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Original article

Evaluation of the effects of tideglusib and calcium sulfate on the healing of experimental bone defects in rabbits

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Abstract

This study aimed to evaluate the effects of tideglusib and bone graft mixture on bone healing. Tideglusib is a drug used in the treatment of various neurological disorders such as Alzheimer's disease. In a relevant study, the positive effect of tideglusib on the Wnt pathway, one of the pathways involved in bone regeneration and dentin tissue regeneration, was demonstrated. Dentin and bone tissues have structurally similar healing mechanisms. Therefore, tideglusib may have a similar effect on the bone tissue. The main goal of bone grafting is to provide bone regeneration and functional healing through remodeling. Bone graft materials are divided into four types based on their source: autogenous, allogeneous, xenogeneous, and alloplastic. Because these graft materials have various advantages and disadvantages, research continues to focus on alternative materials and applications. Sixteen New Zealand rabbits were included in this study. A unicortical 3.5 mm diameter defect was created in the tibia of rabbits under general anesthesia. The groups in the study were as follows: Group 1, left proximal tibia defect area was controlled (defect area was left empty); Group 2, left distal tibia defect area was treated with tideglusib + calcium sulfate; Group 3, right proximal tibia defect area was treated with calcium sulfate only; Group 4, right distal tibia defect area was treated with tideglusib only. Mediolateral (M/L) radiographs of the tibia were taken on the 30th and 60th postoperative days. On the 30th day, the first eight rabbits were sacrificed, and on the 60th day, the remaining eight were sacrificed for histopathological examination. New bone formation in the obtained samples was evaluated by radiological and histopathological analyses. The study concluded that the combination of tideglusib and calcium sulfate significantly enhanced bone healing compared with the other groups ($p < 0.005$). This suggests that tideglusib, either alone or in combination with bone graft materials, could serve as a promising alternative for the repair of bone defects.

Keywords: bone healing, calcium sulphate, graft, rabbit, tideglusib



Introduction

Bone grafts are used to accelerate bone healing in the treatment of trauma-related bone fractures, repair defects caused by cysts, compensate for material loss in bone, and treat congenital deformities (Bulut et al. 2001, Audisio et al. 2015). Bone grafts are classified into autogenous bone grafts, homogenous bone grafts, xenografts, alloplastic grafts, and composite grafts according to the immunologic origin of the applied material. These graft materials have various advantages and disadvantages (Durmuş et al. 2016, İyilikçi 2020). Although bone grafts generally promote increased bone healing, they can have disadvantages such as foreign body reactions and rejection. However, in the case of autografts, there was no foreign body reaction (Nandi et al. 2010, Amini et al. 2012). Studies on alternative materials and their applications continue to increase to minimize and eliminate the disadvantages of bone grafts.

Alloplastic grafts are synthetic graft materials that can be artificially obtained. They have emerged as a result of alternative searches owing to the disadvantages of autogenous, allogeneous, and xenogenous grafts (Laurencin and Freeman 2005). Alloplastic grafts can be divided into three classes: ceramic, metallic, and polymer grafts (Özkurt and Tabak 2011). Ceramics are the most similar to the inorganic structure of bones. Ceramic alloplastic grafts are also known as calcium sulfate, plaster of paris or gypsum (Szponder et al. 2013, Audisio et al. 2015). Calcium sulfate has slow solubility, is crystalline, and can be resorbed between 30-60 days (Sacha Cavelier et al. 2020). Although disadvantages such as allergic reactions and inflammation have been reported in living organisms, the important advantages of calcium sulfate include the fact that the bone formed shows a structure similar to the normal bone structure, does not cause inflammation in the tissue, its porous structure is completely resorbed, it can be used in the presence of infection, it is low cost, and resorbable (Şimşek et al. 2004, Köse 2012, Szponder et al. 2013). It has been reported that calcium sulfate does not inhibit osteoinduction; in contrast, it exhibits osteoconductive properties by forming a matrix that allows osteogenic cells and blood vessels to grow (Şimşek et al. 2004). Owing to these properties, it is currently used for the treatment of bone, osteomyelitis and periodontal defects (Ferguson et al. 2014).

Tideglusib is a chemical used in the treatment of various neurological disorders such as Alzheimer's disease (Martinez et al. 2011, Tolosa et al. 2014). Neves et al. (2017) reported a positive effect of tideglusib on dentin tissue (Neves et al. 2017). It has been reported that this effect is mediated by activation of the Wnt

pathway, which is involved in bone regeneration (Duan and Bonewald 2016). The Wnt pathway is an evolutionarily conserved pathway with well-established and diverse roles in cell proliferation and differentiation as well as in stimulating the natural repair mechanism against tissue damage (Clevers and Nusse 2012). This pathway is also involved in myogenesis and chondrogenesis (Cossu and Borello 1999). An increase in all parameters was observed by activating the Wnt signalling pathway, leading to bone growth (Morvan et al. 2006). In a study conducted by İyilikçi (2020), tideglusib triggered bone formation by increasing the release of bone morphogenetic protein (BMP) and vascular endothelial growth factor (VEGF), and it has been reported to have a positive effect on the healing of defects in calvarial bone with no toxicity (İyilikçi 2020).

Dentin and bone tissues are mineralized structures that are similar in terms of their structure and healing mechanisms (Gual-Vaqués et al. 2018). Based on the positive effects of tideglusib on calvarial bone regeneration and the treatment of dental caries, the study conducted by Guel-Vaquez et al. (2018) evaluated the regeneration efficiency of tideglusib on experimentally induced bone defects in the extremities by comparing it with calcium sulfate, an alloplastic graft, both radiologically and histopathologically. The results obtained from this study will be a resource for the treatment of bone defects and will shed light on studies on the treatment of similar diseases.

Materials and Methods

Sixteen 4-5 month old male New Zealand rabbits, with a mean body weight of 2.5 ± 0.3 kg, were used in this study. Ten days prior to the commencement of the experiment, the rabbits were housed collectively in a controlled environment. The enclosure was furnished with dust-free wood shavings as bedding material, and the rabbits were provided with unrestricted access to commercial pellet feed (Nükleon® rabbit feed, sourced from İvedik/Ankara/Turkey) and ad libitum water. The environmental conditions within the room were meticulously regulated, with a temperature range of $20 \pm 2^\circ\text{C}$ and humidity levels between 50-60%. A consistent light/dark cycle of 12 h was maintained by initiating room lighting at 7 am and terminating it at 7 pm. Importantly, the rabbits were not fasted prior to anesthesia administration. The experimental groups were formed according to the sample studies (Bulut et al. 2001, Audisio et al. 2015, Neves et al. 2017). The study groups were as follows: Group 1, left proximal tibia defect area was the control area (defect area was left empty); Group 2, left distal tibia defect

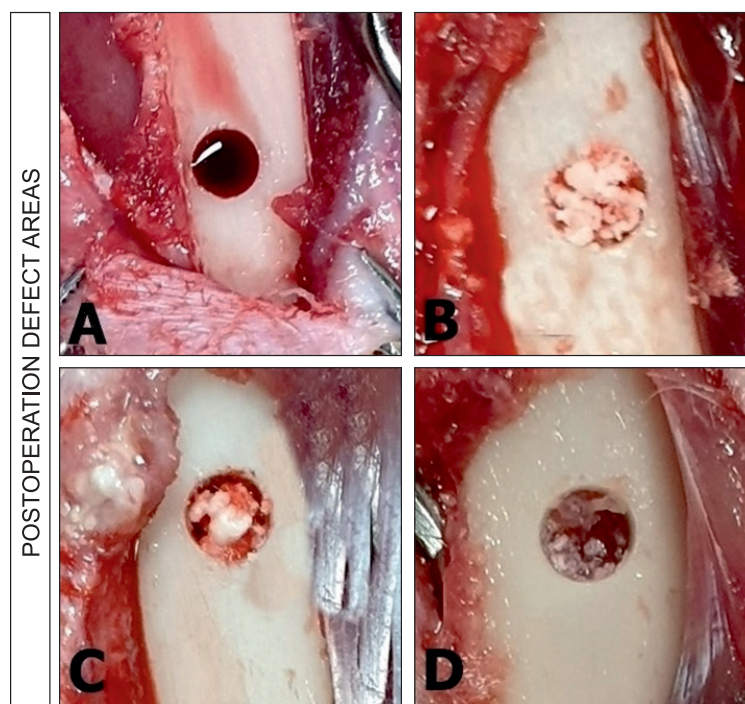


Fig. 1. Postoperative view of bone defects in rabbits in case 12. A) Untreated control defect, B) Defect treated with a combination of tideglusib and calcium sulfate, C) Defect treated with calcium sulfate alone, D) Defect treated with tideglusib alone.

area comprised of tideglusib+calcium sulfate; Group 3, the right proximal defect area was composed of calcium sulfate only; Group 4, the right distal defect area was treated with tideglusib only. Radiographic and histopathological evaluations were performed on the 30th and 60th days. Rabbits were obtained from Harran University Animal Experimentation and Research Center (HRU-HDAM). Care and feeding of the rabbits were performed at the same center. The study was approved by the Harran University Animal Experiments Local Ethics Committee (HRU-Hadyek) with the decision dated 11.12.2020, session number 2020/06, and 01-17 in accordance with the "Regulation on the Working Procedures and Principles of Animal Experiments Ethics Committees." This study was supported by the Harran University Scientific Research Projects Unit (BAP) as a research project (project number 21090).

Sterile commercial preparations of tideglusib (SML 03399- 50 mg, z96% (HPLC) Sigma Aldrich, USA) in granule form and calcium sulfate (Merck 102161 Calcium Sulfate Dihydrate Precipitated For Analysis Supelco® 500 g, GERMANY) in fine gypsum powder form were used in the study. Both tideglusib and calcium sulfate were sterilized using ethylene oxide (ETO) gas sterilization, ensuring complete sterilization without altering the material's properties.

Surgical procedure

The anesthesia protocol included intramuscular injection of 35 mg/kg Ketamine Hydrochloride

(Ketasol, Interhas, 50 mg/ml) 10 min after intramuscular injection of 5 mg/kg Xylazine Hydrochloride (Rompun, Bayer, 23,32 mg/ml). The skin was shaved on the proximal medial aspect of the left and right tibia, and antisepsis was performed using 10% povidone-iodine solution. Four unicortical defects were created on the medial aspect of the tibia, two on the right and two on the left tibia, reaching the medullary canal with a diameter of 3.5 mm and a distance of 1 cm between them with the help of 3.5 mm diameter drills and a rotary motor (micromotor). While creating the defects, the area was continuously wetted with sterile saline to prevent overheating of the bone and to prevent possible bone necrosis. The same procedures were applied to all rabbits, and 64 bone defects (four in each rabbit) were obtained. One defect in each rabbit was left empty (control group) (Fig. 1A), whereas the other defects were filled with tideglusib + calcium sulfate (equally mixed in a 1:1 ratio) (Fig. 1B), calcium sulfate (Fig. 1C), and tideglusib (Fig. 1D). The periosteum and skin in the operation area were closed using 3/0 PGA (3/0 PGA sharp 75 cm KATSAN surgical thread). All patients received 400,000 IU penicillin/streptomycin (Vetimycin, DEVA, Istanbul, Turkey) antibiotic once a day for 5 days, and 4 mg/kg carprofen (RIMADYL, Pfizer, Nebraska, USA) twice a day for 3 days. The dressings of the surgical wounds were checked daily, and the necessary wound care was provided. No lesions other than those in the tibia were created in the forelimbs or hindlimbs during the experimental procedure. The postoperative assessment of lameness in rabbits

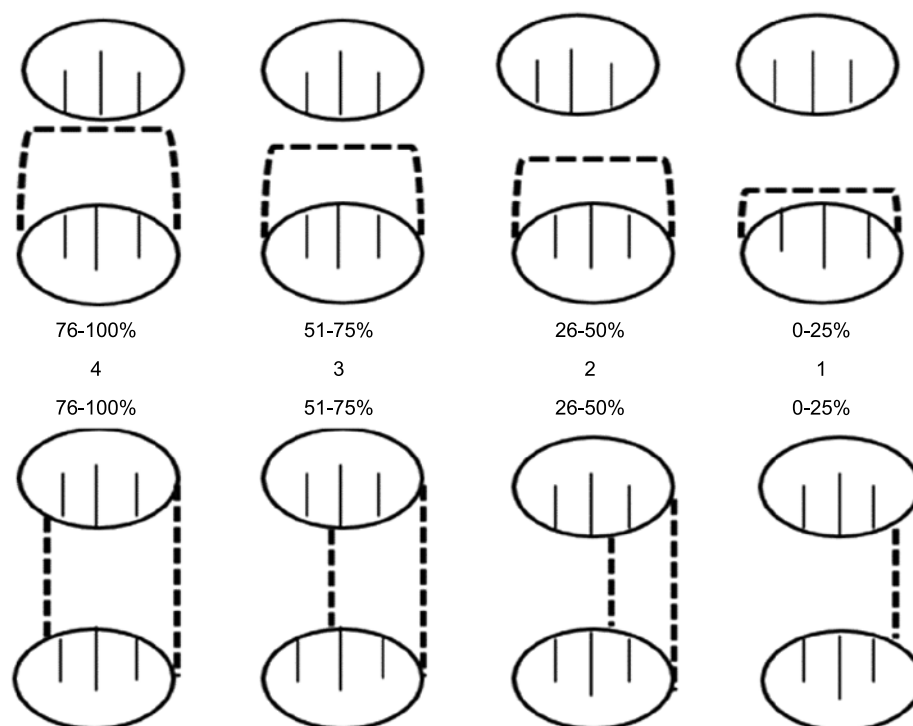


Fig. 2. Schemes to guide researchers in the quantitative evaluation of radiographic images of experimental defects (drawn by Yener).

was conducted using the scoring methodology devised by Brinker and Conner. Lameness was stratified into three distinct categories: mild, moderate, and severe. Mild lameness was characterized by a degree of uncertainty in gait, whereas moderate lameness encompassed the inability to utilize the limb or maintain it in a flexed position without bearing weight. Severe lameness indicated a pronounced impairment in locomotion. Rabbits exhibiting no discernible signs of lameness were deemed to be in a state of full health (Brinker and Conner 2008). Lameness scoring was based on the following criterion: 0, indicated no observable signs of lameness while walking. 1, indicates mild lameness, characterized by mild signs of discomfort or gait irregularity. 2, represented moderate lameness, in which there was a marked disruption of the normal gait pattern that significantly affected the animal's movements. Finally, 3 represented severe lameness, which manifested as marked difficulty in weight-bearing and significantly impeded the animal's ability to walk without obvious discomfort. Simultaneously, the daily food intake, excretory patterns, including defecation and urination, and physical attributes of the rabbits were systematically observed and documented.

Radiographic evaluations

Radiographs were performed with a Hasvet 838 HF50 70KV - 50mA veterinary portable DR X-ray machine and digitally evaluated. The exposure parameters were set to 70 kVp and 50 mA, with an exposure

time of 0.1 seconds. Radiographic evaluations were conducted at standardized time points (30 and 60 days after surgery) to assess new bone formation in the defects. Radiographic images were evaluated according to the modified Lane and Sandhu scoring method (Lane and Sandhu 1987). Each radiograph was scored between 0 and 4:0, with no callus formation; 1, bone formation filled <25% of the fracture site. 2; bone formation fills 25-50% of the fracture site. 3: Bone formation fills 50-75% of the fracture site. 4: Bone formation fills >75% or close to 100% of the fracture site. Based on the radiographic images, the degree of bone formation at the fracture site was scored in longitudinal and transverse sections, as shown in (Fig. 2).

Histological examinations

On the 30th (n=8) and 60th (n=8) postoperative days, the rabbits were euthanized via intraperitoneal injection of thiopental sodium (Pental Sodium 0.5 g I.E. ULUGAY, Istanbul) at a dosage of 200 mg/kg body weight. Bone tissues were fixed in a 10% buffered formaldehyde solution. Bone samples containing the graft site were decalcified in a 10% ethylenediaminetetraacetic acid (EDTA) solution for approximately 10 weeks. Decalcification in the tissues was controlled by changing the solution every week. The tissues in which elasticity was determined were separated into two from the grafted parts and were reduced in size. The reduced tissues were washed under running water for 24 h, and paraffin blocks were pre-

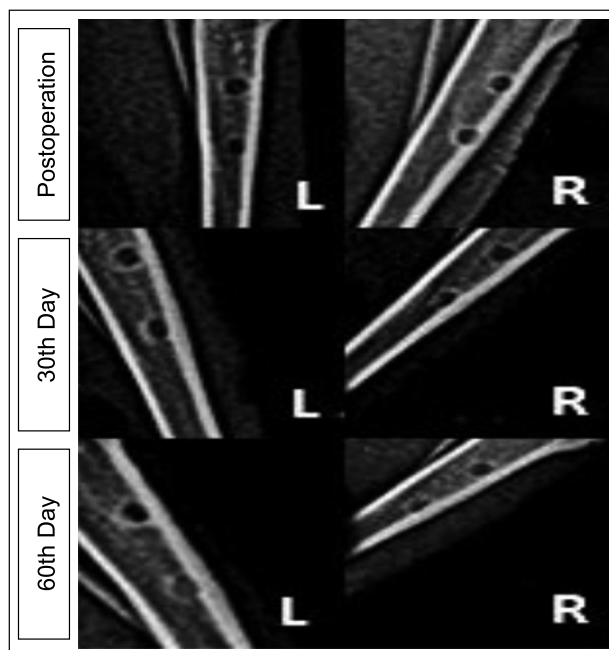


Fig. 3. Radiographic views on postoperative 30th and 60th days (case 12). L) left proximal defect control group, left distal defect tideglusib + calcium sulfate group, R) right proximal defect calcium sulfate group, right distal defect tideglusib group.

pared after routine tissue follow-up. Sections (5 μ m) were obtained from paraffin blocks. Sections were stained with hematoxylin and eosin (H&E) and Masson's trichrome (Luna 1968) and examined under a light microscope (Olympus BX51). During the microscopic examination, the thickness of the newly formed bone tissue was measured using an Olympus DP-73 digital imaging system. Bone healing was evaluated according to the method and histologic scoring parameters reported by Emery et al. (Emery et al. 1996). Each sample was scored from 1-7: 1 for fibrous tissue only, 2 for more fibrous tissue than cartilage tissue, 3 for more cartilage tissue than fibrous tissue, 4 for more cartilage tissue than trabecular bone tissue, 5 for the same amount of immature bone and cartilage tissue, 6 for more trabecular bone than cartilage tissue, and 7 for compact bone tissue.

Statistical analysis

SPSS (version 22.0) was used for the statistical evaluation of this study. The non-parametric Kruskal-Wallis H test was used to determine differences between groups. The non-parametric Mann-Whitney U test was used to compare significant differences between the 30th and 60th day results in each group. Values are presented as mean $\pm$ SEM and median. Differences were considered statistically significant at $p < 0.05$.

Results

Throughout the experimental investigation, no complications occurred during the induction of defects,

implantation of graft materials into the defect sites, postoperative pain management, or emergence from anesthesia. The rabbits exhibited favorable tolerance to the surgical procedures and were fed without any incidents. Meticulous attention was paid to the wound care and dressing procedures during the postoperative period. Uneventful wound healing was noted by the 10th postoperative day, and the sutures were removed in a timely manner. Except for rabbits 7 and 16, no signs of lameness were observed during the post-operative phase. Rabbit 7 initially exhibited moderate lameness in both legs, which resolved completely by the 4th post-operative day. Rabbit 16, however, showed severe lameness in the left leg, which gradually improved to moderate severity by the 10th day, then to mild severity by the 17th day, and ultimately resolved by the 20th day. No instances of lameness were noted in the rabbits euthanized at the conclusion of the 30th and 60th day in the study groups. Statistical evaluation revealed no significant difference in the distribution of lameness scores between the 30th and 60th day groups ($p=0.45$), suggesting that lameness levels were similar at both time points and that the treatment process did not lead to significant changes over time.

Radiographic data obtained on the 30th and 60th days were used for radiologic evaluations (Fig. 3). The radiographic evaluations of the rabbits are presented in Table 1.

All defects were clearly observed on the postoperative radiological findings. However, in radiologic examinations of the tideglusib, calcium sulfate, and tideglusib+calcium sulfate defects, the defect areas

Table 1. Radiologic evaluations of numerical measurements of the healed defect area at the 30th and 60th days according to modified Lane and Sandhu scoring criteria (0 to 4) (20).

Days	n	Control	Tideglusib+ Calcium Sulfate	Calcium Sulfate	Tideglusib	P
30th	8	1.12±0.23 ^{cA} 1	3.37±0.35 ^{aC} 3	2.37±0.51 ^{bA} 2	2.56±0.62 ^{bA} 2.5	,000*
60th	8	2.25±0.26 ^{cB} 2	3.87±0.23 ^{aD} 4	3.12±0.23 ^{bB} 3	3.37±0.23 ^{bB} 3.5	,000*
P		,000 [#]	,002 [#]	,005 [#]	,004 [#]	

The values are given as mean±SEM and median; Different superscripts (a,b) in the same column represent a significant difference ($p<0.01$); Different superscripts (A, B) in the same row represent a significant difference ($p<0.01$); Different superscripts (C, D) in a same row represents a significant difference ($p<0.05$); SEM signifies standard error of the mean. * Kruskal-Wallis H, [#] Mann-Whitney U.

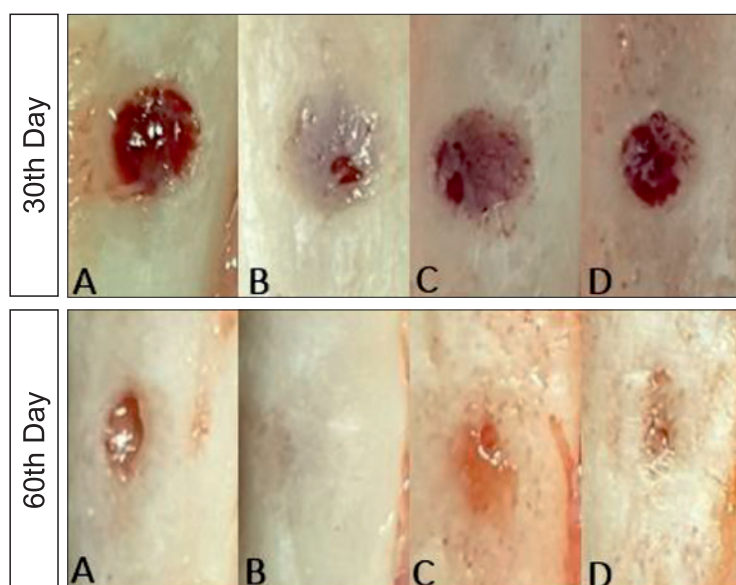


Fig. 4. Postoperative macroscopic images of the defects on the 30th and 60th days in rabbits (case 12). A) Control defect, B) Tideglusib + Calcium Sulfate (T + CS), C) Calcium Sulfate (CS), D) Tideglusib (T).

showed less radiolucent contrast than the control groups. According to radiographic data obtained on the 30th postoperative day, new bone formation was 25% in the control group, approximately 50% in the defect groups containing only tideglusib or calcium sulfate, and 50-75% in the tideglusib+calcium sulfate group. According to radiographic data obtained on the 60th postoperative day, new bone formation was 50% in the control group, approximately 75% in the defect groups containing only tideglusib or calcium sulfate, and approximately 100% in the tideglusib+calcium sulfate group.

In line with the macroscopic findings, examination of the bone defects at the end of 30 days showed that the defects were not completely closed in the control group. More bone formation areas were observed in the defects treated with tideglusib and calcium sulfate than in the control group. In the defect areas where tideglusib and calcium sulfate were used in combination, new bone formation was observed more frequently than in the

other groups. In some cases, most of the defects were closed, and very few cavities were observed. At the end of the 60-day period, the defects in the control group were not completely filled with new bone formation. Most of the bone formation was completed in the defect areas treated with calcium sulfate. In the defect areas containing tideglusib, most of the new bone formation was completed, and the defect area was completely filled in 4 cases. In cases where tideglusib and calcium sulfate were combined, the defects were completely filled with new bone formation; however, some defect traces could be identified in two cases (Fig. 4).

Microscopic images are shown in Fig. 5, and the bone healing evaluations on the 30th and 60th days according to the modified histological scoring criteria (Emery et al. 1996) are summarized in Table 2.

In the histological findings of the 30-day groups, a very small amount of fibrous and cartilage tissue was formed in the control group. In the calcium sulfate group, smaller amounts of fibrous tissue and active

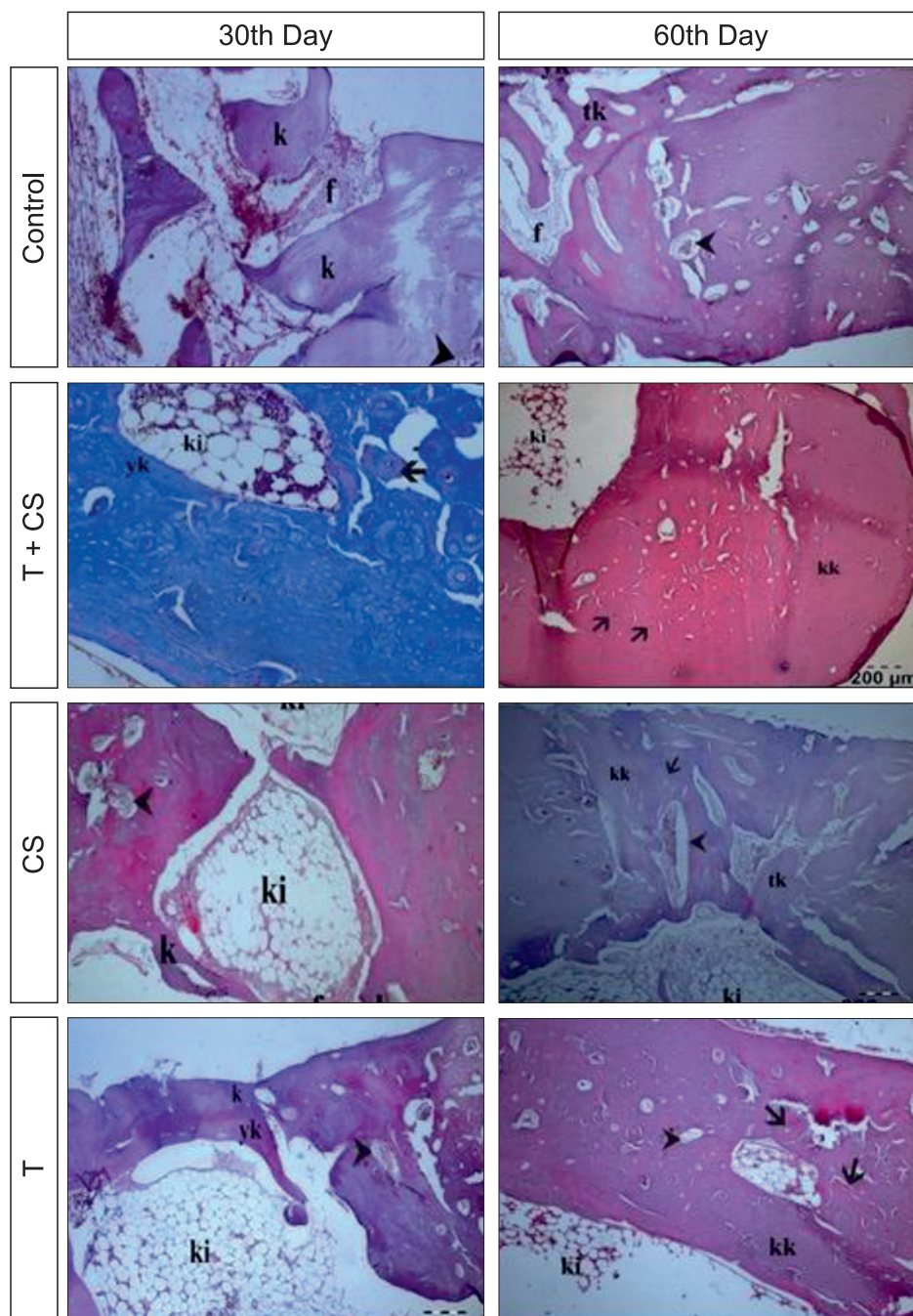


Fig. 5. Histopathological Examination of thirty-day rabbit groups, k: cartilage, f: fibrous tissue, ki: bone marrow yk: new bone, tk: trabecular bone, arrowhead: osteoblast, arrow: Havers canal. Histopathologic examination in sixty-day rabbit groups, yk: new bone, tk: trabecular bone, f: fibrous tissue, kk: compact bone, ki: bone marrow, ok: Havers canal, arrowhead: osteoblast, hematoxylin and eosin stain, scale bars = 200 μ m, x200 (case 12).

osteoblasts were observed among the prominent cartilaginous tissues. In the tideglusib group, cartilage tissue merged with new bone tissue, and a smaller amount of fibrous tissue was also observed. In the calcium sulfate and tideglusib groups, almost complete closure of the defect area was observed. Although new bone tissue formation was predominant in the defect area, small foci of cartilage tissue with trabecular bone were detected in a smaller number of specimens. Haver

canals were partially formed in areas where new bone formed towards the compact bone.

Histological findings in the 60-day groups demonstrated that in the control group, the two ends of the defect area were joined by thin new bone tissue, and in this area, the presence of trabecular bone was more predominant. In the calcium sulfate group, near-complete closure of the defect area just below the area covered by a very thin fibrous capsule was observed.

Table 2. Numerical measurements of the healed defect area on the 30th and 60th days according to modified histologic scoring criteria (0 to 7) (22)

Days	n	Control	Tideglusib+ Calcium Sulfate	Calcium Sulfate	Tideglusib	P
30th	8	1.96±0.40 ^{cA} 2	5.89±0.61 ^{aC} 6	4.14±0.89 ^{bA} 4	4.48±1.1 ^{bA} 4.5	,000*
60th	8	3.93±0.45 ^{cB} 4	6.77±0.40 ^{aD} 7	5.45±0.40 ^{bB} 5.5	5.89±0.40 ^{bB} 6	,000*
P		,001 [#]	,001 [#]	,001 [#]	,001 [#]	

The values are given as mean±SEM and median; Different superscripts (A, B) in the same row represent a significant difference ($p<0.01$); Different superscripts (a, b, c) in the same column represent a significant difference ($p<0.01$); SEM signifies standard error of the mean.

* Kruskal- Wallis H, [#] Mann-Whitney U

In areas of new bone formation close to the compact bone, the Haver's canals were partially formed. The defect repair was almost complete in the tideglusib group. Notably, many Haver's canals were formed in a compact bone. In the calcium sulfate and tideglusib groups, it was observed that the healing process in the defect area ended, and compact bone formation was completed. The healing area was significantly greater than that of the other groups. It was found that the compact bone contained Haver's and Volksman's canal and osteocyte structures in normal bone tissue.

Discussion

Bone defects are one of the most serious problems that negatively affect life, and clinicians have difficulty treating them (Amini et al. 2012). Bone grafting is a method used to treat damaged bone tissues (Santana and Trackman 2006, Amini et al. 2012). Many graft materials and biocompatible alternative materials have been developed to accelerate the healing of bone tissue whose anatomical tissue integrity is disrupted for various reasons, and to strengthen the bone, and studies on this subject continue (Efeoğlu 2003, Özeç and Yeler 2003).

When calcium sulfate is used as a graft, capillaries and perivascular mesenchymal tissues in the bone can easily invade owing to their osteoconductive crystalline structure (Sacha Cavelier 2020). They allow the proliferation of connective tissue cells and contribute to rapid healing of soft tissues at the graft site (Yahav et al. 2020). Studies have shown that calcium sulfate can be easily used in combination with local antibiotics or drugs containing growth factors to treat diseases related to the bone tissue (Ferguson et al. 2014).

Tideglusib, a member of the thiazolidinedione family, is an irreversible inhibitor of GSK-3 β . This drug contributes to bone regeneration by inhibiting GSK-3 β and activating the Wnt/ β -catenin pathway (Domínguez et al. 2012, Arioka et al. 2014). Studies investigating the

efficacy of tideglusib in osteogenesis in combination with autogenous and xenogenous grafts have been reported in the literature (Holmen et al. 2005, Arioka et al. 2014). In our study, calcium sulfate and tideglusib, an Alzheimer's disease drug, were used simultaneously.

In experimental studies conducted to investigate bone healing, tideglusib has been studied either alone or in combination with various grafts. In a study on rats, a 5 mm diameter defect was created on the calvarial bone, and tideglusib was combined with an autograft and a gelatin sponge. Cone beam computed tomography imaging was performed and it was reported that the healing of tideglusib combined with both materials was better than the control group and statistically significant ($p<0.05$). In the same study, tideglusib application in calvarial bone defects was reported to increase bone mineral density and new bone areas by decreasing apoptosis and increasing osteoclastogenesis (Aysan et al. 2020). In another study conducted by İyilikçi (2020) on the healing of defects in calvarial bone, xenografts and autografts were used alone or in combination with tideglusib (İyilikçi 2020). The results of the study showed that there was a significant increase in new bone formation in the groups in which tideglusib was used with grafts for all parameters examined, and that tideglusib synergistically enhanced the effect of grafts.

In a study conducted by Comeau-Gauthier et al. (2020), the efficacy of tideglusib alone on the cortical defect in the femur bone was investigated and 7, 14 and 28 days postoperative evaluations were conducted and it was noted that tideglusib increased bone healing compared to the control group and it was statistically significant ($p=0.04$) (Comeau-Gauthier et al. 2020). In the present study, in the group in which tideglusib was used alone in the defect created in the tibial bone, it was observed that its efficacy on healing was higher in radiographic evaluation, and in the radiographic evaluations made on the 30th and 60th days, the filling of the defects with new bone formation was observed

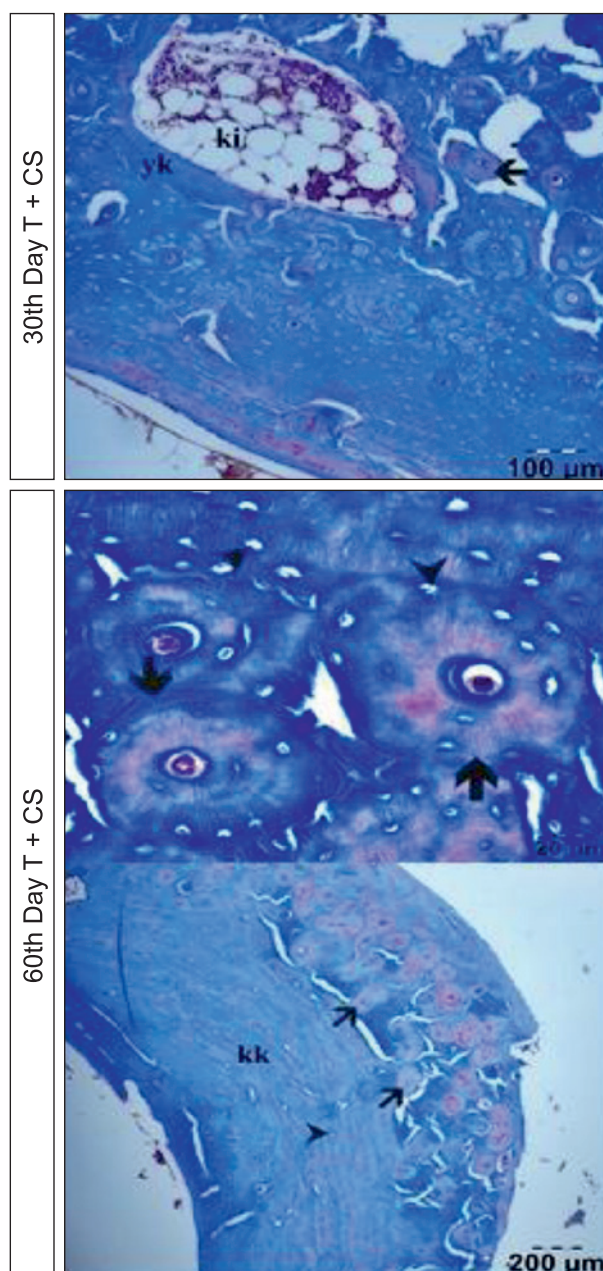


Fig. 6. Day 30 and day 60 Tideglusib + Calcium Sulfate group, yk: new bone, kk: compact bone, ki: bone marrow, arrow: Havers canal, arrowhead: osteocyte, Masson's Trichrome, day 30, $\times 100$, day 60 (middle), $\times 400$, day 60 (bottom), $\times 40$ (case 12).

as 50% and 75%, respectively. Radiologically, it was statistically significant that tideglusib contributed positively to bone healing on the 30th and 60th day ($p < 0.001$).

In a study conducted on rabbits, a 1 cm osteotomy operation was performed on the tibia bone, and bone healing was evaluated at 2, 4, and 6 weeks after calcium sulfate was applied separately and in combination with chitosan, a chitinous substance. In this radiographically evaluated study, it was reported that the corticalization in the bone continued in the 6th week, and the calcium sulfate group was better than the chitosan group; however, the healing in the group in which the two were combined was better than that in both groups (Cho et al. 2005).

Jiang et al. (2020) carried out a study investigating the effect of calcium sulfate in combination with aspirin, which is mentioned in the literature as a painkiller and blood thinning drug, on defects by creating defects with a diameter of 3.5 mm in rat tibia (Jiang et al. 2020). Radiography and micro-computed tomography evaluations of the study were performed at weeks 4 and 12 and it was reported that calcium sulfate combined with aspirin significantly increased the bone volume/total volume scale compared to the control group, and the difference was statistically significant ($p < 0.05$).

In parallel with the literature, in studies in which graft materials and drugs used as alternative graft materials were combined (Cho et al. 2005, Hua et al. 2010,

Papadimitriou et al. 2015, Aysan et al. 2020, Zhao et al. 2020), it was also found in the present study that healing was better compared to the other groups.

In a study examining the efficacy of tideglusib on bone healing in calvarial defects, collagen type 1 and osteocalcin, which increase osteoblast activity, were significantly higher in tideglusib-containing groups than in control groups ($p < 0.05$) (Dumas et al. 2009). In the present study, in the histopathologic examinations on the 30th and 60th day, it was observed that the healing in the defect area where tideglusib was used alone and combined with calcium sulfate was better than in the control group ($p < 0.001$). This is consistent with the results obtained from the radiological findings.

In a study involving the combination of calcium sulfate with an alternative graft material (Papadimitriou et al. 2015), simvastatin, a drug used in the treatment of hyperlipidemia, inorganic bovine bone graft, hydroxyapatite, and collagen sponge were utilized. In this study, defects were created in rabbit femurs and histopathologically evaluated at weeks 4 and 8. New bone formation was reported to be higher in the bovine inorganic bone graft+calcium sulfate group at the 4th week and higher in the simvastatin+calcium sulfate group at week 8th. As a result of the study, it was reported that simvastatin, a drug used in cholesterol treatment, could be safely used locally in bone defects.

In another study by Yang et al. (2012), calcium sulfate was combined with polyamino acid (PAA) a drug used to support the immune system in cancer treatment by creating unilateral femoral condylar defects in rabbits and evaluated by histological studies at weeks 4, 8, 12 and 16 (Yang et al. 2012). Western blot analysis revealed that the calcium sulfate + PAA group had significantly increased BMP-2 and VEGF formation compared to the control group and contributed to ossification and bone marrow formation.

In the present study, the histopathologic evaluation of healing in terms of new bone formation in the defect area in the calcium sulfate + tideglusib group was better than that in the other groups. Furthermore, on the 30th day, it was found that the defect area was almost completely closed and Haver's canals started to form partially in the areas where new bone formations were formed towards the compact bone ($p < 0.001$). On the 60th day, the healing process in the defect area ended and compact bone formation was complete ($p < 0.001$).

Conclusion

In conclusion, based on the findings obtained from this study and the literature, it was deduced that tideglusib is a graft material that can be used alone or in

combination with bone grafts in the repair of bone defects to minimize labor, time, and economic losses by accelerating the repair time of bone defects. Additional in vivo studies to evaluate the mechanism of action of tideglusib on bone formation will provide a better understanding of its contribution to bone healing. The present study will help researchers in future studies.

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