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Clinical Evaluation of Antibiotic Loaded Calcium Phosphate Beads as Treatment Options for Avian Soft Tissue and Orthopaedic Cases

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ABSTRACT

The process of wound and fracture healing comprises several stages, some of which necessitate the use of enhancers. The aim of the present study was to assess the efficacy of gentamicin-clindamycin (GEN/CLI) loaded in calcium phosphate (CaP) beads as potential treatment modalities for contaminated wounds and fracture healing in avian patients. Six (6) avian patients of various species with contaminated wounds, open and closed fractures, or lumps requiring surgical intervention were enrolled in the study. The CaP beads were loaded with gentamicin and clindamycin antibiotics before being implanted during surgical procedures. A radiographic and a modified Southampton surgical wound scoring system were used to assess bone fractures and wounds, respectively. All scores were checked for coherence, and the findings were compared across days using the one-way analysis of variance (ANOVA) with the Tukey-Kramer (HSD) test. There were significant differences in the wound healing progression based on the modified Southampton ranking periodically at $p < 0.05$. The radiographic assessment demonstrated the biocompatibility of CaP beads, which aided in the formation of new bone tissue while causing no adverse effect. The use of GEN/CLI-loaded CaP beads demonstrated promising results in enhancing both wound and fracture healing in avian patients. The treatment was well-tolerated, promoted new bone formation, and showed no adverse effects, indicating good biocompatibility. The statistically significant improvement in healing progression supports the potential of this approach as an effective therapeutic option. Given the current lack of research in this area, especially concerning avian species, the findings highlight the clinical value of GEN/CLI-loaded CaP beads as a suitable and beneficial adjunct in the surgical management of contaminated wounds and fractures in birds, with potential relevance to other animal species facing comparable clinical conditions.

Introduction

Antibiotic loaded bone cement beads are used provisionally to provide high local antibiotic concentrations and fill the dead space after debridement.[1,2] Fortification in combination with or the replacement of the norm of systemic antibiotics was the basis for the development and practice of a local antibiotic delivery system in the treatment of bone and soft tissue infections. In the current study, synthetic calcium phosphate (CaP) was used as the antibiotic carrier system. On the basis of composition, synthetic CaP presently used as biomaterials are classified as calcium hydroxyapatite (HA). Calcium phosphate has a chemical composition that is suitable for bone remodelling kinetics.[3-5] Therefore, CaP bone cement simulates biodegradable materials by serving as an

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osteoconductive matrix compared to polymethylmethacrylate (PMMA) beads, and these biodegradable materials do not require to be removed when they are used to fill dead spaces.[2,6] The combination of gentamicin and clindamycin has a synergistic bactericidal effect on over 90% of the bacteria common to infections in orthopaedic cases.[7-9] Reports on the usage of gentamicin-clindamycin (GEN/CLI) loaded CaP beads are scarce in laboratory animals to none especially in avian patients. We hypothesize that the local delivery of these antibiotics via CaP beads may offer therapeutic and prophylactic advantages in promoting bone healing, reducing soft tissue and bone infections in avian patients with contaminated wounds and fractures. This approach may be particularly effective in the avian context, where species-specific responses and surgical challenges differ from those in human joint arthroplasty that have shown limited benefit. While CaP beads alone have been studied in humans and mammals, their application in avian species remains largely unexplored. Therefore, the objective of this study lies in evaluating the dual role of antibiotic loaded CaP beads in promoting bone regeneration and preventing bone and soft tissue infections in birds, thereby addressing a significant gap in avian orthopedic and surgical care.

Materials and Methods

Animals

All avian patients presenting with relevant conditions at the University Veterinary Hospital (UVH), Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM), Selangor, Malaysia, were diagnosed and subsequently included in the study during the treatment phase. As the study involved routine veterinary medical procedures conducted on four wild-ranging birds and two client-owned pet avian patients, formal approval from an ethics committee was deemed unnecessary. However, informed consent was obtained from the owners of the client-owned pet avian patients through a signed consent form. Nevertheless, the avian patients underwent a thorough examination in strict adherence to the Malaysian Veterinary Surgeons Act of 1974 and the Animal Welfare Act of 2015. A total of six avian patients, each belonging to a different species, were included in this comprehensive evaluation (Table 1).

GEN/CLI Loaded CaP Bead Preparation

The implant material utilised in the study consisted of a biodegradable osteopaste, specifically a calcium phosphate-based synthetic bone construct (CaP SBC), supplied by GranuLab (M) Sdn. Bhd., Malaysia. The CaP beads were prepared in a controlled and sterile environment located in the operation theatre. The preparation was carried out by incorporating 0.4 g of clindamycin hydrochloride (Clinacin 150 mg, Chanelle Pharma, Ireland), equivalent to 300 mg, and 2.75 ml of gentamicin sulphate (Genta-Kel 10%, KELA Laboratoria NV, Belgium), equivalent to 275 mg, into 4 g of CaP SBC.[7,8,18] The combinations were subsequently thoroughly mixed using a sterile spatula for a duration of 5–10 minutes until they achieved a semisolid consistency. The resulting mixture was then carefully placed into silicone bead moulds measuring 3-5 mm in size. The duration required for the curing process of each individual bead is estimated to be approximately 40–45 minutes. The beads were subsequently positioned under aseptic conditions, specifically within a sterile blue film. A 4-gram CaP SBC powder yields an estimated quantity of approximately 60 beads, each measuring approximately 5 millimetres in diameter.

Surgical Implantation

All avian patients were induced and maintained under general anaesthesia using isoflurane (Isoflurane USP 100%, Piramal Healthcare Limited, India) in combination with 100% oxygen. Bacterial swab samples were aseptically collected from all the avian patients for bacterial culture and bacterial sensitivity test, both prior to and during the surgical intervention. Contaminated compound and closed fracture wounds, along with the abnormal mass, were appropriately addressed. A total of five beads, with a diameter of 3-5 mm, were implanted, taking into careful consideration the dimensions of the wounds.

Surgical Wound Assessment

The assessment of the surgical wound was conducted on the second day post-operation and subsequently on alternate days until full recovery was achieved. The evaluation of the surgical wound was carried out using a modified Southampton wound scoring system.[10] In this study, only primary grading's were used, thus changing it as a modified Southampton wound scoring system (Table 2).

Radiographic Assessment and Evaluation of Fracture Healing

Bone healing was evaluated by means of sequential radiographs taken prior to and after the fracture repair procedure, at two and six weeks following the surgical intervention. Ventrodorsal (VD) and lateral radiographic images were obtained from selected individuals. The X-ray settings for avian patients were set at 44 kilovolt peaks (kVp) and 2 milliampere seconds (mAs), with an exposure time of 0.04 milliseconds. The assessment of the fracture healing process was conducted in a descriptive manner, utilising a radiological grading system (Table 3).[11]

Table 1. Case details and outcomes of six avian patients treated with combination of GEN/CLI loaded CaP beads.

Case	Signalment	Diagnosis	Bacteria culture	Outcome
P1	1-y-old, 0.47 kg, African grey parrot	Bilateral periorbital abscess	*Pseudomonas aeruginosa*	Complete healing at day 10 No reoccurrence
P2	10-y-old, 5 kg, black swan	Gred 4 pododermatitis	*Escherichia coli*	Complete wound healing at day 14
P3	Adult, 0.82 kg, spotted wood owl	Compound comminuted fracture at the right proximal ulna	No growth	Resolution of wing droop at day 14 Normal wound healing at day 10
P4	9-m-old, 2.5 kg, Chinese goose	Compound comminuted fracture at 1/3 right proximal radius-ulna	*Escherichia coli, Enterococcus fecalis, Streptococcus bovis-equinus complex*	Resolution of wing droop at day 32 Normal wound healing at day 8
P5	Adult, 1.2 kg, crested serpent eagle	Self-mutilation wound	*Escherichia coli, Enterococcus fecalis*	Poor and delayed wound healing Start of normal healing at day 21 Complete wound healing at day 48
P6	Young, 0.47 kg, barn owl	Severe (1/3 distal left radius-ulna malunion and elbow luxation)	No growth	Normal wound healing at day 12

Table 2. A modified Southampton wound scoring system.

Grade	Appearance
0	Normal healing: without bruising / erythema
1	Normal healing with bruising / erythema: mild contusion, noticeable contusion, mild erythema
2	Erythema including other signs of inflammation: at one point, around sutures, along wound, around wound
3	Serous or serosanguineous exudates: at one point only (< 2 cm), along wound (> 2 cm), large volume, prolonged (> 3 days)
4	Major complication: purulent exudate, at one point only (< 2 cm), along wound (> 2 cm)
5	Deep or severe wound infection with or without tissue necrosis

0 and 1 = Normal healing; 2 and 3 = Minor complication; 4 and 5 = Severe wound infection

Table 3. Radiological scoring system for fracture healing.

Criteria	Observation
Fracture line	Visible Partially visible Absent (Clinical Union)
Axial alignment	Poor Fair Good
Callus formation	No Callus Minimal callus Extensive callus
Callus remodelling	No remodelling Early sign Partial Complete

Statistical Analysis

All scores were tested for their coherence, and results were compared across days using the one-way analysis of variance (ANOVA) with the Tukey-Kramer (HSD) test. The statistical analysis was conducted using JMP-10 Pro software.

Results

Evaluation of Wound Healing

In the present study, variations in the progression of wound healing were observed among individual patients (Figure 1), as assessed using a modified wound grading scale that categorizes wound healing based on the severity and is subsequently quantified as a wound score (WS). There were significant differences in the wound score progression based on the modified Southampton wound scoring system ranking periodically at $p < 0.05$ (P1 = 1.14 ± 0.45; P2 = 1.57 ± 0.36; P3 = 1.71 ± 0.42; P4 = 1.29 ± 0.42; P5 = 4.57 ± 0.20; P6 = 1.57 ± 0.48). On day 2, all the patients (P) had WS-2 and 3 except P5, who had WS-4 and 5. This is most likely due to extensive wound contamination and suture breakdown in P5, whereas other patients experienced healing with serous or serosanguineous exudates. A noteworthy proportion of wounds (83%, 5/6) patients exhibited a parallel progression through the distinct phases of wound healing. The inflammatory phase starts between day 2 and day 8, and the granulation phase starts between day 8 and day 12. Subsequently, from day 10 onwards, a state of normal wound healing was observed, characterised by a WS-0 and 1. No significant ($p > 0.05$) disparities in wound healing appearance were observed between primary intention and tertiary intention healing, except for P5. The progression of WS from day 2 to day 14 demonstrates an optimal wound grading of grade 3, which subsequently improves to grade 0. The grading of wounds at grade 2 typically commences between days 4 and 6 in majority of the avian patients, before transitioning to grade 1. Wound grade 1, characterized by the appearance of a contusion with normal healing, typically persists for approximately 2 to 4 days before transitioning into the normal healing process associated with grade 0. The typical healing process for a grade 0 wound commences between days 10 and 14 following the initial injury, with scab detachment or peeling commonly observed by day 14. Patient P5 showed consistently the highest WS with grade 4 and 5 wound appearance following the surgical correction. Wound grade 4 and 5 denotes wounds that exhibit notable complications, including dehiscence and bacterial infections. A progressive dissemination within the wound leads to tissue necrosis, necessitating the implementation of wound debridement on day 13. Consequently, the wound healed by secondary intention. Notably, complete healing in P5 occurred by day 48.

Assessment of Bone Healing

Only two patients, P3 and P4, met the criteria to be considered suitable candidates for bone healing assessment (Figure 2). Both cases underwent a surgical debridement procedure followed by external coaptation using the figure of 8 bandage technique to effectively immobilise the fracture sites. Post-operatively, in the first and second weeks, secondary union with a visible fracture line, fair to good axial alignment, and minimal callus formation were seen in both birds. At five and six weeks following the surgical procedure, the patients

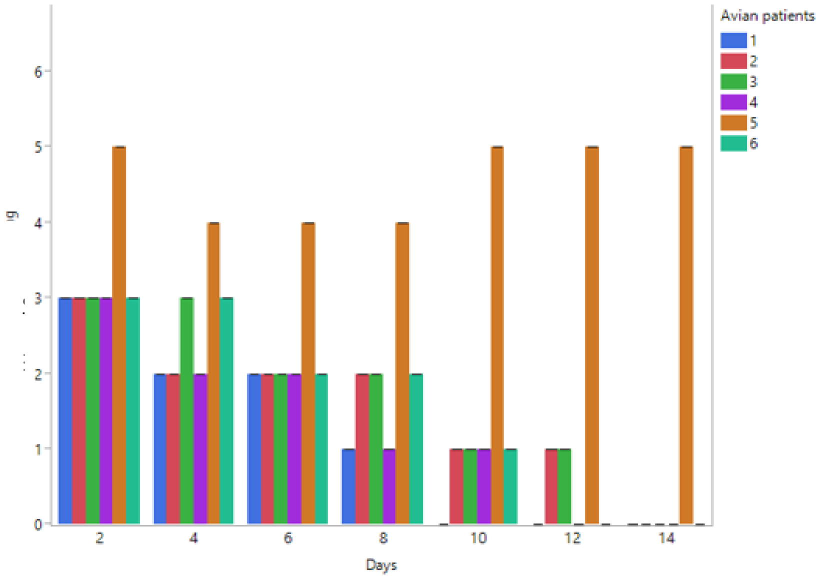

Figure 1. Bar graph illustrating the progression of wound score (WS) for six avian patients from day 2 to day 14, assessed using the modified Southampton wound scoring system. Statistical analysis using one-way ANOVA with Tukey-Kramer (HSD) post hoc test indicated a significant difference in healing progression among patients ($p < 0.05$), with patient P5 consistently demonstrating higher wound scores throughout the observation period (mean ± SD: P5 = 4.57 ± 0.20), indicating delayed healing compared to the other avian patients.

Figure 2. Pre-operative and post-operative ventrodorsal (VD) radiographs illustrate the progression of fracture healing for patients; P3 (left column) at 0, 2, and 6 weeks, and P4 (right column) at 0, 1, and 5 weeks. Figures E and F demonstrate evidence of secondary bone union, characterized by partially visible fracture lines and progressive callus formation.

exhibited secondary union, which was characterised by a partially visible fracture line. Additionally, the patients displayed good axial alignment and extensive callus formation, indicating a robust healing response. Furthermore, complete bone remodeling was observed, indicating a successful recovery process. Additionally, it should be noted that the 3mm CaP beads that were implanted in P3 experienced degradation and were no longer visible by day 15. In contrast, the 5mm CaP beads that were implanted in P4 only partially degraded and remained visible up until day 35.

Discussion

The process of wound healing in different avian patients is subject to various factors, including the nature of the wound, its extent, and its anatomical site.[12] These findings were comparable to those that Guo and Dipietro published.[13] The progression of wound healing observed in this study aligns with the previously described reports on physiological stages of wound healing.[14,15] The healing sequence starts with inflammation, followed by granulation, and culminating in remodelling was evident in most patients. The exception, P5, underscores how contamination, suture failure, and infection can significantly hinder healing, necessitating secondary intention approaches. These findings mirror those by Guo and DiPietro, who described similar complications delaying wound healing in compromised tissues.[13] The positive synergetic bactericidal effects of GEN/CLI loaded CaP beads in reducing bacterial burden supports findings from earlier research that demonstrated antibiotic-impregnated biomaterials enhance wound healing by controlling infection compared to wounds that did not receive antibiotic beads.[16-19] Additionally, The temporal progression of wound score seen in this study WS-2 and 3 to WS-0 and 1 over approximately two weeks is consistent with healing timelines observed in other animal models including goats, rabbits, rats, and sheep.[20-23]

The GEN/CLI loaded CaP beads have demonstrated favourable biocompatibility, exhibiting a propensity to stimulate osteogenesis without eliciting any adverse effects on the bone regeneration mechanisms.[24] According to Tully's findings, it has been observed that the process of complete secondary unions accompanied by bone remodelling, commences at the fourth week for internal fixation and at the eighth week for external fixation.[12] The application of CaP beads were employed to fill the dead space, thereby facilitating optimal conditions for a complete union and subsequent bone remodelling without any osteosynthesis. The evaluation of these interventions was conducted at weeks five and six following the surgical procedure and showed, a remarkable bone remodelling. Sakamoto *et al*. reported similar outcomes, with CaP successfully reconstructing cranial defects and preventing MRSA infection in rat models.[22] However, in contrast, CaP beads were utilised in this study as opposed to CaP paste, as described by Sakamoto *et al*.[22] Furthermore, the observed variability in degradation rates between 3mm and 5mm beads in patient P3 and P4 indicates that the bead size and porosity play critical roles in biodegradation and antibiotic elution profiles.[23-25]

The use of GEN/CLI loaded CaP beads demonstrated promising potential in enhancing both wound and fracture healing in avian patients. The treatment was well-tolerated, exhibited favourable biocompatibility, and supported new bone formation without adverse effects. Given the current paucity of data regarding the application of GEN/CLI loaded CaP beads in avian species, the result of this investigation contributes valuable knowledge to the field. The combination of antibiotics delivered through CaP beads offers a synergistic effect that may help address challenges such as bacterial contamination and antibiotic resistance. Hence, this method presents to be a viable and beneficial adjunct in the surgical management of contaminated wounds and fracture repair in avian patients, with potential applicability to other animal species involving similar clinical challenges.

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Conflict of Interest

The authors declare no conflict of interest.

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