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Effect of allogenic bone transplantation from the iliac crest and mandible on fracture healing in rat tibia

Efecto del trasplante óseo alogénico de la cresta ilíaca y la mandíbula sobre la consolidación de fracturas en tibia de rata

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ABSTRACT

The aim of this study was to compare the healing performance of non-vascularized bone allografts harvested from the mandible and the iliac crest in an experimental rat tibial fracture model. The rats selected as subjects were divided into four groups: the jaw allogeneic bone (n = 7), the iliac allogeneic bone (n = 7), the fracture control group (n = 7), and the donor group (n = 4). The donor rats were first sacrificed, and 5 mm thick and 5 mm wide block grafts were obtained from the corticocancellous bone portion of the mandible and iliac bones, both right and left. These grafts were securely fixed with Kirshner wires between two bone fragments obtained by cutting the corticocancellous bone portion of the rats' right tibias with a disc under serum irrigation. In the control fracture group, a fracture was created only in the tibia and securely fixed with Kirshner wires. After an eight week healing period, all rats were sacrificed, and their bone tissues were collected for analysis. Healing at the fracture line was assessed by the percentage of new bone formation for each sample. Data were analyzed using the Kruskal Wallis and Mann Whitney U tests. The percentage of new bone formation in the mandibular allogeneic transplantation group was higher than in the control and iliac crest groups ($P < 0,05$). New bone formation in the iliac crest allotransplantation group was lower than in the control group ($P < 0,05$). Non-vascularized allografts of mandibular origin showed higher new bone formation in the experimental rat tibial fracture model. These findings suggest that the donor site may play a significant role in determining the biological behavior and regenerative capacity of bone allografts.

Key words: Allograft transplantation; non-vascular allograft; new bone formation; tibia fracture; rat.

RESUMEN

Este estudio tuvo como objetivo comparar el rendimiento de curación de aloinjertos óseos avasculares obtenidos de regiones donantes de cresta ilíaca y mandibular en un modelo de fractura de tibia creado en ratas hembras. Utilizando para ello, ratas seleccionadas como sujetos de estudio se dividieron en cuatro grupos: hueso alogénico mandibular (n = 7), hueso alogénico ilíaco (n = 7), grupo control de fractura (n = 7) y grupo donante (n = 4). Las ratas donantes fueron sacrificadas y se obtuvieron injertos en bloque de 5 mm de grosor y 5 mm de ancho de la porción corticocancelosa de la mandíbula y los huesos ilíacos, tanto del lado derecho como del izquierdo. Estos injertos se fijaron firmemente con agujas de Kirschner entre dos fragmentos óseos obtenidos mediante el corte de la porción corticocancelosa de la tibia derecha de las ratas con un disco bajo irrigación sérica. En el grupo control de fractura, se creó una fractura únicamente en la tibia, la cual se fijó firmemente con agujas de Kirschner. Tras un periodo de cicatrización de ocho semanas, todas las ratas fueron sacrificadas y se recolectaron sus tejidos óseos para su análisis. La cicatrización en la línea de fractura se evaluó mediante el porcentaje de formación de hueso nuevo en cada muestra. Los datos se analizaron mediante las pruebas de Kruskal-Wallis y U de Mann-Whitney. El porcentaje de neoformación ósea en el grupo de trasplante alogénico mandibular fue mayor que en los grupos control y de cresta ilíaca ($P < 0,05$). La neoformación ósea en el grupo de trasplante alogénico de cresta ilíaca fue menor que en el grupo control ($P < 0,05$). Los aloinjertos no vascularizados de origen mandibular mostraron mayor neoformación ósea en el modelo experimental de fractura tibial en ratas.

Palabras clave: Trasplante de aloinjerto; aloinjerto no vascular; formación de hueso nuevo; fractura de tibia; rata.

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INTRODUCTION

Bone graft applications are frequently used in orthopedics and traumatology for cases of delayed fracture healing or bone defects. Non-vascularized bone allografts are preferred due to their ease of surgical access, reduced donor-site morbidity, and extensive experience. However, the osteoinductive (stimulating bone formation) and osteoconductive (providing a framework for bone tissue to grow onto) properties of these grafts are thought to vary depending on the donor site, graft structure, bone density, and host environmental conditions. The ideal bone graft should possess the properties of osteogenesis (specifically, containing viable osteoblasts and osteoprogenerator cells), osteoinduction (stimulating bone precursor cells to form bone), and osteoconduction. However, while autografts (grafts taken from one's own body) are still considered the "gold standard," they have disadvantages such as donor-site morbidity, limited availability, and the need for additional surgery [1].

Allografts (bone tissue taken from another person) are an important alternative to overcome some of these problems, but they introduce new challenges such as donor-host compatibility, immune response, graft resorption, and lack of vascularization. For example, one evaluation reported that non-vascularized bone grafts can be used with success rates close to 90 %, but they also have significant complication rates [2].

Donor site selection stands out as a critical factor affecting graft quality and performance. Clinically, morbidity has been reported to be approximately 4 % for fibular grafts harvested from regions such as the and around 40 % for ilium grafts [3].

Experimental and clinical data indicate that the donor site influences not only the graft size but also its cortical/cancellous tissue ratio, vascularization potential, mechanical stability, and immune system interaction. For example, grafts harvested from the craniofacial region may have advantages in surgical access but may have limitations in providing adequate mechanical support in long bone defects [4 , 5].

In this context, examining the impact of donor site differences on bone healing in animal models is of fundamental scientific and clinical importance. This study compared the healing performance of non-vascularized bone allografts obtained from mandible and iliac crest donor sites in a female rat (*Rattus norvegicus*) tibia fracture model. This comparison aimed to reveal the impact of donor site selection on tibial bone healing in experimental fracture model.

MATERIAL AND METHODS

Animals and experimental design

This study was approved by the Fırat University Local Ethics Committee for Animal Experiments (Approval No: 2024/01-09, Date: 09.01.2024) and conducted in accordance with ethical standards. The rules regarding subject welfare specified in the Declaration of Helsinki were strictly adhered to throughout all experimental stages of the study. A total of 25 female Sprague–Dawley rats (220–250 g) were used in the study. To ensure standardization, vaginal smears were taken to ensure that all rats were in the same estrus stage. The animals were housed at 22 ± 2 °C, under a 12-h light/12-h dark cycle, and with free access to standard pellet food and water. Four rats served as donors during the surgical procedures; the remaining 21 rats were randomly divided into three equal groups (n = 7):

Fracture control group (n = 7): A tibia fracture was created, but no graft was applied.

Mandibular allogeneic bone graft group (n = 7): Non-vascularized mandibular bone allografts were placed at the tibia fracture line.

Iliac allogeneic bone graft group (n = 7): Non-vascularized iliac bone allografts were placed at the tibia fracture line.

Donor tissue preparation

Mandibular and iliac bone segments were removed from donor rats under sterile conditions (FIGS. 1 and 2). The resulting bone fragments were purified from muscle, periosteum, and soft tissue, then washed in phosphate-buffered saline and stored at -20 °C (Arçelik, 2533D, Türkiye) . Before transplantation, the grafts were thawed at room temperature in a sterile environment and were ready for use. All grafts were non-vascularized and prepared with similar sizes and shapes (approximately 5 mm thick and 5 mm long) and placed in the defect area created between the fragments.

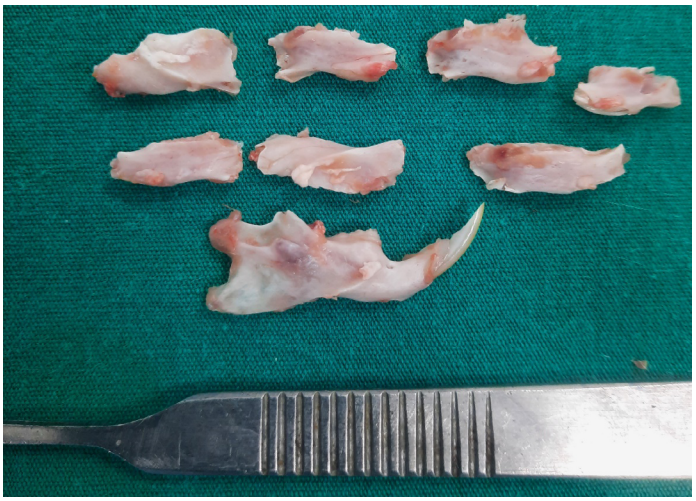

FIGURE 1. Mandibular bone grafts were prepared by cleaning the surrounding soft tissues after euthanizing the donor rats

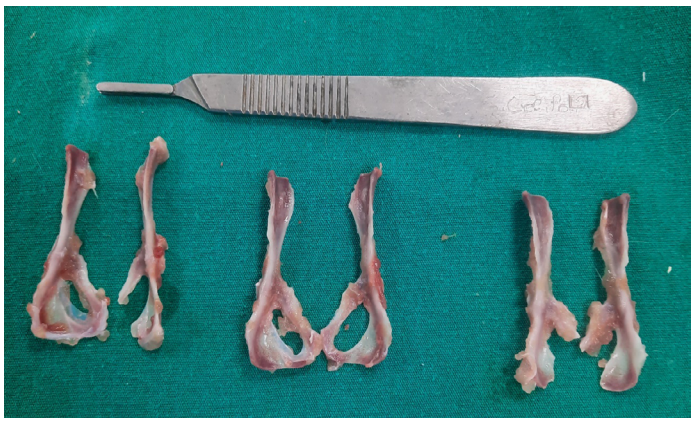

FIGURE 2. Iliac bone grafts were prepared by cleaning the surrounding soft tissues after euthanizing the donor rats

Experimental fracture model

All surgical procedures were performed under sterile conditions and general anesthesia. A combination of intraperitoneal Ketamine hydrochloride (50 mg/kg) and Xylazine (10 mg/kg) was used for anesthesia. A standard fracture model was created in the mid-diaphyseal region of the right tibia of each rat. In experiments where non-vascularized bone transplants (mandibular or iliac bone allograft) were performed, a 5 mm long bone fragment was cut and removed with a rotary instrument. Osteotomy was performed using a low-speed micromotor (Dremel 3000, Germany) with a 0.8 mm cutting tip and cooled. The fracture line was stabilized intramedullary with a 0.8 mm K-wire.

In the graft groups, a mandibular or iliac bone allograft, prepared to the appropriate size, was placed over the fracture line (FIG. 3). The soft tissues surrounding the fracture line were closed primarily. Meloxicam 1 $mg \cdot kg^{-1}$, s.c. (Bavet Meloxicam, Istanbul, Türkiye) was administered as an analgesic and Cefazolin sodium 40 $mg \cdot kg^{-1}$ i.m. (Iespor 250, I.E. Ulagay, Türkiye) as an antibiotic to all rats postoperatively. At the end of the eight-week follow-up period, euthanasia was performed with intraperitoneal high-dose anesthetic.

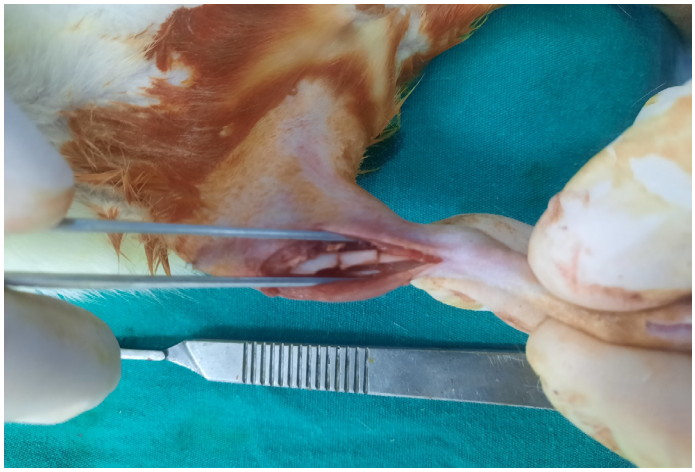

FIGURE 3. Image after transplantation of bone graft taken from donor bones to the tibia bone using Kirschner wire

Histological procedures and evaluation

At the end of the eight-week follow-up period, euthanasia was performed by intraperitoneal administration of a high-dose anesthetic, in accordance with international animal welfare guidelines. Deep anesthesia was induced using an overdose of ketamine combined and xylazine. Sufficient depth of anesthesia was confirmed by the absence of corneal reflexes and pedal withdrawal. Death was verified by the complete cessation of respiratory movements and cardiac activity. Donor rats were euthanized using the uniform protocol prior to graft harvesting. The tibiae obtained after euthanasia were fixed in a 10 % neutral formalin solution for three days (d). Following fixation, the specimens were carefully cleaned of surrounding soft tissues such as muscle, tendon, and fascia. The cleaned specimens were decalcified in a 10 % formic acid solution for approximately one week.

Decalcified tissues were processed through ascending alcohol, xylene, and paraffin series using an automatic tissue processing device (Leica TP1020, Germany). The samples were then embedded in paraffin in the longitudinal plane and blocked (Leica EG1150H-C, Germany). 3-µm-thick sections were obtained from the prepared blocks using a rotary microtome (Leica RM2125RTS, Germany). The sections were stained with hematoxylin-eosin (H&E) (Leica Autostainer XL) and evaluated under a light microscope (Olympus BX42, Japan).

The histological assessment of bone healing was based on new bone formation (NBF). For this purpose, the entire healing tissue area at the fracture site was digitally measured in each specimen. The newly formed bone tissue area was then determined, and the “new bone formation rate” (%) was calculated for each animal by dividing this value by the total healing area. The resulting rates were subjected to comparative statistical analysis between the groups.

RESULTS AND DISCUSSION

In the control and experimental groups, varying degrees and types of callus formation were observed at the fracture line. In all groups, callus tissue was found to partially or completely cover the fracture site. In the control group, callus formation was irregular and profuse, with cartilage formation predominant in these areas, and foci of neovascularization and areas of fibrous tissue were also present (FIG. 4). In some samples, areas of necrotic bone that had not been fully resorbed were noted.

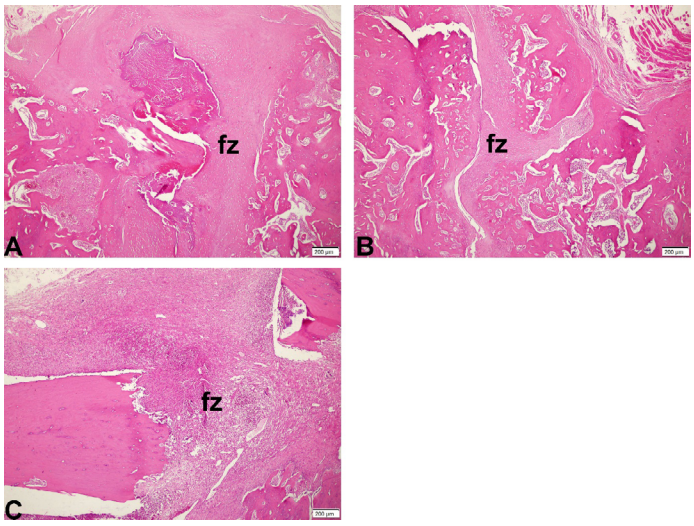

FIGURE 4. General view of the healing area in the fracture zone (fz) in the Fracture Control (A) and treatment groups (B: Jaw Transplant and C: Iliac Transplant). 4X, HxE, X=10 magnification

In the jaw (mandible) allograft transplantaion group specimens, the callus tissue was found to have a more organized architecture, and areas of NBF were more frequent and prominent within the healing callus. In these specimens, the osteoid matrix was more organized, and the trabecular pattern was formed early.

In the iliac allograft group, significant lymphohistiocytic and neutrophilic cell infiltration was detected in the healing area in three animals. This intense inflammatory response negatively affected callus organization, resulting in inadequate bone union (nonunion) in the affected areas (FIG. 5) (TABLE I).

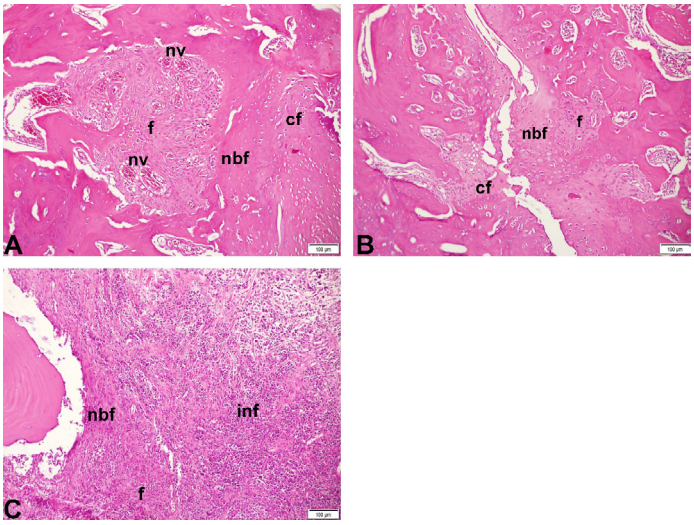

FIGURE 5. New bone formation (nbf), fibrosis or fibrous callus (f), cartilage formation (cf), neovascularization (nv) and inflammation (inf) areas in the fracture control (A) and experimental groups (B: Jaw transplantation, C: Iliac transplantation). 10X, HxE, X=10 magnification

TABLE I
New bone formation ratios (%) of the groups after the experimental period

Groups	NBF (%) Medyan/ Mean	Min.	Max.	P*
Control (n=7)	44 / 46	40	53	
Jaw Allogenic Bone Transplant (n=7)[a1]	57 / 55,43	41	63	
Iliac Bone Allogenic Bone Transplant (n = 7)[a2,b]	39 / 37,57	33	41	0.001

.*Kruskal- Wallis Test (P < 0.05). [a]: Statistically significantly different compared with the controls. [b] Statistically significantly different compared with the Jaw Bone Allogenic Transplant Group. [a,b] : Mann-Whitney U Test. [a1]: 0.017, [a2] : 0.001, [b:] 0.001 (P < 0.05). New bone formation (NBF)

In histomorphometric analysis, NBF rates showed statistically significant differences between the groups (Kruskal–Wallis test, P = 0,001). The mean NBF rate was calculated as 46 % in the control group, 55.43 % in the mandibular allograft group, and 37.57 % in the iliac allograft group (TABLE I). In pairwise comparisons, the mandibular allograft group had a significantly higher NBF rate than the control group (P = 0.017). The iliac allograft group showed a significantly lower NBF rate than both the control group (P = 0.001) and the mandibular group (P = 0.001).When evaluated together with histopathological findings, the best bone healing was observed in the jaw allograft group, while significant inflammation and limited new bone formation were noted in the iliac grafts.

The observation of a higher rate of new bone formation in the mandibular allograft group, together with more pronounced inflammation and insufficient union in the iliac crest group, is consistent with the concept that the embryological origin of the donor bone may influence the balance between graft resorption and incorporation. While the mandible is predominantly of intramembranous origin, the iliac crest is derived from endochondral bone.

Experimental and clinical studies have reported that intramembranous bone grafts tend to preserve volumetric integrity more effectively and exhibit a more predictable remodeling pattern, particularly in craniofacial applications, whereas endochondral grafts are often associated with greater resorption [4]. Within this context, the more balanced behavior of mandibular grafts—characterized by structural support combined with controlled remodeling during the early healing phase—may provide a biological explanation for the enhanced new bone formation observed at the tibial fracture site.

This study compared the effects of non-vascularized bone allografts derived from the mandible and iliac crest on bone healing in a rat tibia fracture model. After eight weeks of follow-up, the rate of NBF was significantly higher in the mandibular allograft group, while a pronounced inflammatory response and inadequate bone union were observed in the iliac allograft group.

The findings demonstrate that the donor site has a significant impact on graft biology. Mandibular bone is a tissue with strong osteoinductive capacity due to its high corticocancellous ratio and relatively rich osteoprogenitor cell content. The literature has reported that mandibular bone offers advantages in the regenerative process due to the high vascular potential and growth factor content in its trabecular structure [5 ,6 ,7]. The high NBF rates in this study are consistent with these biological characteristics.

The low NBF values of iliac grafts can be explained by an inflammatory response due to inadequate tissue compatibility and vascularization. Lymphohistiocytic and neutrophilic cell infiltrations observed at the graft site reflect immune-mediated resorption and fibrosis processes [7]. Similarly, Allsopp *et al*. [8] reported that the host immune response delays bone formation and increases osteoclastic activity in non-vascularized iliac allografts.

Vascularization is a critical factor for the integration of bone grafts. In non-vascularized grafts, the revascularization process is delayed, resulting in decreased osteocyte viability and the development of necrosis in the graft core [9]. This is particularly evident in grafts with high cortical density (e.g., iliac crest). Mandibular grafts are thought to vascularize more rapidly due to their thinner cortical structure and dense cancellous content [10]. Data obtained from this study show that mandibular non-vascularized grafts have better healing.

Ozcan *et al*. [11] implanted non-vascularized allografts taken from rat tibias into the tibias of rats with implants of different surfaces. The researchers reported that the non-vascularized tibial allografts integrated with the bone tissue and provided a three-dimensional bone tissue reconstruction around the implant. In this study, non-vascularized allografts taken from both the iliac crest and the mandible were successful in reconstructing the bone tissue.

In other experimental study Ozcan *et al*. [12], using jaw, tibia, and femur allografts from donor rats, found no statistically significant difference between the biomechanical osseointegration values of the tibia and femur grafts. They used the nonvascularized grafts with implant integration. The biomechanical osseointegration values of the jaw allografts were found to be higher than those of both the tibia and femur transplant groups. Osseointegration and bone healing were successful in all three non-vascularized transplant groups. In this study, bone healing was found to be statistically significantly higher in the mandibular allograft transplant group compared to the iliac crest group.

Taken together, the present findings suggest that the donor site may influence graft biology; however, this influence should be interpreted within the context of multiple interacting variables rather than as an isolated determinant. Factors such as embryological origin, microarchitectural characteristics (cortical-to-cancellous bone ratio) and the specific allograft processing protocol are likely to collectively shape graft behavior during fracture healing [13]. Thus, the association of mandibular allografts with increased iliac allografts and new bone formation with enhanced inflammatory response and incomplete union should not be construed as evidence of inherent or universal superiority of mandibular bone. Rather, these observations should be regarded as experimental indications that donor-specific graft properties, along with processing-related variables, may play an important role in modulating fracture repair outcomes. Additional controlled studies are required to clarify the relative impact of each factor.

The capacity of an allograft to promote fracture healing extends beyond merely providing an osteoconductive scaffold, it also depends on its potential to facilitate early vascularization and host cell migration. The comparatively higher inflammatory response observed in iliac-derived grafts represents an expected phenotype in situations where the coupling between subsequent remodeling and early angiogenesis is disrupted. Moreover, studies using small animal models have reported that bone harvested from the iliac region can achieve fracture union by the eighth week under suitable conditions; yet, in the absence of supporting histological or morphometric analyses, interpretation of the underlying biological mechanisms is relatively limited [14].

These findings demonstrate that donor site selection (mandible versus iliac crest) represents more than a simple difference in tissue source; rather, it constitutes a biological measures capable of jointly influencing host inflammatory response and early fracture bridging through factors such as the graft's microarchitectural properties, embryological origin and processing-related parameters [13 , 15].

Collectively, these data demonstrate the concept that donor site–specific graft characteristics may contribute to early fracture healing dynamics.

CONCLUSION

This experimental study indicates that the donor site is an important determinant of the osteoinductive and osteoconductive capacity of non-vascularized bone allografts .Non-vascularized allografts of mandibular origin were distinguished by a more uniform callus structure and higher new bone formation in the tibial fracture model. Nevertheless, the limited sample size and the evaluation of only histological parameters are major limitations of the study. Further studies are recommended to include different graft types, biomechanical testing, and long-term follow-up.

Funding

There is no funding.

Ethics approval and consent to participate

The present study was performed in line with the principles of The Declaration of Helsinki. Approval was granted by the Fırat University Experimental Animal Ethics Committee (Approval date: 09.01.2024, Protocol no: 2024/01-09; Elazig, Turkiye).

Conflict of interest

The authors declerate there is no conflict of interest.

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