The effectiveness of varying of Tideglusib gel concentrations in ridge preservation of osteoporotic models

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ABSTRACT
Background: Repairing impaired hard tissues in osteoporotic patients posed challenges with restricted treatment modalities. Meanwhile, Tideglusib is regarded as an exciting treatment for Alzheimer's disease; its applications for ridge preservation in osteoporotic rabbits are not investigated. The aim of this research was to investigate the effects of Tideglusib gel in osteoporotic rabbits with empty sockets. Thirty-two male rabbits weighing 2.5 - 3 kg were used for the study. Then the mandibular first molar of each rabbit was extracted. The rabbits were allocated into 4 study groups: 1. Control normal rabbit with socket healed normally; 2. Osteoporotic rabbit with socket healed normally; 3. Osteoporotic rabbit with socket received 5 µM Tideglusib gel; 4. Osteoporotic rabbit with socket received 10 µM Tideglusib gel. At four weeks, the rabbits were then slaughtered. To assess the bone development in the extraction sockets, histopathologic and histomorphometeric analysis were carried out.
Results: After 4 weeks of socket augmentation, a significant increase in mean % of mature bone (MB) in the control group and two treated groups. While Group 2 showed the significantly lowest mean % increase in MB. Also, a significantly mean % increase of collagen deposition was generated in all study groups. However, group 2 showed the statistically significantly lowest mean % increase. Group 4 demonstrated significantly higher new bone formation than those of groups 2 and 3.
Conclusion: Tideglusib gel could accelerate bone healing through an increase in the trabecular area and elevate collagen deposition in the extracted socket of a rabbit. A specific dose- dependent response of Tideglusib gel was observed in histological and morphometric parameters, indicating that 10 µM of Tideglusib gel is considered an effective rational therapy for post-extraction socket repair, particularly in the osteoporotic model.

1. Introduction

Following tooth extraction, reduction of width and height of the alveolar ridge occurred. It is due to bone remodeling during the healing process and losing its physical stimulation, which is an irreversible phenomenon. Six months following tooth extraction, 50% of the initial alveolar ridge width will probably be lost (1).

Therefore, alveolar ridge preservation (ARP) is intended especially to reduce bone loss following extraction in order to maintain the hard and soft tissues at the extraction site. This maintenance is essential to facilitate the effective insertion of implants (2).

Osteoporosis is a disease caused by an imbalance in the interaction between osteoblasts and osteoclasts that results in the loss of bone mass. This reduced bone mineral density causes premature fracture. Also, osteoporosis can delay the regeneration of defective bone (3).

The development of the bone regeneration process, especially in osteoporosis, is challenging; consequently, it is essential for the use of biomolecules to promote the body's healing processes in order to restore tissue to its original condition and function (4).

Tideglusib is a non-ATP-competitive, irreversible, and selective inhibitor of glycogen synthase kinase 3-β (GSK-3β) (5). It prevents apoptosis, decreases tau phosphorylation, and irreversibly inhibits GSK-3 in neurons and neuroblast cells.

It also triggers the Wnt/-cat signaling pathway for healing and promotes stem cells.

(6) The activation of Wnt/β-cat signalling is an immediate early response to tissue damage that is essential for stimulating the cellular-based repair in all tissues (7).

In the dentistry literature, tideglusib was extensively studied in a variety of trials.

According to a previous study, in contrast to MTA, tideglusib has been suggested to promote reparative dentine production and increase mineralization at the site of injury (7)

Furthermore, another investigation proposed the use of bioactive glass encapsulated by tideglusib in pulp therapy, which promotes more rapid dentin production and better repair. (8)

According to another research, tideglusib at a dose of 100 nM had a beneficial effect on the growth of stem cells and increased the number of viable pulpal cells (9). Using clinically healthy animals, some in vitro studies have demonstrated the favorable impact of tideglusib powder on bone regeneration when combined with bone grafts. (10,11)

But no studies focused on bone regeneration and bone repair of varying concentrations of Tideglusib gel for ridge preservation under osteoporotic conditions. This study was conducted to assess and compare new bone formation within empty sockets of rabbits under osteoporotic conditions treated with different concentrations of Tideglusib gel using histological examinations, including hematoxylin and eosin (H&E), Masson's trichrome special stain, and histomorphometeric analysis.

Material and methods

Sample size calculation:

Sample size was estimated using G*power 3.1.9 software with an alpha level of .05, a power of 95%, and a total number of 32 rabbits was required based on data reported by Lektemur Alpan A et al. [(10).]

Thirty-two adult male rabbits, weighing 2.5 - 3 kg, were selected. The study's care of animals methods adhered to experimental protocols authorized by the ethics committee of the Faculty of Dental Medicine for Girls, Al-Azhar University(Code: REC-PD-24-31) .

The animals were equally divided into four groups: eight rabbits in each group: the first group, a control group in which normal rabbits whose sockets were left empty to heal normally; the second group, in which osteoporotic rabbits with alveolar sockets were left empty to heal normally. Group 3: Osteoporotic rabbit with an alveolar socket filled with 5 µM Tideglusib gel. Group 4: in which the osteoporotic alveolar socket was filled with 10 µM Tideglusib gel.

Osteoporosis experimental model:

For group 2,3, and 4; Osteoporosis was experimentally induced by receiving only daily methylprednisolone hemisuccinate (MPH) injections of 1.5 mg/ kg/day for 28 days (12). Then the rabbits were anesthetized and mandibular left first molar was extracted.

Tideglusib gel formulation

Tideglusib powder (Sigma-Aldrich business) amount was calculated using the molarity calculator equation found at https://www.selleckchem.com/products/tideglusib.html. and dissolved in dimethyl sulfoxide solution (DMSO) to obtain the 5 µM and 10 µM stock solutions. Then, to create poloxamer 407 gels, mix poloxamer 407 into distilled water while stirring consistently. To obtain 5µM and 10µM of tideglusib gel, combine the prepared gel and two different concentrations of tideglusib stock solution.(13) In groups 3 and 4, Tideglusib gel was placed inside the socket. At week 4 after socket preservation, rabbits were sacrificed by slaughter in all groups.

Histopathological Analysis

Following the extraction of the mandible with the socket, specimens were decalcified and fixed in paraffin and were sliced in a line with the extracted socket's axis at a thickness of 5 µm. In accordance with approved procedure, fixed samples have been handled to create non-demineralized sections (14). Sections were stained with H&E and Masson's trichrome (15,16) and examined using a light microscope, and then the slides were analyzed histomorphometrically using computer software.

Qualitative histomorphometric analysis:

Five microscopic fields for each slide were photomicrographed by a digital camera mounted on a research light microscope (Leica Qwin 500, England) at the Pathology Department, Faculty of Dental Medicine for Girls, Al Azhar University. Then the percentage area (%) of new mature bone and collagen deposition within the total specimen area was automatically calculated.

The results

Hematoxylin and Eosin (H&E) staining

In group 1 (the control group), there is a newly formed bone that contains numerous osteocytes with many Haversian canals, and Volkmann's canal fills the normal extracted socket.(Fig. 1 A). By contrast, in group 2, a network of woven bone is made up of tiny bone matrix segments that connect to one another that is occupied with many osteocytes surrounded by wide lacunae. Also, these are bone spicules dispersed in fibrous connective tissues (Fig. 1B).

While in group 3, there are moderately large bone trabeculae that are filled with many osteocytes and Haversian canals. This bone trabeculae is lined by plumb osteoblasts that are surrounded by fibrous connective tissues (Fig. 2 C). Whereas in group (4), large bone trabeculae occupied the socket area; these trabeculae contain numerous osteocytes with wide lacunae and many Haversian canals. The bone trabeculae are lined by many osteoblasts, and there are many blood vessels in the fibrous connective tissues around them. (Fig. 2 D).

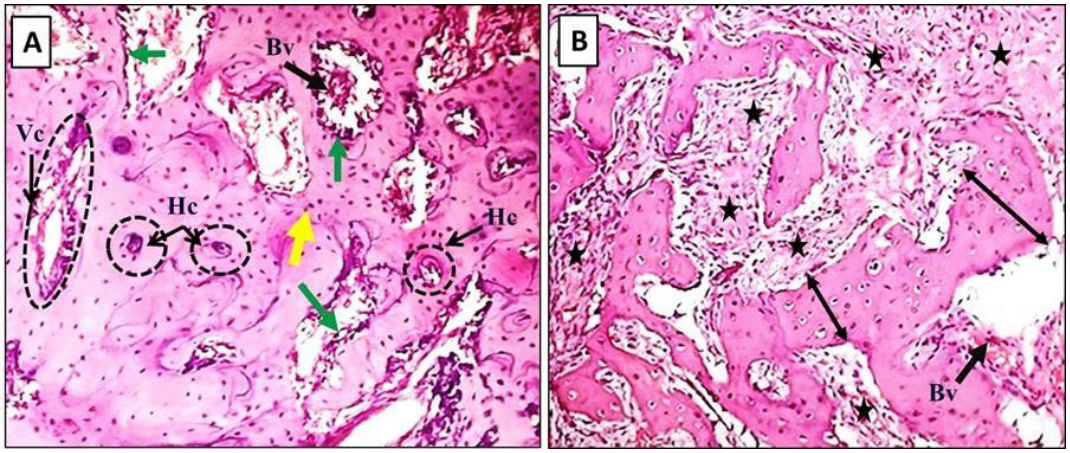

Figure (1): Histological micrograph (A) showed group 1 with newly formed compact bone. (B) a histological micrograph of group 2; showed small spicules (double head arrow) and fibrous connective tissues (black stars); Haversian canals (Hc) (central canal) and Volkmann's canal (Vc) (perforating canal); osteoblasts (green arrows), blood vessels (Bv) (H&E Orig. Mag. X 100).

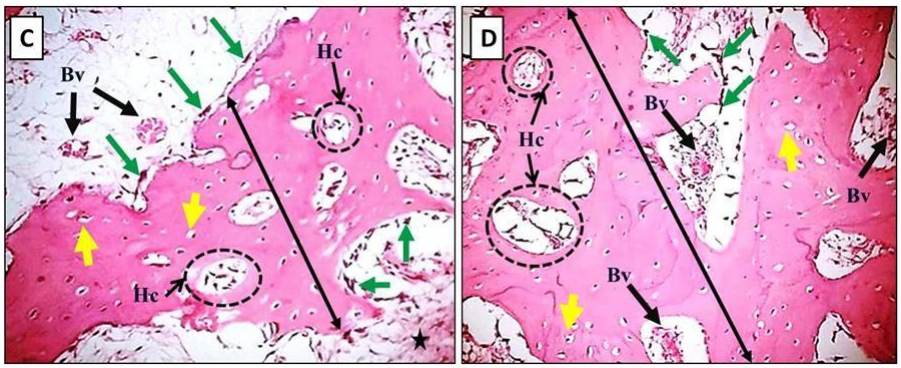

Figure (2): Histological micrograph of group 1 (C); showed moderately large bone trabeculae (double-headed arrow). (D) A histological micrograph of group 4 showed large bone trabeculae (double-headed arrow); osteocytes (yellow arrows) and Haversian canals (Hc) (central canal), osteoblasts (green arrows), and blood vessels (Bv) (H&E Orig. Mag. X 100).

Masson's trichrome staining:

Masson's trichrome was used to stain the socket healing process. Notably, the collagen of newly formed bone will appear green, and mature bone development was established by the appearance of red stains. Group 1 exhibits a socket almost filled by normal architecture trabecular bone with osteocytes and blood vessels (Fig. 3 A). In contrast, group 2 displayed large areas of immature bone with a few number of matured spicules of bone (Fig. 3 B). (Fig. 4 D). Group 3 showed an increased maturation and thickness of the bone (Fig. 4 C). Group 4 showing thick, comparatively maturing trabecular bone that fills the socket partially to entirely.

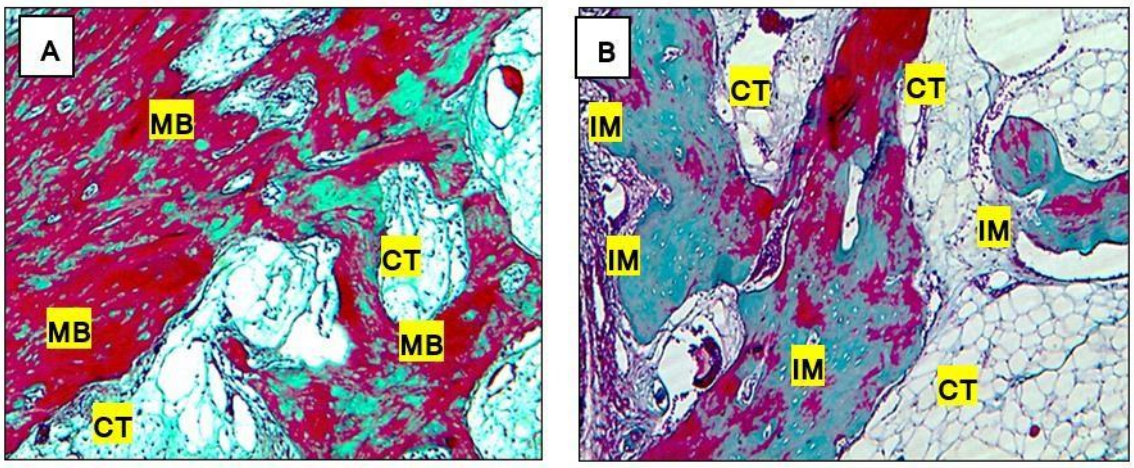

Figure (3): Histological micrograph of group 1 (A); showed an extraction socket that normally healed with mature trabecular organization (MB). (B) histological micrograph of group 2; showed immature bone (IM) mixed with mature bone trabeculae and surrounded by fatty connective tissues (CT) (Masson's trichrome stain, original magnification x40).

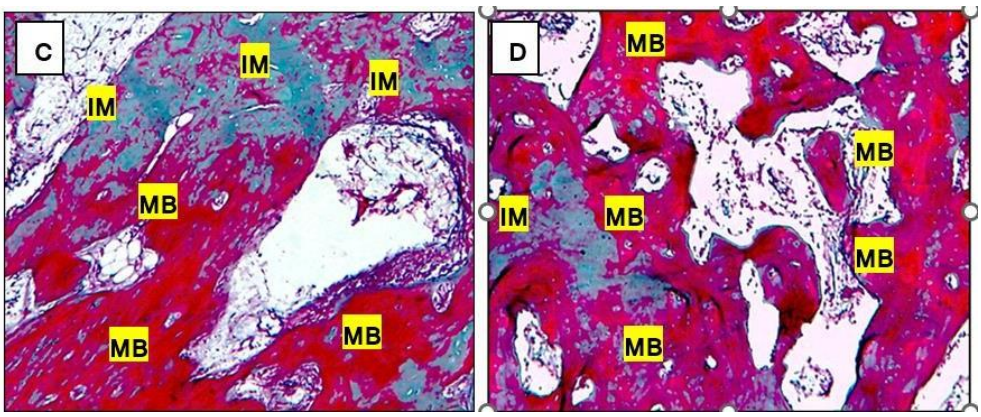

Figure (4): C; histological micrograph of group 3 showed mature trabecular organization (MB) mixed with large areas of immature bone formation (IM), and (D) group 4 showed the mature trabecular organization (MB) interrupted with immature bone (IM) (Masson's trichrome stain, original magnification x40).

Table (1): Comparison of means and SD for % of MB and collagen deposition between four groups.

	Mature bone (MB)area %	**Collagen deposition %**
	Mean± SD	Mean± SD
Group 1	51.7±4.4[a]	29.29±3.28 [a]
Group 2	17.1±1.3[d]	19.12±2.25 [b]
Group 3	29.6±4.5 [c]	22.69±5.78 [a]
Group 4	45.8±3.1 [b]	20.57±5.21 [a]
***P*-value**	0.000	0.03

The percentages of newly formed bone were 51.7±4.4%, 17.1±1.3%, 29.6±4.5, and 45.8±3.1% in the four groups, respectively. While the corresponding collagen deposition % in the four groups was 29.29±3.28%, 19.12±2.25, 22.69±5.78, and 20.57±5.21% (Figure 5).

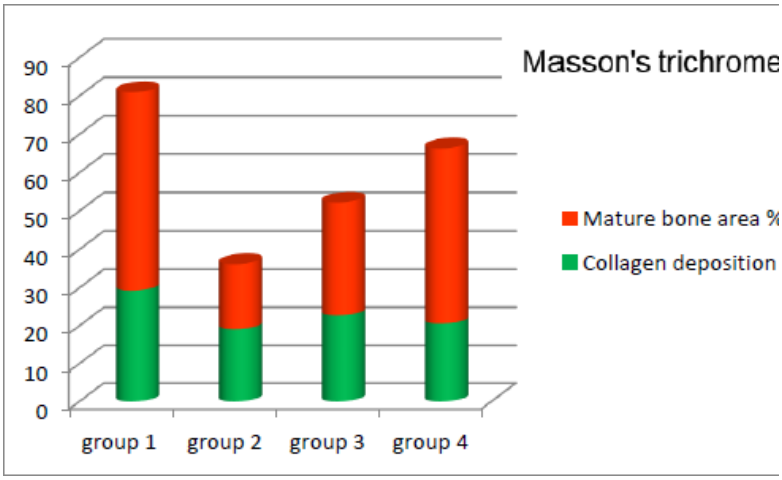

Figure (5): Bar chart showing the histomorphometric results of study groups.

By comparing four groups at the end of the study, we found that group 1, followed by group 4, showed the statistically significantly highest mean % increase in MB, then group 3. By comparing the two treated groups, group 4 showed a significantly much higher new mature than group 3, indicating that 10 μM Tideglusib gel concentration is more effective than 5 μM. In contrast, Group 2 showed the statistically significantly lowest mean % increase in MB (P value < 0.000).

Analysis of overall percentage of collagen deposition revealed that a significantly mean % increase of collagen deposition was generated in all study groups. However, group 2 showed the statistically significantly lowest mean % increase (P value < 0.03).

Statistical evaluation

Data are shown as mean ± standard deviation. ANOVA test was used to compare between groups, followed by Tukey's post hoc test for pairwise comparison. A *p*-value of less than 0.05 was considered significant.

Discussion

The animal model that has been used in this study was rabbits. Their size, haversian remodeling, and epiphyseal plate closure make them a viable model of osteoporosis (17). In particular, the study of bone anabolic drugs and the preservation of cortical bone strength depend heavily on Haversian remodeling (18). In this study, glucocorticoids at a dose of 1.5 mg/kg/day were used to induce osteoporosis, as this method produced substantial loss of bone in the lumbar spine and other anatomical areas, while greater dosages of about 2 mg/kg/day were fatal (12).

Tideglusib was chosen in this current research since tideglusib is a small molecule that overcomes most of the shortcomings of growth factors. It is protein stable, has a low propensity to trigger an immunological reaction (20) and a low risk of cross-species contamination(21).

In the current study, Masson staining were chosen to evaluate the collagen deposition and new bone formation., Collagen deposition plays a crucial role in bone remodeling and healing because collagen is the main organic component of bone matrix (22, 23).

The current study offers pertinent data regarding how current treatment improves osteoporotic bone of the socket. After four weeks, most of the socket of control and group 4 were healed by appropriate new mature bone tissue. while group 3 exhibited nearly equivalent mature and immature bone formation. Only a few bone areas were seen in the osteoporotic group, and the socket was mostly filled with fibrous connective tissue containing fat cells.

In further quantitative analysis of collagen deposition, % showed a significantly much higher new collagen fibers (immature bone) in control and treated groups than in the osteoporotic group. The osteoporotic group showed the slowest rate of bone healing when compared with other groups. This is in accordance with a previous study that revealed that severe bone remodeling and a retardation of osteoblast differentiation occur when osteoporosis develops with delaying the development of new bone in the extraction socket and osteotomy site (24).

Our findings suggest that Tideglusib gel improves bone formation, which may be explained by Tideglusib activating the WNT/ß-catenin signaling pathway by inhibiting GSK-3ß (25).

This pathway regulates the postnatal bone synthesis (26). According to Arioka et al., local administration of GSK-3 inhibitors may promote bone formation by promoting osteoblastogenesis and inhibiting osteoclastogenesis (25).

Moreover, another possible mechanism by which tideglusib may enhance bone development is through its capability to activate the PI3K/Akt signaling pathway. Previous studies have revealed that Tideglusib can signal this pathway in myotubes, hypoxic-ischemic brain injury (27-29). The differentiation and calcification of osteoblast precursor cells into mature osteoblasts has been attributed to the PI3K/Akt pathway (30). Also, it's an activation increased the expression of Ocn and ALP, to facilitate osteoblast differentiation and osteogenesis (31). ALP is an early molecular marker while, Ocn is a marker of late-stage osteogenesis and is tightly bound to osteoblast differentiation and maturation and ECM mineral deposit (32).

The pathologic results of this investigation were consistent with prior research that found that animals given lithium chloride, an inhibitor of GSK-3, showed an increase in trabecular area and thickness in addition to bone mineral density (33).

Conclusion

Tideglusib gel could accelerate bone healing through an increase in the trabecular area and elevated collagen deposition in the extracted socket of a rabbit with osteoporosis induced by glucocorticoid. Additionally, Tideglusib gel showed a dose-dependent effect in histomorphometric and histopathological parameters, indicating that 10 μM of Tideglusib gel is considered more effective rational therapy than 5 μM of Tideglusib for post-extraction socket repair, particularly in the osteoporotic model. However, effective concentrations of Tideglusib gel required for clinical application in humans need further studies.

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