B-5203
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RESEARCH ARTICLE Indian Journal of Animal Research

Evaluation of β-tricalcium Phosphate (β-TCP) and Platelet Rich Plasma (PRP) Combination for Fracture Healing in Dogs Stabilized with Intramedullary Pinning

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10.18805/IJAR.B-5203

ABSTRACT

Background: The use of synthetic bone substitute,β-tricalcium phosphate (β-TCP) as an osteo-conductive material and platelet-rich plasma (PRP) as an osteo-inductive material is the alternate strategies for replenishing bone loss. Thus, the present study was conducted to evaluate the bone healing efficacy of β-TCP and PRP combination in dogs with long bone fracture repaired with threaded intramedullary pinning.

Methods: Fifteen dogs were selected, which were allotted to two groups irrespective of age, breed and sex. In six dogs of Group I intramedullary pinning was performed, while in nine dogs of Group II β-TCP and PRP was applied at fracture site after stabilising with intramedullary pin. Clinical and radiographic examinations were conducted preoperatively and postoperatively on 15th, 30th and 60th day.

Results: The mean weight bearing and radiographic union scores were better in group II animals as compared to group I at different time intervals. The present findings suggest that β-TCP and PRP effectively enhances the bone healing in dogs with long bone fracture.

Keywords: Fracture, β-Tricalcium Phosphate, Platelet Rich Plasma, Intramedullary Pinning

INTRODUCTION

Fracture is defined as the loss of continuity in the bone and/or cartilage and is the most frequent orthopaedic disorder encountered in small animal practice (Prabhukumar *et al.*, 2019). The ultimate goal of fracture treatment is to restore the function of the affected limb quickly with least complications (Shahar, 2000; Minar *et al.*, 2013). The gap between the fracture ends has to be filled through bone graft or its substitute, otherwise may often lead to delayed union, malunion, non-union or rounding of fracture ends. (Ruhaimi, 2000; Lu *et al.*, 2021). Besides analogous bone grafts, the use of synthetic bone substitute, β-tricalcium phosphate (β-TCP) as an osteoconductive material and platelet-rich plasma (PRP) as an osteo-inductive material is the alternate strategies for replenishing bone loss. Some authors reported positive effects on use of β-TCP and PRP for fracture healing in their studies (Szponder *et al.*, 2018; Singh *et al.*, 2020) and others lack of positive effects (Plachokova *et al.*, 2006; Rabillard *et al.*, 2009; Lu *et al.*, 2021). Thus, the present study was conducted to evaluate the efficacy of β-TCP and PRP for fracture healing in dogs with long bone fracture.

MATERIALS AND METHODS

The present study was conducted on 15 dogs referred to the department for management of long bone fractures which were randomly allotted to two groups irrespective of age, breed, sex and body weight.

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How to cite this article: Kumar, N.S., Arora, N., Tiwari, D.K., Saharan, S., Sharma, S., Poonia, A. and Kumar, S.(2024). Evaluation of β-tricalcium Phosphate (β-TCP) and Platelet Rich Plasma (PRP) Combination for Fracture Healing in Dogs Stabilized with Intramedullary Pinning. Indian Journal of Animal Research.
doi: 10.18805/IJAR.B-5203.

Submitted: 22-07-2023 **Accepted:** 27-03-2024 **Online:** 23-05-2024

Sterilized β-TCP granules having a particle size of 355-500μ were used at fracture site as an osteoconductive material to fill the bone defect. PRP was prepared according to method suggested by Wang *et al.* (2014). In the present study, initial mean platelet count of whole blood was

Group	Treatment plan
Group I (n=6)	Internal fixation using threaded intramedullary pinning
Group II (n=9)	Internal fixation using threaded intramedullary pinning along with β-TCP and PRP at fracture site

317.11± 23.90 and after PRP preparation the count increased to 902.56± 94.48. The affected limb was aseptically prepared as per the standard procedure.

Anaesthesia

All the animals were fasted overnight and premedicated with intramuscular injection of Atropine Sulphate @ 0.04 mg/kg body weight, pre-emptive analgesia with Inj. Meloxicam @ 0.2 mg/kg body weight and prophylactic antibiotic Inj. Ceftriaxone @ 25 mg/kg body weight prior to surgery. After 5 min, mixture of Inj. xylazine hydrochloride @1mg/kg body weight and Ketamine hydrochloride @ 5 mg/kg body weight was administered intramuscularly. After tracheal intubation anaesthesia was maintained with isoflurane at minimum alveolar concentration (MAC) of 1.0-3.0 % for the rest of the surgical procedure.

Surgical technique

In the present study, femur, tibia and humerus were approached for intramedullary pinning as described by Piermattei and Greeley (1979). Once the fracture site was exposed, fracture fragments were elevated using bone hook and secured with bone holding forceps. In both the groups intramedullary end threaded Steinmann pin of sufficient diameter (60-70% of medullary cavity diameter) was inserted through the medullary cavity in a retrograde fashion for femur and humerus and normograde manner for tibia. Moreover, anatomical reduction and the proper pin position was confirmed intra-operatively using C-Arm. In group II, following anatomical reduction and internal fixation, 1 cc of sterile beta-tri calcium phosphate bone graft placed in a sterile petridish and mixed with 0.5 ml of platelet rich plasma was applied using a spatula at the fracture gap and remaining PRP was injected at fracture site. The muscles and skin were closed as per standard protocol.

Post-operative care

The dogs were administered with inj. Meloxicam @ 0.5 mg/kg body weight, I/M and inj. Ceftriaxone @ 25 mg/kg body weight, I/M for 5 days and modified Robert Jones bandage was applied after the surgery to support the operated limb and advised leash walk for two weeks. Skin sutures were removed after 15 days of surgery.

Post-operative evaluation

Bone healing was evaluated by clinical and radiographic examinations preoperatively and postoperatively on 15th, 30th and 60th day, postoperatively. Weight bearing while standing and walking was assessed as per the method suggested by Sahu *et al.* (2017). Radiographic examinations were done to evaluate bone formation scores and bone union score as per Lane and Sandhu (1987), and the stage of bone union was evaluated as per Hammer *et al.* (1985). Clinical outcome on the basis of functional limb usage was evaluated using classification system adopted by Fox *et al.* (1995).

Statistical analysis

The statistical analysis of data was done by one-way-ANOVA with linear repeated measure via SPSS software. All the data values were expressed as Mean ± Standard error of mean (Mean ± S.E.). P-value less than 0.05 considered as statistically significant.

RESULTS AND DISCUSSION

This investigation was carried out to evaluate the bone healing efficacy of β-TCP and PRP combination in dogs with long bone fracture repaired with threaded intramedullary pinning. The bone formation scores (BFS) in group II was found significantly higher as compared to group I at different time intervals (Table 1). Similarly, the bone union scores (BUS) were non significantly higher in group II as compared to group I (Table 2). Stage of union was achieved earlier in group II as compared to group I (Fig. 1). The increase in the mean radiographic scores in group II due to application of osteoconductive material, β-TCP and osteoinductive material, PRP at the fracture site might have led to increase in the bone formation rate. Goel *et al.* (2013) and Preethi *et al.* (2021) also reported the osteoconductive abilities of β-

Table 1: Bone formation scores at different time intervals in both the groups (Mean± S.E.).

Time interval	0th day	15th day	30th day	60th day
Group I	0.00^{a}±00	0.67^{bA}±0.21	1.67^{cA}±0.21	2.83^{dA}±0.40
Group II	0.00^{a}±00	1.89^{bB}±0.12	3.11^{cB}±0.21	4.00^{aB}±0.00

(Means with different superscripts (a, b)/ (A, B) varies significantly (p<0.05) within group/ between the group)

Table 2: Bone union scores at different intervals in both the groups (Mean± S.E.).

Time interval	0th day	15th day	30th day	60th day
Group I	0.00^{a}±00	1.33^{b}±0.42	1.67^{b}±0.33	2.67^{b}±0.42
Group II	0.00^{a}±00	2.00^{ab}±0.0	2.44^{b}±0.31	3.33^{c}±0.35

(Means with different superscripts (a, b) varies significantly (p<0.05) within group)

Table 3: Weight bearing scores while standing at different time intervals in both the groups (Mean ± S.E.).

Group	0th day	15th day	30th day	60th day
Group I	$0.00^{a}\pm0.00$	$1.00^{bA}\pm0.26$	$1.83^{c}\pm0.31$	$2.33^{c}\pm0.33$
Group II	$0.00^{a}\pm0.00$	$2.25^{bB}\pm0.31$	$2.56^{bc}\pm0.26$	$2.89^{c}\pm0.12$

(Means with different superscripts (a, b)/ (A, B) varies significantly ($p<0.05$) within group/ between group respectively).

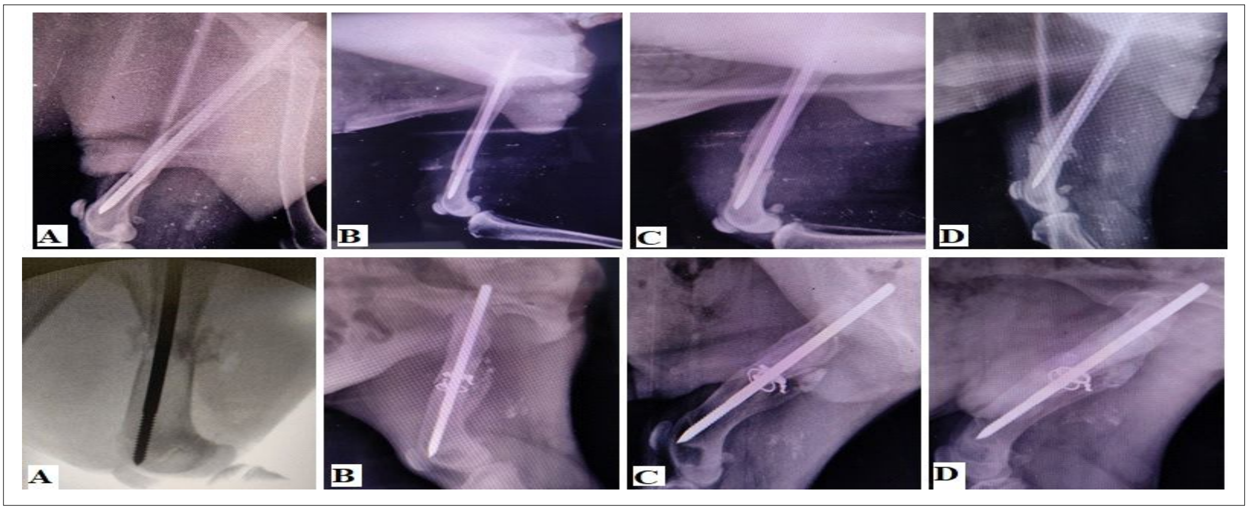

Fig 1: Radiographs showing fracture healing in group I (upper row) and II (lower row). Immediately after surgery (A) and on 15th (B), 30th (C), 60th (D) post-operative day.

TCP which was safe and effective therapeutic option for fracture healing in large osteo-periosteal defects. Addition of PRP to β-TCP enhance healing process, which is suggestive of beneficial effects of growth factors present in the PRP as reported based on effects of hydroxyapatite tricalcium phosphate (HA/β-TCP) on healing of bone defects in the skull of rabbits (Szponder *et al.*, 2018; El Backly *et al.*, 2014) and by incorporating PRP into osteoconductive material as a source of growth factors, bone production was accelerated by the activation of stem cells and the cellularity within the defects was improved. In contrast to above findings, many authors reported no beneficial effect of PRP and β-TCP on fracture healing in dogs (Rabillard *et al.*, 2009; Özdemir and Ökte, 2012).

The mean weight bearing score while standing (Table 3) improved gradually over a period of time in both the groups ranging from 0.00 ± 0.00 on day 0 to 2.33 ± 0.33 on day 60th in group I (Fig. 2) and 0.00±00 to 2.89 ± 0.12 in group II (Fig. 3), respectively. The mean weight bearing scores were better in group II during the entire post-operative period. Moreover, all animals showing non-weight bearing lameness in both the groups on day of presentation. Animals tend to carry the damaged leg off the ground because of pain in the affected fracture area due to inflammation and damage to the surrounding muscles (Gupta, 2015). Significant increase in the weight bearing scores in group II was observed on 15th day as compared to group I. Moreover, all the animals were found touching either the toe or the paw on the ground in group II, whereas in group I, majority of the animals were carrying the limb off the ground. An improvement in the weight bearing score was observed in group I and most of the animals were found touching the toe or placing the paw on the ground on 30th day. Majority of the animals started full weight bearing on the affected limb on 30th day in group II. Animals of both the groups showed full weight bearing on 60th day postoperatively.

Similarly, the weight bearing scores while walking (Table 4) was non significantly higher in group II as compared to group I at different intervals. Similar findings were also observed by Singh *et al.* (2020). The improvement in the weight bearing scores (while standing and walking) was due to early and optimum fracture healing in dogs of group II as supported by radiographic scores which can be related to local application of β-TCP and PRP at the fracture site. Similar results were observed by Singh *et al.* (2020) in dogs treated with combination of β-TCP and PRP. This suggests the positive effects of PRP in bone union soft tissue healing surrounding the fracture site. Many authors reported the beneficial effects of PRP on healing of soft tissue trauma (Boyan *et al.,* 2007; Wang *et al.*, 2013).

In group I, functional usage of the limb was excellent in one case, good in two, poor in two and fair in one. In group II, excellent in five cases, good in three and fair in one. This finding also coincides with the fact that animals of group II achieved early callus formation and early weight bearing, hence an early return of limb function with full range of motion. Similar findings were observed by Singh *et al.* (2020).

β-TCP aid in bone healing by regulating osteogenic process like mesenchymal stem cell differentiation into osteoblasts, growth of new blood vessels, the release of angiogenic growth factors, and blood clot formation (Lu *et*

Table 4: Weight bearing scores while walking at different time intervals in both the groups (Mean ± S.E.).

Group	0^{h} day	15^{th} day	30^{th} day	60^{th} day
Group I	0.00^{a}±0.00	1.17^{a}±0.65	2.33^{b}±0.76	2.67^{b}±0.71
Group II	0.00^{a}±0.00	2.89^{b}±0.62	3.67^{bc}±0.35	3.78^{ac}±0.24

(Means with different superscripts (a, b) varies significantly (p<0.05) within group).

Fig 2: Post-operative weight bearing on day 15^{th} (A), 30^{th} (B) and 60^{th} (C) in group I.

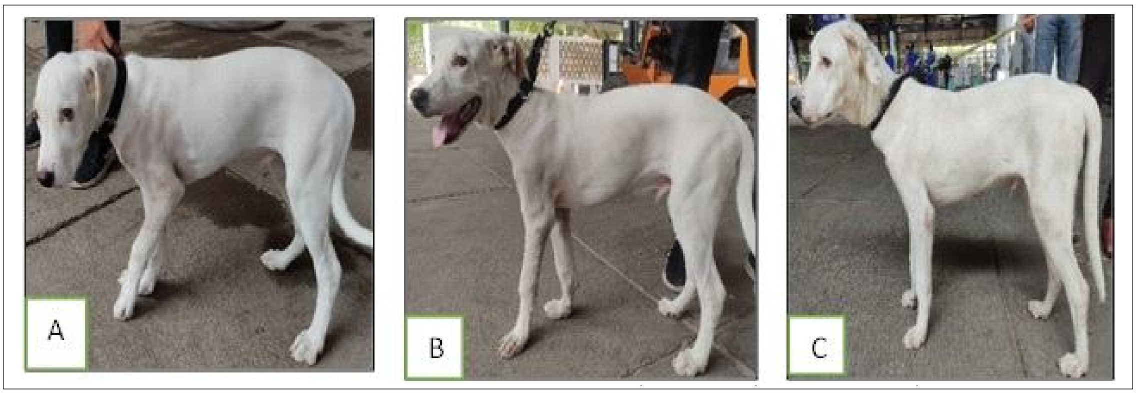

Fig 3: Post-operative weight bearing on day 15^{th} (A), 30^{th} (B) and 60^{th} (C) in group II.

al., 2021). PRP could offer positive outcomes in bone grafting techniques, since it contains various growth factors *viz.,* platelet derived growth factor (PDGF), transforming growth factors (TGF- β1, TGF-β2), insulin like growth factor (IGF), epidermal growth factor (EGF) and epithelial cell growth factor (ECGF). These growth factors promote angiogenesis, stimulate the growth and chemotaxis of chondrocytes, osteoblasts, and mesenchymal cells (Gracia *et al*., 2012). Osteoblast differentiation, proliferation and metabolic activity are intimately associated with platelet-derived growth factors (PDGF) and other proteins secreted by platelets, which activate intracellular signalling pathways for matrix formation and cellular differentiation. Platelet-rich plasma has a major impact on the early inflammatory phase of fracture healing, which includes the formation of a fracture hematoma and the proliferation of mesenchymal stem cells produced from the bone marrow (Canbeyli *et al.*, 2018).

CONCLUSION

On the basis of the above findings, it is concluded that the weight bearing scores, radiographic scores and functional usage of limb was better in dogs with long bone fractures treated with intramedullary pinning alongwith β-TCP and PRP.

Conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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