decreased pain sensation due to laser irradiation. The latter has been found to increase the secretion of andorphin which help in reducing the pain (21). Additionally, low energy laser irradiation has been found to enhance the healing process through the improvement of bone formation (22). The complete functional use of the broken limb was attained at the end of the 6th P.O. weeks and this could be attributed to the combined effects of marrow transplantation and laser irradiation on healing and functional use. Radiographically, external callus formation was more intense than in the other two groups during the 1st and 2nd P.O. week, as indicated by almost disappearance of fracture line. This could be due to the effect of marrow transplantation and laser irradiation in stimulating periosteal reaction. In support of this, is the findings of (22 & 23), that the low energy laser irradiation enhances fracture repair through improvement of bone formation. Additionally, it improves the productive function of the bone marrow, the functional and reaparative roles of cells, oxygen supply, cell permeability, and phagocytic activity (24 & 25). The fracture line disappeared completely at the end of the 3rd week, which means that the gap between the ends of the broken bone have been filled with bony material (26 & 27). Laser irradiation enhances calcium decomposition and increases the activities of osteoblasts and fibroblasts and brings more blood to fracture sites (27 & 28). At the end of the 4th P.O. week, the external callus was decreased in size and bone remodeling has advanced, giving the fracture bone a normal shape. By the end of the 5th P.O. week, the fractured bone regained its normal shape and this could be due to the effects of laser irradiation, namely the enhancement of blood supply and lymph drainage from the fracture site. The remodeling process and resorption of the callus occurred at the 6th, 7th, and 8th P.O. weeks, which meant that laser irradiation has enhanced these processes. At the 12th P.O. week, the external callus disappeared and the fractured bone assumed a normal shape, and this indicated completion of remodeling process.

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