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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Histological evaluation of transverse fracture healing in pigeon (*Columba livia*) ulna

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Abstract

This study was conducted on 12 adult pigeons (*Columba livia*) of six months age. The birds were randomly divided into three groups of four birds in each. A transverse fracture was created at mid shat of left ulna. The fracture was fixed with external skeletal fixation and all operations performed under Isoflurane anaesthesia. All birds were studied for three, six and 12 weeks. This study was approved by Animal Care and Use Committee of University Putra Malaysia. The purpose of this study was to find out the histological assessment of healing of ulna fracture stabilized with external skeletal fixation in bird model. Results at three weeks showed cartilage and cancellous bone union with a mean value of 2.75±0.63. At six weeks cancellous and compact bone union was seen with a mean value of 4±0.0. At 12 weeks significant bone union along with callus remodelling was observed at P<0.05. It was concluded that histological assessment of fracture healing showed that ESF provided excellent fracture healing and could be useful for fracture stabilization in avian clinical fracture management.

Keywords: Fracture; assessment; histology; bone union; ESF; pigeon

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Introduction

Fracture is a breakage in the continuity of bones that occurs due to the impact of a strong force on bones (Chitty and Lierz, 2008; Doneley, 2010). Primary bone healing occurs through rigid stabilization and fracture healing begins with Haversian system (Hatt, 2008). But primary bone healing in birds is lacking and it does not ensue in case of a gap or if movement is present in bones (Bennet and Kuzma, 1992), hence rigid fixation play important role for fracture fixation. Fracture is a frequent clinical problem in birds and needs successful fixation (Bennett, 1997; Redig, 2001). Various fracture conditions in birds conferred by trauma, falls and impacts with hard objects during flight (Bennet and Kuzma, 1992; Chitty and Lierz, 2008). For example, tibiotarsal fractures are commonly encountered by falcons during training and tracking (Muller and Nafeez, 2006). The fractures are mostly open and comminuted owing to the fact that bones in birds have less soft tissue coverage (Doneley, 2010). Proper fracture treatment is commendable to enable the birds to assume their normal functions as soon as possible. In addition, limited information is available on assessment of fracture healing in birds using histological method (Bennet and Kuzma, 1992). Histology is a basic technique that is used for the assessment of bone healing, bone union and cortex remodelling (An et al., 1999). Histologically there are two stages of bone healing; the primary (direct) and secondary (indirect) (Hatt, 2008). A good bone healing process can take place when a broken bone is fixed with rigid stabilization (Koivukangas, 2002). Indirect fracture healing is the process of bone healing which occurs through endochondral ossification. This healing process takes place by chondrogenesis that supports the formation of woven and lamellar bones, which in turn serves as a model for endochondral ossification.

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(Gerber and Ferrara, 2000; Frandson et al., 2003). Moreover, the bone is remodelled and then the woven bone hardens into lamellar bone by the action of the osteoclasts to resorb the old bone and then by the osteoblasts to form new bone (Marsh and Li, 1999).

An external skeletal fixator (ESF) has been successfully used for fracture fixation in birds and it imparts meagre damage to bones and the blood supply (Hatt, 2008). If such application is not used to stabilize broken bones of birds, facture will not heal and there will be delayed union (Kaderly, 1993). The accomplishment of quick bone union in bird fracture repair is key factor for the survival of fractured birds. Furthermore, choice for fracture treatment is somewhat limited for the birds because of their unique anatomical nature of their bones (Bennett, 1997). Thus, there is a need for a safe and effective fracture fixation to hasten the bone healing process. The ESF has been proven to be successful stabilization procedure for fracture treatment in avian species and thus recommended for clinical fracture cases (Redig and Cruz, 2008). The hypothesis of this study was that the ESF would stabilize the transverse fracture and clinical union in the pigeon ulna model. The purpose of this study was to find out the histological assessment of transverse ulna fracture healing and stabilization with external skeletal fixation in the pigeon model.

Materials and Methods

Study design

This study was approved by the Animal care and use committee of the Faculty of Veterinary Medicine, University Putra Malaysia (AUP/10 R118). In this study, pigeons (*Columba livia*) were selected as the model animal because they are generally used for fracture repair research (Gull, 2011). In fact, most of the experimental studies on fracture repair and bone healing have been carried out on pigeons (Bush et al., 1976: Jalila, 2002; Gull, 2011). Pigeons in good physical form were purchased from the local market two weeks prior to the experiment and were acclimatized. They were kept at the experimental house at the Faculty of Veterinary Medicine, Universiti Putra Malaysia. Feed and fresh tap water was made available *ad libitum* throughout the study. All the experiments were performed at the Postgraduate research room, University Veterinary Hospital, Faculty of Veterinary Medicine, Universiti Putra Malaysia. A total of 12 six-month-old pigeons weighing 292.50±12.88 grams (means±SD) were used in this study. The birds were divided randomly into three groups of four birds in each. They were observed for three, six, and 12 weeks post-surgery for histological assessment of fracture healing.

Anaesthesia and monitoring of patient

The birds were off feed six hours prior to surgery. All pigeons were anaesthetized through Isoflurane inhalation anaesthesia using a SurgiVet anaesthesia machine with 4% to 5% Isoflurane (Piramal Healthcare, India) mixed with O_2 (1-1.5 L/min) and using a SurgiVet facemask (Degernes, 2008). Then, 20 ml/kg body weight of lactated Ringers solution was injected subcutaneously. Later, the birds were intubated with a small, semi rigid un-cuffed plastic endotracheal (ET) tube (2.5 mm to 4 mm length), and the Isoflurane anaesthesia was maintained at 1.5-2.5% with O_2 (1-1.5 L/min) by using a modified Jackson Rees (MJR) non-rebreathing anaesthesia circuit system (Degernes, 2008).

Surgical procedure

The left wing was prepared using 40% Chlorhexidene solution and was painted with 10% Povidone solution. The birds were kept in a state of sternal recumbency on the surgical table over a heating pad (Conair Corp, East Windsor, NJ, USA) to maintain body temperature. For osteotomy, the left wing was extended and the dorsal approach was used to expose the ulna bone (Martin and Ritchie, 1994). A two centimetre longitudinal incision was made on the skin from cranial to dorsal between the radius and ulna for easy approach (Fig. 1). Next, the extensor metacarpi ulnaris, extensor metacarpi radials and extensor digitorum communis muscles and fascia were retracted (Doneley, 2011). The ulna bone was exposed gently and a Non-Critical Size Defect (NCSD) was created at the mid-shaft of the left ulna (Fig. 2). The fracture was induced using ESF pins with a mini pin driver inserted in the dorsal and lateral cortex and four holes were gently created around the cortex. Finally, the bones were separated using a bone cutter (Jalila, 2002). A similar surgical procedure was applied in all the NCSD groups.

Fracture stabilization with ESF and acrylic column

The fracture was reduced and stabilized by inserting an external skeleton fixation (ESF) Type-1, size 0.045″ (Imex, Veterinary Inc., TX, USA) in the cortex of the left ulna bone of the pigeon (Fig. 3). Four threaded ESF pins were fixed perpendicularly one by one to the long axis of the bone's cortex. A mini Jacobs chuck was used to insert and fix the pins, two proximally and two distally, to the fracture site (Redig, 2001). When the ESF pins were firmly in place, the fascia and muscles were sutured with Safil-violet 5/0 (Barcelona, Spain) and the skin was closed by an interrupted pattern. After that, a latex Penrose tube (3/8″ wide and 7 cm. long) was inserted over the top of the ESF pins parallel to and above the ulna bone. Then the latex Penrose tubing was filled with a 10 ml. mixture of acrylic material using a plastic syringe.

When the acrylic material had dried completely, the ESF pins over the column were cut using a pin cutter (Fig. 4) (Redig, 2001).

Post-operative care

Butorphanol tartrate 0.2mg/kg (2mg/ml, Fort Dodge Animal Health, USA) was injected subcutaneously once only for post-operative analgesia. Terramycin antibiotic ointment (Pfizer, Inc. USA) was applied on the incision and the ESF pin site. The wound was covered with a melolin (Smith and Nephew, Ltd.) absorbent pad and a woven gauze sponge to prevent any infection. Then the wing was wrapped with a figure-of-eight bandage. After three days, the figure-of-eight bandage was removed and the wing was covered with a coban bandage. An oral antibiotic tablet, Noroclav 30 mg (50 mg Amoxicillin and Clavulanic Acid), was given to the birds once a day for seven days.

Histological evaluation of transverse fracture healing in pigeon ulna

The operated birds were sacrificed at three, six and 12 weeks for radiographic and histological examinations using intravenous injections of 0.3ml of pentobarbital (182.0 mg, Lure Cedex, France). The specimens were removed and fixed in a 10% buffered formalin solution. After decalcification and dehydration, 5µm sections were cut and slides were stained with hematoxylin and eosin (H&E). The fracture healing scoring system is described in Table 1.

Statistical Analysis

All procedures were performed at 95% confidence level. The statistical analysis was performed using the SPSS system (v 20.00, Inc., Chicago, IL, USA).

Results

NCSD fracture healing after three weeks

After three weeks post-fracture fixation, a histological assessment of the fracture healing showed that 25% of the birds showed fracture healing with fibrous connective tissue union, 50% of the birds showed cartilage cancellous bone union, and 25% of the birds showed cancellous bone union (n= 4). 50% of the birds showed poor bone alignment while 50% had fair alignment. A minimal callus size was found in 25% of the birds, an average callus in 25%, while 50% of the birds showed extensive callus. Figures (5 and 6) show that there was no remodelling of callus of callus at three weeks.

NCSD fracture healing after six weeks

After six weeks post-fracture fixation, 100% of the birds showed bone union with cancellous bone formation (n=4/4). This mineralization arose at the cortex and developed from the endosteum proximally to the opposite side. 50% of the birds showed poor bone alignment, 25% showed fair bone alignment and 25% showed good alignment (n=4birds). A minimal callus size was found in 25% of the birds, while 75% showed extensive callus. No remodelling of callus was observed and there was formation of newly woven bone that had bridged the callus in the medullary cavity and had united the bone (Fig. 7).

Table 1: Modified histological assessment parameters of non-critical sized defect healing in pigeons. Source: (Marino et al., 1979; Jalila, 2002)

Criteria	Subjective observation	Score
a) Bone Union	Fibrous Clot	0
	Fibrous connective tissue and cartilage	1
	Cartilage only	2
	Cartilage and cancellous bone	3
	Cancellous bone	4
	Compact bone (Clinical union)	5
	Remodelled compact bone	6
b) Axial Alignment	Poor	0
	Satisfactory (Fair)	1
	Good	2
c) Callus size	Minimal	0
	Average	1
	Extensive	2
	Remodelling	3

Table 2: Histological assessment of non-critical sized defect healing in pigeon ulna. Score Mean ±SE (n=4 birds/week in rows)

Assessment Parameters	Three Weeks	Six Weeks	Twelve Weeks
Bone Union	2.75±0.63[a]	4± 0.0 [ab]	5.25±0.25[b]
Alignment	0.5±0.29[a]	0.75± 0.48[a]	2.00±0.00[b]
Callus Size	1.25± 0.48[ns]	1.75± 0.25[ns]	1.50±0.64[ns]

[NS]. Not significantly different at P<0.05. [a,b]Means with different superscripts in a row differed significantly (P<0.05).

NCSD fracture healing after 12 weeks

After 12 weeks post-fracture fixation, 75% of the fracture was united nicely with compact bone union. A noticeable remodelling of the bony cortex was found in 25% of the birds (n=4). All the birds showed good bone alignment and minimal callus was found in 25% of the birds, average callus in 25%, extensive callus in 25%, and 25% of the birds showed remodelling of the callus (n=4). Figure 8 shows the complete remodelling of callus and Figure 9 shows healing with no remodelling.

The mean summary results of the histological assessment of NCSD healing revealed that cartilage and cancellous bone union was observed in the three-week group with a mean value of 2.75 ± 0.63. Cancellous and compact bone union was seen in the six-week group with a mean value of 4 ± 0.0, and lastly a compact bone

Fig.1: Photograph showing skin incision for osteotomy in pigeon ulna

Fig. 2: Showing creation of transverse fracture in Pigeon ulna

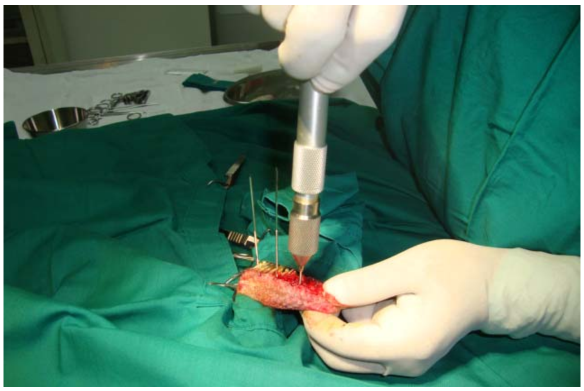

Fig. 3: Photograph showing fracture fixation with ESF type-1 in pigeon ulna

union along with a callus remodelling was noted in the 12-week group with a mean value of 5.25 ± 0.25. Good bone alignment was observed at 12 weeks post-fracture fixation as compared to the three-week and six-week bird groups (Fig. 10 & 11).

Fig. 4: Photograph showing ESF fixed with acrylic column in pigeon ulna

Discussion

The histological results indicated that the fractures of the ulna bones that were fixed with ESF demonstrated good cortical union. This investigation revealed that bone union was achieved in all groups, but compact bone union and remodelling was apparent at 12 weeks post-fracture fixation. This could be mainly attributed to the rigid stabilization of the fracture with ESF. The NCSD fracture managed with the ESF showed healing with primary bone union as well as indirect bone union or callus formation. In this study, bone healing for the three-week group occurred with fibrous connective tissue formation and excessive callus formation. Cortical union and bridging of callus were evident at six weeks. Internal and external callus formation was histologically observed in all the birds at three and six weeks. The same findings were observed by Rodriguez-Quiros et al. (2001). In this study, the ulna fracture was managed by rigid stabilization with ESF and provided good bone healing and remodelling after three months. The findings are in accordance with those by Griffon (2003), who mentioned that direct fracture healing occurs by rigid stabilization and complete remodelling takes a few months. However, fracture healing in birds has not been well researched and only limited literature is available. There have been no experimental studies on fracture healing by rigid fixation in birds. While primary fracture healing in birds is lacking, primary bone healing occurs in mammals in cases of rigid stabilization and bone union directly in the Haversian system without callus formation. Primary bone healing will not occur if there is a space at the fracture site. Secondary fracture healing occurs by inflammation, soft callus formation, hard callus formation and remodelling of callus (Bennett and Kuzma, 1992). The present investigation also depicted in the histological assessment that the fracture healed at three weeks by callus formation, and

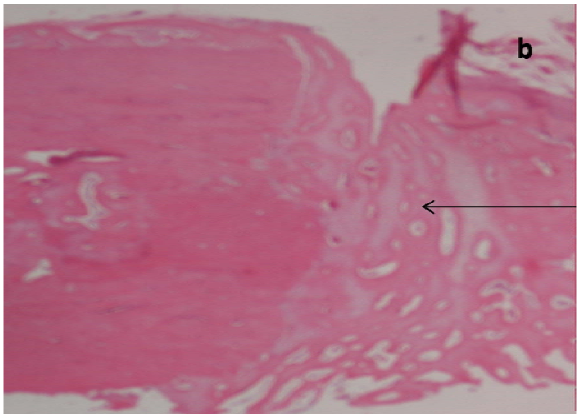

Fig. 5: Photomicrograph showing corticocancellous bone union at 3 weeks Arrow shows woven bone formation (H&E(x4)

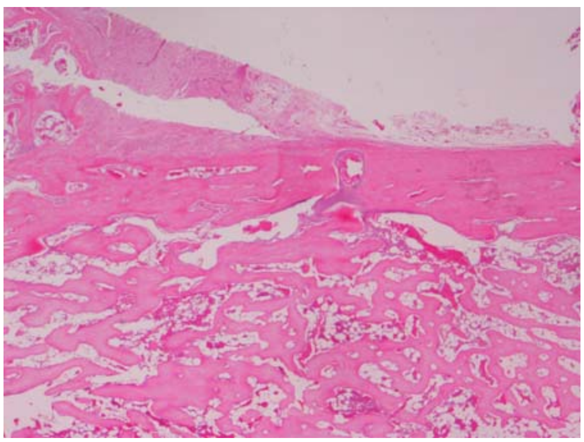

Fig. 6: Photomicrograph showing trabecular bone formation at fracture site after 3 weeks of fracture fixation- Pigeon-12 (H&E(x4)

Fig. 7: Photomicrograph shows repair of ulna fracture healing at 6 weeks in pigeon with callus formation

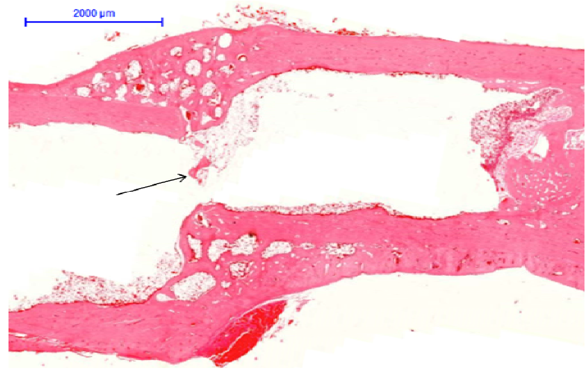

Fig. 8: Photomicrograph shows complete fracture healing and complete callus remodelling with rigid fixation and cortical union at 12 weeks (H&E staining)

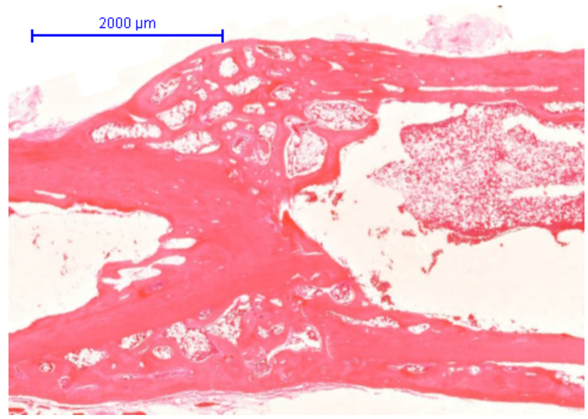

Fig. 9: Photomicrograph illustrates fracture healing, bridging of internal and external callus formation and no callus remodelling at 12 weeks (H&E staining)

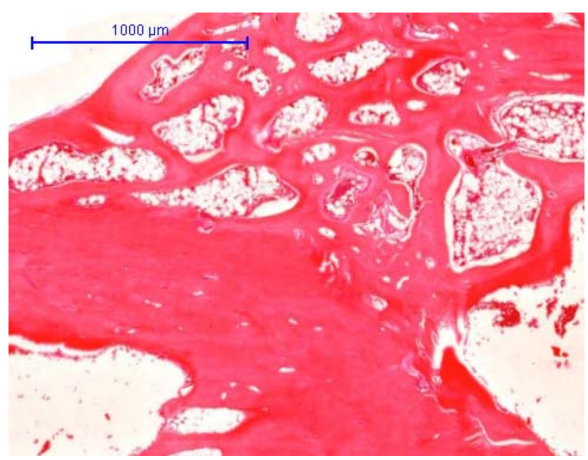

Fig. 10: Photomicrograph shows new bone formation and bridging of internal and external callus formation and bone union at 12 weeks (H&E staining)

Fig. 11: Photograph shows complete bone union after 12 weeks

remodelling occurred at 12 weeks post-fracture fixation. It was clear from this study that the ESF mechanically stabilized the broken ulna bone and provided good fracture healing. The histological results of this study suggest that the bone united nicely at 3 weeks and better healing occurred at 6 weeks. It shows that complete fracture healing occurred between 3 to 6 weeks but remodelling was observed in the 12-week bird group. Similarly, the findings of this study are in agreement with those of Marsh and Li (1999), who mentioned that ESF provided sufficient stability for rapid endosteal healing without external callus formation.

The results of this study are in agreement with the observation of Rosenthal et al. (1994) regarding the stabilization of cockatoo femur and tibiotarsus fracture using the ESF Type-1 fixator connected with an acrylic bar. Such a fixation method provides excellent fracture repair and an early return to function. The ideal fracture stabilization devices should provide firm support and should be flexible for fracture healing in birds (Orosz, 2002). However, ESF pins are light in weight and cost-effective for fracture repair in birds (Doneley, 2011). Many fracture fixation techniques that have been described in the literature could be useful for the repair of fractures in birds (Altman et al., 1997). However, ESF is the most frequently used method for fracture stabilization in birds. This technique has its advantages as there is less damage to vascularity and it provides good stability (Hatt, 2008). Similarly, the ESF provided good stability in the current study where the ESF pins were fixed in the thin cortex where they had minimum interference in the bone cavity (Redig, 2001). In this study, transfixation pins were also inserted in the cortex of the pigeon's ulna safely (Doneley, 2011). A radiographic assessment of the fracture healing showed that bone union, the fracture line, axial alignment, callus formation and callus remodelling were achieved between three and six weeks in all the groups with the ESF pins and a similar finding was observed at 4.6 weeks in the ulna transverse fracture of the pigeons when the fracture was stabilized with the IM-ESF tie-in technique (Jalila, 2002). Similar results were found in raptors by Redig et al. (2000), who reported that clinical union was observed at 4.4 weeks after fracture stabilization with the ESF-IM fixator tie-in technique, and he further found earlier clinical union at three weeks post-fixation in birds. Similarly, radiographic examination of fracture healing showed a higher endosteal callus formation and less periosteal callus formation in birds (Walsh et al., 1997). Furthermore, endosteal callus formation was observed at three weeks and six weeks. In their study, Bennett and Kuzma, (1992) reported that ESF provided good bone alignment and strength, which promotes bone healing with minor callus formation. The ESF Type-1 has been proven to be effective for fracture fixation and is better tolerated by all birds.

Conclusion

The ESF pins used for the fracture fixation in this study are easy to apply and this is a suitable technique for ulna fracture fixation in bird models. It is concluded that ESF may advance bone healing and could be useful for fracture fixation in avian clinical fracture management.

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