EFFECT OF GLYCOGEN SYNTHASE KINASE ANTAGONISTS-3 ON BONE REGENERATION IN COMPARISON TO INJECTABLE PLATELETS RICH-FIBRIN: HISTOMORPHOMETRIC AND BIOMARKERS RESULTS

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ABSTRACT

Introduction: Teeth with periapical lesions heal after non-surgical endodontic intervention. However, sometimes persistent symptoms requires peri-radicular surgery in order to remove the pathological tissues and endorse healing. Tideglusib, is a glycogen synthase kinase 3 inhibitor which treats Alzheimer disease, it has been investigated for its efficiency in bone regeneration. The aim of the study was to determine the effects of Tideglusib on bone regeneration compared to injectable platelet rich fibrin in rabbits' tibial defects.

Methodology: Twenty four adult male New Zealand rabbits, weighing about 2.5- 3.5 kg, were used in this study. Full-thickness flap was elevated to expose tibial bone. A 4 mm bone defect was created on each rabbit tibia. The defects were divided into four groups: Group (CN): control received no treatment (n = 12), Group (CG): control received Gelatin sponge (n = 12), Group (G/i-PRF): Gelatin sponge + i-PRF (n = 12), Group (G/TDG): Gelatin sponge+ Tideglusib (n =12). Rabbits were sacrificed four week post-operatively, then Enzyme-linked immunosorbent assay for osteogenic markers, Histopathological, Histochemical and Histocmorhometric analyses were performed.

Results: Statistically significant difference was recorded between groups (p<0.05), as Group (G/TDG) showed the highest genes expression for all biomarkers and newly formed bone area percentage (%) and mature bone area % followed by Group (G/i-PRF), then Group (CG) and the lowest value was observed with Group (CN).

Conclusion: Tisuglusib is biocompatible with the potential to upregulate osteogenic marker genes with a robust anabolic effect on the regeneration process of tibial bone defect in rabbits compared with i-PRF

KEYWORDS: Tisuglusib; injectable platelet-rich fibrin, Gelatin sponge; Bone regeneration

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INTRODUCTION

The goal of endodontic treatment is to remove inflamed vital and necrotic pulp remnants from the pulp space and to prevent the occurrence of peri-radicular diseases and promote healing of any existing peri-radicular pathosis. Teeth with periapical lesions usually heal satisfactorily after non-surgical endodontic treatment [1]. However, surgical endodontics is considered an important treatment modality for the management of persistent periapical infections as well as procedural errors, resorptive defects and calcifications that cannot be treated by conventional endodontic techniques [2]. It has been reported that 16% to 64.5% of endodontically treated teeth are associated with periapical radiolucent lesions [3], and that regeneration and healing of periapical bony defects is considered a significant problem in endodontic surgery [4].

The key to tissue regeneration is to initiate a cascade of healing events by the aid of growth factors, extracellular matrix and bone morphogenetic proteins instead of routinely used synthetic bone grafts where synthetic bone grafts promote regeneration by osteoconduction, whereas these biological modulators promote regeneration by osteoinduction [5]. Therefore, blood concentrates; including platelet-rich fibrin (PRF), platelet-rich plasma (PRP) and injectable platelet-rich fibrin (i-PRF), are now widely applied in teeth and bone regeneration as they provide the needed biologic modulators for the reparative process [6].

i-PRF is a platelet concentrate formed at a low centrifugation speed which results in large amounts of platelets and leukocytes with higher release of growth factors compared to other blood concentrates, thus it can promote excellent hard and soft tissue regeneration [7]. Following its application, the human liquid fibrinogen in i-PRF is gradually transformed to a growth factor-rich PRF clot, which allows continuous growth factor release over 10 to14 days. i-PRF promotes human mesenchymal stem cells (MSCs) proliferation and migration,

and triggers their osteogenic differentiation [8,9]. It was also reported that i-PRF has a high anti-inflammatory and anti-microbial activity against many pathogens, which can contribute to faster tissue regeneration [10,11].

Reparative dentinogenesis and osteogenesis were shown to be enhanced by the activation of a signaling pathway called the Wnt/β-catenin signaling pathway. GSK-3 inhibitors including Tideglusib were found to be able to stimulate the Wnt/β-catenin signaling pathway and therefore could repair bone and dentine defects[12,13]. Tideglusib is a potent anti-inflammatory and neuroprotective drug used in the treatment of neurological disorders such as Alzheimer disease. It is a non- adenosine tri phosphate (ATP) competitive inhibitor of glycogen synthase kinase 3 (GSK-3) which was shown to promote dentine and bone formation and regeneration [14]. In addition, Tideglusib does not interfere with regenerative precursor cell recruitment and commitment; consequently, it could be used directly at a fracture site or bony defect [13].

As per our knowledge, there are no studies in literature communicating the effect of GSK-3 Antagonists and i-PRF on healing of bone defect. Thus, the aim of the study was to assess and compare the effect of Tideglusib and i-PRF on bone regeneration in tibial bone defects in rabbits.

MATERIAL AND METHODS

Ethical approval

The present study was approved by the Institutional Animal Care and Use Committee (IACUC)-Cairo University (Committee approval number CU III F 54 22).This research was done in compliance with the ARRIVE guidelines and regulations.

Sample size calculation

According to the finding of El-kabbany A & Abdelsalam E,[15] the minimum estimated sample

size using PS version 3.1.2, with an alpha (a) level of 0.05 (5%) and a beta (b) level of 0.20 (20%), i.e., power = 80% and an estimated difference between the experimental and control means of 2 to reject the null hypothesis was 12 tibial defects per group.

Materials

i-PRF

After anesthetizing the rabbits, approximately 10 ml of rabbit venous blood was drawn and transferred immediately to a sterile plastic tube without anticoagulant, which was immediately centrifuged at 700 rpm for 3 minutes at room temperature (60g force), the upper plasma layer (1ml) was collected and designated as i-PRF.

Tidelglusib

50 nM Tideglusib (TDG; Sigma-Aldrich, St. Louis, Missouri, USA) was dissolved and diluted in dimethyl sulfoxide.

Gelatin sponge (Gelita -Spon standard, Eberbach, Germany) is formed of low endotoxin gelatin that provides excellent compatibility. The scaffold was cut with a sterile scalpel to obtain circular sections with a diameter that fit in the surgically induced bone defects to be placed in the medullary cavity of bone defect areas.

Experimental animals

The experiment was carried out on a group of 24 adult male New Zealand white rabbits weighing 2.5 to 3.5 kg and bred at Cairo University's Faculty of Medicine's animal house. The animals were randomly housed in separate cages at a controlled room temperature 25±2°C with 12/12 hour light/dark cycle, fed ad libitum with the addition of hay and had unlimited access to water. Animals were quarantined for 7 days before to surgery to ensure they were clear of any infectious diseases.

Surgical procedure

The surgery was performed under sterile conditions with sterile instruments. Surgical procedures were performed under general anesthesia with a combination of ketamine chlorhydrate (Ketamine®, Amoun CO.) (0.08 mL/100 g body weight) and xylazine 2% (Xyla-Ject®, Phoenix™, Pharmaceutical Inc.) (0.04 mL/100 g body weight) The incision site was shaved and sterilized with iodated alcohol, an approximately 3 cm long incision was made on the medial side of the tibiae. The periosteum was reflected to allow direct approach to the rabbit tibiae. A cylindrical 4 mm intra-bony defect was created till bone marrow was reached in each tibia using a surgical trephine bur mounted on a low-speed hand piece with 2000 rpm under continuous irrigation with sterile saline to reduce thermal damage. By employing the same size trephine bur head, the size and depth of bone defects were standardized.

Each rabbit received two bone defects (one in each tibia), for a total of 48 bone defects. Tibial defects were divided into four groups based on treatment applied.

The two experimental groups includes; Group (G/i-PRF): bone defects received gelatin scaffold that was soaked with i-PRF and were placed in the medullary cavity. Group (G/TDG): bone defects received gelatin scaffold that was soaked with Tideglusib and were placed in the medullary cavity of the bone defect area and covered with sutured periosteum.

Control groups; Group (CN): bone defects received no intervention and left to heal spontaneously as negative control. Group (CG): bone defects received gelatin sponge immediately in the medullary cavity of bone defect areas to serve as positive control and then covered with sutured periosteum.

The muscular layer was sutured with resorbable catgut #4.0 and the skin was sutured with interrupted #4.0 silk sutures. All the procedures were performed by the same trained surgeon.

Post-surgery, animals were administered antibiotic Amikacin (Amikacin®, Amoun pharmaceutical company) at a dose of 10 mg/kg every 12 hours and for one week. Throughout the monitoring period, all animals were checked daily.

Animal sacrifice

After four weeks, the animals were terminated using an intra-cardiac overdose of 10% sodium thiopental, as suggested by the Universal Declaration of Animal Rights. All tibiae were dissected free of soft tissues, and bone specimens from each group were sliced with a disc under continual irrigation to encompass all defect sites.

Enzyme-linked immunosorbent assay (ELISA)

Just before animal euthanization, blood samples were collected from the jugular vein, 5ml of blood was placed into plain tubes. The serum was separated from the whole blood by centrifuging the blood for 20 minutes at 3000 rpm. Serum was transferred by micropipette to Eppendorf tube and stored in a deep freeze at -2°C then analyzed using microplate reader for; rabbits bone Alkaline Phosphates (ALP) (Beyotime Biotechnology, Co., Ltd., Shanghai, China), Osteocalcin (OCN) (Beyotime Biotechnology, Co., Ltd., Shanghai, China) and Osteopontin (OPN) (rabbit osteopontin ELISA Kit, USA) by measuring absorbance (Optical density OD) at 450 nm.

Histopathological and Histochemical examination

Bone tibiae specimens were preserved in 10% formal saline solution for 48 hours and subsequently demineralized in 10% ethylene diamine tetra-acetic acid (EDTA) (El-Gomhouria co.) solution for 4-5 weeks, with weekly renewals. After decalcification, specimens were dehydrated in ethyl alcohol (70% to 95%), embedded in paraffin wax, serially sectioned into sections of about 5 mm, and mounted on glass slides for Hematoxylin and Eosin (H&E) staining and Masson's trichrome staining (MT) for illuminating collagen fibers and newly formed bone.

Histomorphometric analysis

Percentage of total newly formed bone area was calculated from the H&E stained sections, whereas mature bone area percentages were calculated from the MT stained sections. The histomorphometric data were obtained using image analysis software Image J (NIH) using objective lens of magnification x200 non-overlapping fields from H&E-stained and MT sections respectively from each specimen.

Histopathological and Histomorphometric analysis evaluations were reviewed twice by the same pathologist at different times.

Statistical analysis:

Shapiro-Wilk and Kolmogorov-Smirnov tests were used to check whether the data were normal, and the data showed a parametric (normal) distribution. For each category, the mean and standard deviation were determined. To compare more than two groups, a one-way ANOVA was performed. This was followed by a Tukey post hoc analysis, and Pearson correlation was used to examine the relationship between total newly formed bone area percentage and alkaline phosphatase, Osteocalcin and Osteopontin gene expression. IBM SPSS Statistics Version 20 for Windows (IBM Corporation, New York, USA) was used for the statistical analysis and the outcome assessor was blinded in this trial.

RESULTS

The means and standard deviations (SD) for the comparison between groups for ALP (U/l), OCN (ng/ml) and OPN (ng/ml) gene expression and comparison between groups for newly formed bone area percent (H&E stain) (%) and mature bone area percent (MT stain)(%) are shown in (Table 1, 2).

TABLE (1) Descriptive statistics and comparison between groups for alkaline phosphatase (U/l), Osteocalcin (ng/ml) and Osteopontin (ng/ml) gene expression (ANOVA test and Tukey's post hoc test).

Parameter	Group	Mean±SD	Std. Error	95% Confidence Interval for Mean	P-value
Alkaline phosphatase (U/l)	Group (G/i-PRF)	148.50±5.09 [A]	1.47	(145.45, 151.55)	0.000*
	Group (G/ TDG)	152.25±5.56 [A]	1.61	(149.20, 155.30)	
	Group (CN)	128.50±5.49 [C]	1.58	(125.45, 131.55)	
	Group (CG)	139.0±4.81 [B]	1.39	(135.95, 142.05)	
Osteocalcin (ng/ml)	Group (G/i-PRF)	5.99±0.525 [A,B]	0.151	(5.747, 6.236)	0.000*
	Group (G/ TDG)	6.19±0.438 [A]	0.126	(5.947, 6.436)	
	Group (CN)	3.93±0.233 [C]	0.0672	(3.684, 4.173)	
	Group (CG)	5.59±0.43 [B]	0.125	(5.347, 5.836)	
Osteopontin (ng/ml)	Group (G/i-PRF)	20.72±0.41 [B]	0.144	(20.36, 21.07)	0.000*
	Group (G/ TDG)	24.3±0.844 [A]	0.244	(23.95, 24.65)	
	Group (CN)	16.96±0.448 [D]	0.129	(16.61, 17.31)	
	Group (CG)	18.675±0.56 [C]	0.161	(18.32, 19.03)	

*Significance level P<0.05, *significant*

Means with different superscript letters are significantly different.

TABLE (2) Descriptive statistics and comparison between groups for total new bone area percent (%) and mature bone area percent (Masson Trichrome)(%) (ANOVA test and Tukey's post hoc test).

Parameter	Group	Mean±SD	Std. Error	95% Confidence Interval for Mean	P-value
Total new bone area percent (%)	Group (G/i-PRF)	53.4±8.30 [A]	2.40	(49.56, 57.16)	0.000*
	Group (G/ TDG)	59.8±7.0 [A]	2.02	(55.95, 63.54)	
	Group (CN)	31.9±5.25 [C]	1.51	(28.10, 35.70)	
	Group (CG)	39.4±5.01 [B]	1.45	(35.55, 43.14)	
Mature bone area percent (Masson Trichrome) (%)	Group (G/i-PRF)	44.18±4.3 [B]	1.24	(42.22, 46.14)	0.000*
	Group (G/ TDG)	50.178±2.29 [A]	0.661	(48.22, 52.14)	
	Group (CN)	37.4±2.01 [C]	0.580	(35.44, 39.36)	
	Group (CG)	36.41±4.19 [C]	1.21	(34.45, 38.36)	

*Significance level P<0.05, *significant*
Means with different superscript letters are significantly different.

ELISA

ALP, OCN and OPN gene expression varied significantly ($p<0.05$) among the four groups. A significant higher ALP expression in Group (G/TDG) as compared to Group (CG) and Group (CN) . A significantly higher expression was also observed in Group (G/i-PRF) as compared to both Group (CG) and Group (CN) and Group (CG) as compared to Group (CN), while no statistically significant difference ($p>0.05$) was detected between Group (G/TDG) and Group (G/i-PRF). Moreover, Group (G/TDG) showed a statistically significant ($p<0.05$) higher mean OCN as compared to both Group (CG) and Group (CN), Group (G/i-PRF) recorded a significantly higher mean value as compared to Group (CN), while statistically significant difference ($p<0.05$) was observed between Group (G/TDG) and Group (G/i-PRF). OPN gene expression was highest in Group (G/TDG), followed by Group (G/i-PRF), then Group (CG) and finally Group (CN).

Histopathological and Histochemical examination

H&E stained bone specimens was analyzed to confirm bone regeneration as shown in Figure (1), Group (G/i-PRF); trabecular bone became thickened and marrow cavities were connected. Still minute remnants of undegraded scaffold were seen. Islands of woven bone were intermingled with areas of osteons containing osteocytes and lamellar bone (Fig.1A). Group (G/TDG); thick more organized trabecular bone with osteoblast lining the marrow cavities filled the entire defect. Haversian system (osteonal pattern) and lamellar bone were seen denoting advancement in the healing process. Limited areas of woven bone were noticed (Fig. 1B).

In Group (CN); minimal amount of newly formed bone trabeculae dispersed in rich highly cellular granulation tissue and chronic inflammatory infiltration and blood vessels were observed (Fig.1C). Group (CG); a net of growing woven bone trabeculae enclosing haphazardly arranged osteocytes in their wide lacunae and lined with osteoblast cells were still surrounded by highly vascular granulation tissue. Undegraded scaffold remnants were also identified (Fig.1D).

MT stained bone specimens were analyzed to confirm bone maturation as shown in Figure (2), MT stains collagen fibers and newly formed bone matrix in blue; however, the mature well-calcified bone was stained red. The Group (G/i-PRF) showed adequate stain reaction (Fig.2A), and Group (G/TDG); showed bone tissue with marked elevated calcification expressed in the red color and superior bone trabeculae arrangement (Fig.2B). Group (CN) showed intense stain reaction with increased expression of the blue stain (Fig. 2C) and Group (CG) revealed a moderate stain reaction (Fig.2D). Overall, these observations were consistent with those from H&E-stained sections.

Histomorphometric analysis

The total newly formed bone area % calculated from the H&E stained sections recorded statistically significant difference between groups($p<0.05$). Higher percentage was observed in Group (G/TDG) and Group (G/i-PRF) as compared to Group (CG) and Group (CN), while the difference between Group (G/TDG) and Group (G/i-PRF) was not statistically significant (p>0.05). Total newly formed bone area % showed positive correlation with each of ALP (0.786), OCN (0.717) and OPN gene expression (0.784).

For the mature bone area % calculated from the MT stained sections the highest value was recorded in Group (G/TDG), followed by Group (G/i-PRF), then Group (CN) and finally Group (CG).
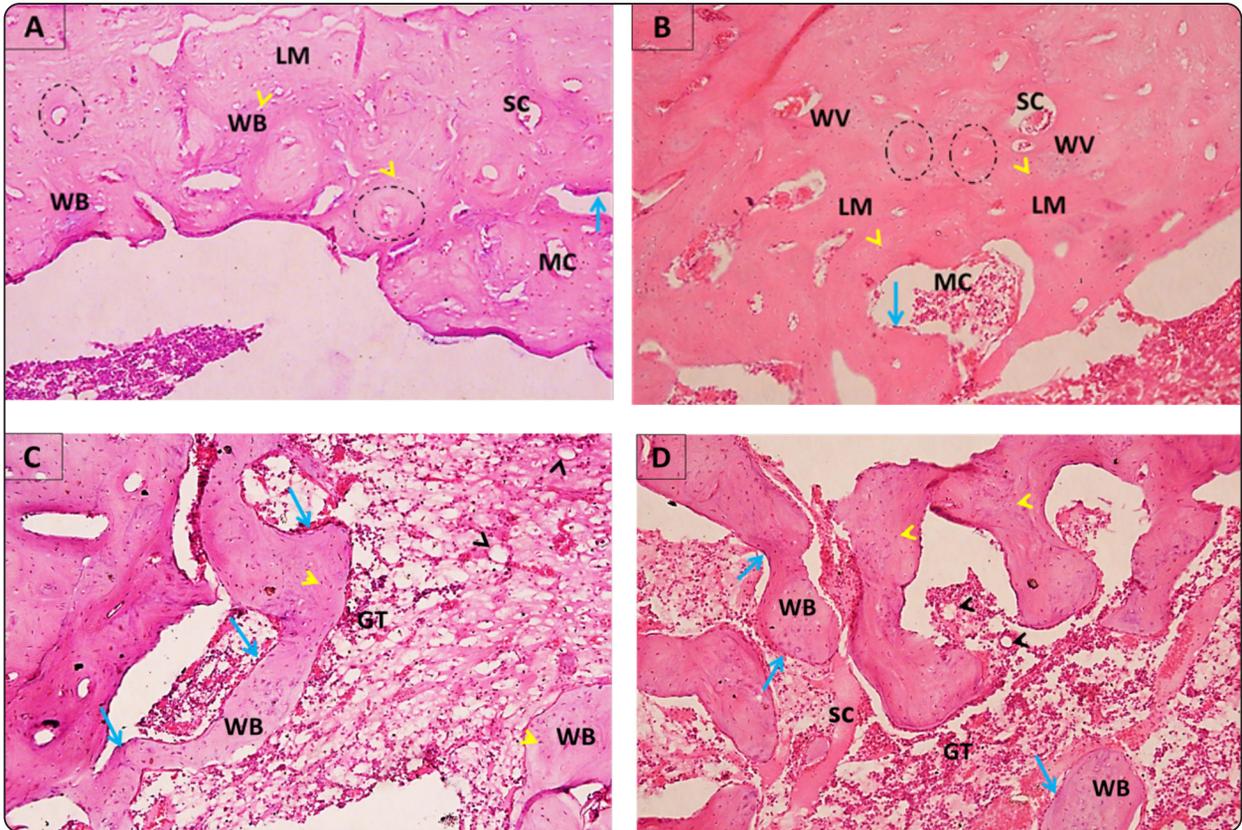
Fig. (1) Photomicrographs of specimens four weeks post-operatively in H&E-stained sections (x200). A: Group (G/i-PRF) showed woven bone filled most of the defect with some areas of lamellar bone and Osteons enclosing osteocytes. B: Group (G/TDG) showed the highest amount of new bone formation. Areas of woven bone intermingled with areas of lamellar bone and osteons. C: Group (CN) showed minimal amount of newly formed woven bone trabeculae lined by osteoblasts and enclosing osteocytes. Granulation tissue filled the defect with chronic inflammatory cell infiltrates and blood vessels. D: Group (CG) showed woven bone trabeculae spaced with moderate amount of granulation tissue containing inflammatory cells and blood vessels. Remnants of scaffold were seen in all experimental groups (SC). Woven bone trabeculae (WB), Marrow cavities (MC), Osteon (Dotted circle), Lamellar bone (LM), osteoblasts (blue arrows), osteocytes (yellow arrow head), Granulation tissue (GT), blood vessels (Black arrow head) (H&E, orig. Mag. 200x).
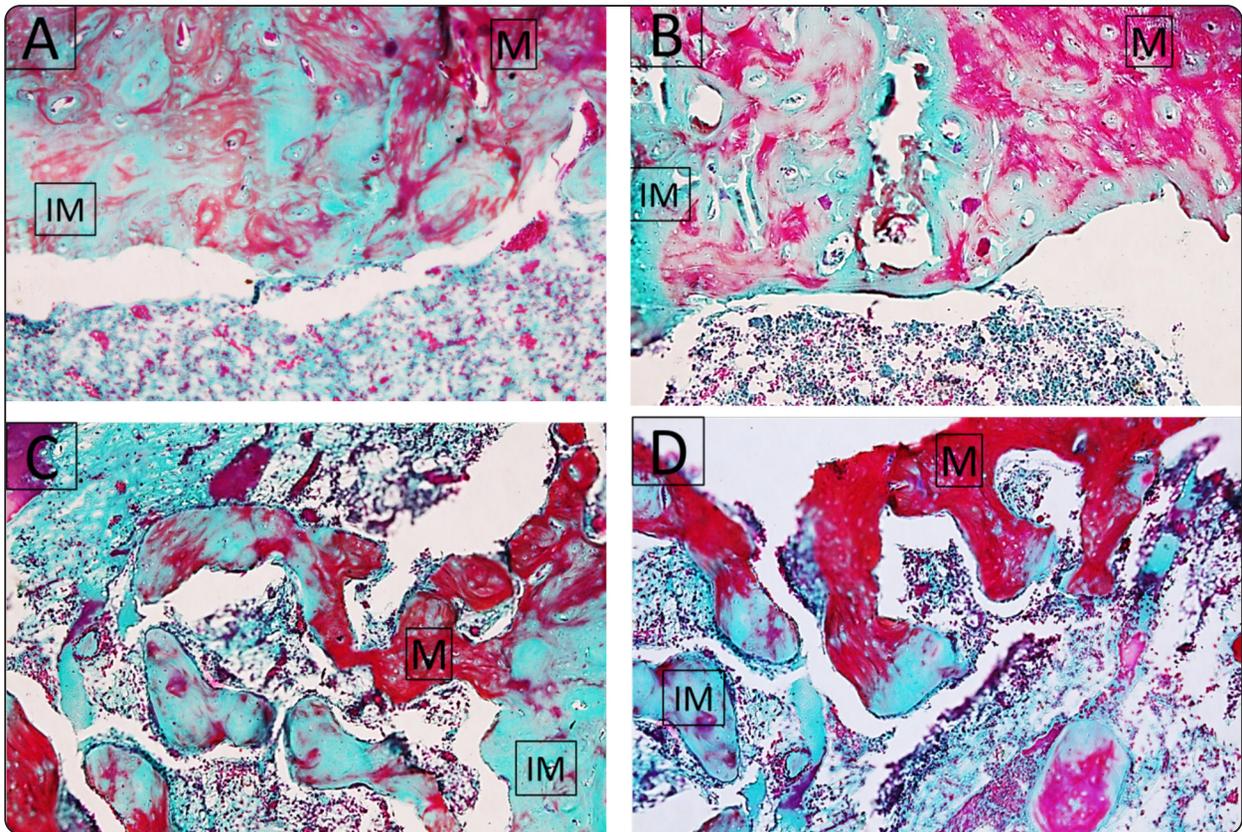
Fig. 2: Photomicrographs of specimens four weeks post-operatively in MT-stained sections (x200). A: Group (G/i-PRF). B: Group (G/TDG). C: Group (CN). D: Group (CG). Immature newly formed bone (Collagen fiber) was stained with blue (IM) and mature newly formed bone was stained with red color (M) (MT, orig. Mag. 200x).

DISCUSSION

Endodontic surgery should be considered the last option for saving a tooth before extraction by any dentist. Following surgery, a complex healing process involves a number of reactions such as clotting, inflammation, granulation tissue creation, collagen synthesis and tissue remodeling [16,17]. The purpose of this study was to assess and compare the bone regenerative effect of Tideglusib compared to i-PRF, both loaded on a gelatin scaffold, in an induced tibial bone defect in rabbits through ELISA, Histopathological examination, Histochemical and quantitative histomorphometric analyses.

A novel injectable liquid PRF formulation has been developed by decreasing centrifugation rates. i-PRF can be used for injectable purposes, which may enhance the wound-healing processes with improved vascularization. In addition, i-PRF has more benefits than PRP since it has a higher concentration of leukocytes, releases growth factors slowly and continuously, promotes cell migration and transforming growth factor expression [18].

Wnt signaling plays an important role in the formation and maintenance of many organs and tissues, as well as bone. Multiple investigations have found that endogenous Wnt signaling plays an important role in osteoblastogenesis [19,20]. GSK-3 participates in adaptive changes via numerous protein kinases, including pro-inflammatory cytokine and interleukin synthesis, in events such as cellular proliferation, migration, inflammation and immunity, cell proliferation, and cell proliferation. GSK-3 activation inhibits the Wnt/b-catenin signaling pathway [21,22].

Tideglusib is a small molecule and function as a selective and irreversible non-ATP competitive GSK-3 inhibitor and is used to treat neurological disorders such as Alzheimer disease (AD). Additionally, it promotes dentine regeneration [23]. 50 nM was selected since studies have indicated that dosages above 50 nM are cytotoxic [23], and this dose was sufficient to promote bone repair in a critical-sized defect model [24]. Also, 50nM of Tideglusib were able to activate endogenous stem cells to form reparative dentine via activating the Wnt signaling pathway in 4 week, resulting in increased mineralization and dentin formation[23]. As a result, existing research suggests that Tideglusib may be effective in both speeding and boosting bone healing.

In this study, gelatin sponge was used as vehicle to deliver low doses of small molecule GSK-3 antagonists and i-PRF [23,26]. Scaffolds in regenerative medicine are selected according to several physical and biological factors. An ideal scaffold should have certain mechanical and geometrical properties which allow proper transport of nutrients and oxygen, promoting bone cells' survival and in addition to enhancing resident stem cells differentiation and homing [27]. Gelatin is a denatured form of collagen and comprises number of biological functional groups as amino acids, which makes it appropriate for hard-tissue applications [28].

New Zealand rabbits were chosen as the animal model for our research because they are inexpensive, easy to obtain and handle and give ample space for the formation of big bone cavities. Similarities in bone mineral density and fracture toughness of mid-diaphyseal bone have been established between rabbits and humans [29,30]. Furthermore, rabbits are classified as tiny animals, which is preferable from an ethical standpoint. The rabbits were roughly three kg in weight to be able to endure surgical stress and have a higher survival rate [30].

Defects were drilled in both tibiae for proper evaluation of bone regeneration as it has a proximal epiphysis with a slightly convex wide medial surface that is free of muscle insertion, making it suitable for drilling bone defects [31].

Sohn et al., 2010 [32] claimed that the remodeling phase in rabbits is about three times faster than in humans and it was reported that two to four weeks would be a sufficient interval to measure healing progress in rabbits, thus this investigation was limited to four weeks [24,25].

Non-collagenous proteins play a key role in multiple physiological and pathological conditions, including wound healing, inflammation, tumor growth, calcification and bone remodeling [33]. In the current study, ALP, OCN and OPN were investigated. ALP is abundant in osteoblasts and is involved in the mineralization of newly created bone 42, and a number of Wnt proteins can induce ALP in mesenchymal cells [34]. OCN is one of the major non-collagenous protein found in the bone matrix, which is synthesized by osteoblasts; [35] circulating OCN Serum levels are generally proportional to osteoblast activity, and thus OCN is an established measure of bone formation [36]. Moreover, studies have shown that OPN, plays a role in bone metabolism and homeostasis. OPN is an important factor in in biological activities such as proliferation, migration and adhesion of several bone-related cells [37].

The immunological results were confirmed with the histopathologic and morphometric results. The highest expression of ALP, OCN and OPN was observed in Group (G/TDG), which was also validated by Histological and histochemical examination which revealed the ability of Tideglusib to significantly boost new bone formation compared to all other groups [24,25]. This was coinciding with a study investigating the effect of a GSK-3 inhibitor on bone metabolism in ovariectomized rats, where the inhibition of GSK promoted increased bone volume as well as increased the expression levels of non-collagenous proteins[38], the findings of Gambardella

and colleagues [39] who determined that subcutaneous injection of GSK-3 inhibitors in mice could promote bone formation via stimulating MSC proliferation, which in turn leads to osteogenic differentiation. It was demonstrated that Wnt proteins are capable of inducing ALP in mesenchymal cells [24] . Moreover, Popelut et al.,[40] revealed that WSP activation could improve implant osseointegration by increasing osteoblast activity, inhibiting osteoclast activity, or differentiating pluripotent stem cells.

Strikingly, Tideglusib effectiveness in increasing bone formation and bone mass could be explained to be as a result of increased levels of ALP, OCN and OPN supported by the histomorphometrical, and histochemical results.

Group (G/i-PRF) revealed thickened trabecular bone with interconnected marrow cavities. Mostly, islands of woven bone intermingled with areas of osteons and lamellar bone were seen , enhanced mature bone area % and upregulated ALP, OCN and OPN gene expression compared to control which was consistent with El-kabbany A & Abdelsalam E,[15] who reported a significant increase in the amount of newly formed bone with the i-PRF group, and in accordance with a Hidajat et al. [41], where i-PRF were able to enhance the quality of fracture healing of a long bone in rabbits in terms of union quality, cortex growth and remodeling and bone graft and new bone union . i-PRF bone regenerative potential may be attributed to the increased concentration of platelets, leukocytes, and growth factors that initiate the migration and proliferation of osteoblasts, prompting tissue regeneration [42,43] . i-PRF may play an vital osteoinductive role in mature and dense bone formation via growth factors release [44] .

In Group (CG); growing woven bone trabeculae network surrounded by highly vascular granulation tissue and undegraded scaffold remnants were observed. This could be attributed to the ability of biodegradable and biocompatible proteins, such as gelatin sponge, to promote osteoblast activities, allowing cell growth and migration into sponge porosities[45]. Clinically, Gelatin was proved to be temporary defect filler and an agent in wound dressing because of its biodegradability and biocompatibility [46].

Group (CN) showed minimal amount of newly formed bone trabeculae dispersed in rich highly cellular granulation tissue. These findings were consistent with Ou et al.,[47] who discovered that femoral defects in rabbit showed fibrous connective tissue with only a few bone trabeculae formed four weeks after surgery, and Santos et al.,[48] reported the presence of granulation tissue only in the defect area with no signs of new bone formation in the negative control group 4 weeks post-operatively.

Group (G/TDG) and Group (G/i-PRF) exhibited better osteoconduction and were progressively replaced by higher amount of new formed bone than Group (CG) and Group (CN).

CONCLUSION

Collectively, these observations indicate that local application of Tideglusib can stimulate the expression of osteogenic markers which enhanced the quantity and the maturity of the newly formed bone in tibial bone defects, which suggest that Tiduglusib can be used for bone regeneration and Further research is needed to understand the effect of Tideglusib on osteoblastogenesis and osteoclastogenesis.

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