Evaluation of electroacupuncture in bone healing of radius-ulna fracture in dogs

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Abstract

Acupuncture promotes peripherical sensory stimulation and local and distant release of neuropeptides and can influence bone healing. The aim of this controlled and prospective clinical study was to evaluate the effects of electroacupuncture (EA) in bone healing and bone mineral content of canine radius-ulna fracture after closed reduction and external immobilization. Ten dogs with radius-ulna fractures were randomly allocated to 1 of 2 treatment groups. Group 1 dogs (n=5) received percutaneous EA immediately after closed reduction and external immobilization; after this first treatment, the frequency of application was twice a week during 4 weeks. Group 2 dogs (n=5) did not receive EA treatment. Radiographic score (RS) system and Radiographic optical densitometry (ROD) were used to assessment of fracture healing at the first day of clinical evaluation (M1), 30 days (M30) and 45 days after treatment (M45). Values of $P<0.05$ were considered significant. Mean±SD ROD values (mmAl) between groups 1 and 2 did not differ significantly ($P=0.15$) at all periods, respectively M1 values (4.94±0.94; 4.3±1.14), M30 (5.19±1,24; 4,91±1.45) and M45 (5.16±1.12; 5.31±1.71). Mean±SD RS values of group 1 at M30 (2.8±0.83) was significantly different ($P=0.003$) from M45 (4.6±0.54). However, mean±SD RS values of group 2 at M30 (4.4±1.51) was not significantly different ($P=0.30$) from M45 (5.16±0.75). Comparisons of mean±SD RS values between groups 1 and 2 showed no significant difference at M30 ($P=0.07$) and M45 ($P=0.19$). Results demonstrated that EA treatment did not accelerate bone healing and did not enhance bone mineral density in canine radius-ulna fracture during the 45 days of follow-up.

Key words:
Electroacupuncture.
Bone healing.
Fracture.
Radigraphic optical densitometry.
Dogs.

Introduction

Radius and ulna fractures are very frequent in small animals.[1] The incidence can occur in 8,5% to 18% of bone fractures in dogs and cats. The most common causes are car accident and minimal trauma such as fall or jump, mainly in small breeds.[2]

Non-union, delayed union or problems in angulations or rotation are very common complications in distal radius and ulna fractures, mainly in toy breeds. This fact is due to a decreased vascular density at the distal diaphyseal-metaphyseal junction, compared with large breed dogs and associated with poor prognosis for fracture healing in small-breed dogs. Also biomechanical factors may predispose to continued bone fragment instability after fracture reduction, including the local anatomy and fracture type.[3] Nerve injury may be associated with the initial fracture[4] and the involvement of radial nerve must be considered to be evaluated in dogs with radius and ulna fractures[1].

Although open reduction is recommended in those cases, the owners frequently select closed reduction and external immobilization because of financial restraints and convenience. However, one must have a good ability to apply for this technique and there must be a constant

supervision of the patients in order to have favorable results.[5] Campos et al.[6] evaluated the effectiveness of external immobilization by bandage in 43 miniature breed dogs with distal radius-ulna fracture. They found success rate of 91% of the dogs with bone consolidation after a mean of 45 days and only 9% of the dogs presented non-union. These results demonstrated superior success rate when compared with the literature. [2, 3, 5, 7, 8]

In the evaluation of fracture healing, many factors that influence or contribute to complications must be considered. This knowledge could partially explain the radiographic appearance in follow-up evaluations. A normal vascular ingrowth at the fracture site occurs within the first 10 days after injury. This ingrowth is seen radiographically as slight demineralization of fragment ends. The available soft tissue in the region of the fracture is the principal source of neovascularization for healing bone – extraosseous blood supply. Antebrachial diaphyseal fractures in miniature breeds heal more slowly because there is a decreased soft tissue support and poor vascular recruitment in the region. The stability and degree of postreduction apposition are other important factors that must be considered.[4] Identification of innervations in bone tissue was demonstrated by imunohistochemical studies and this fact leads to associate the neural influence and bone healing in fractures.[9,10,11,12]

Acupuncture was recognized as a veterinary specialty in Brazil.[13] Acupuncture promotes peripherical sensory stimulation and local and distant release of neuropeptides. This fact occurs because of the involvement of Central and Peripherical Nervous Systems.[14] Several scientific researches have been done in the last 20 years, enhancing the acceptance and incorporation of this procedure to the treatment protocol of many diseases. Acupuncture is related to a variety of physiological effects in diverse internal systems. It can be combined with other medical or surgical treatments, especially in resistant diseases.[15] Acupuncture can influence healing and regeneration of tissues, including bone fractures[16,17,18] although routinely the principal indications are neurological and/or musculoskeletal related diseases with a mean of 79.6% of recovery.[19]

Shen et al.[17] related the effect of acupuncture in fracture healing of rabbit radius due to the pituitary-thyroid axis. They found in the second week a significant increase ($P<0.01$) of the serum T4 and TSH contents in the treatment group compared with the control group. They also found in the fourth week a significant increase ($P<0.05$) of the serum T3 content in treatment group compared with the control group. The acupuncture points Large Intestine (LI) 4, LI11, Spleen (SP) 6/ Gallbladder (GB) 39 and Stomach (ST) 36 were used with electroacupuncture (EA) stimulation.

Sharif and Bakhtiari[18] evaluated acupuncture treatment in experimentally canine radius fracture following external immobilization with plaster of paris cast. The acupuncture points LI11, LI4, ST36, Triple Heater 5 were used during 10 minutes daily for two weeks. After 90 days, they observed a positive and stimulatory effect on callus formation resulting to comparatively more bony tissues with trabeculae formation and with least fibrocartilage in the experimental animals. It was concluded that acupuncture therapy is quite effective in bone healing and faster remodeling of callus. However, it was not performed evaluation at the earlier stages of bone healing.

Radiographic optical densitometry (ROD) is an analytic method to measure bone mineral content through radiographic images.[19,20,21,22] It is also a noninvasive method to make serial evaluation of the bone after therapeutic interventions.[23,24] The optical density is measured by image processing software that compare the grayscale of an aluminium penetrometer with the shade of a selected bone portion, both radiographed simultaneously. The optical density values are given by millimeters of aluminium (mmAl).[23]

Improvement of bone healing rate accelerates the return of musculoskeletal function in fracture cases. Therefore, it is relevant the knowledge of methods that stimulate bone healing, especially in common cases of closed reduction of radius and ulna fractures and external immobilization. The aim of this controlled and prospective clinical study was to evaluate the effects of EA in bone healing and bone mineral content of canine radius-ulna fracture after closed reduction and external immobilization.

Material and Method

This research was approved by the Bioethic Commission of School of Veterinary Medicine (SVM)/University of São Paulo (USP), protocol number 537/2004. All owners received and signed a consent form prior to the treatment.

From October 2004 to February 2005, 10 dogs were evaluated with radius and ulna fractures at the Veterinary Hospital of SVM/USP. The animals were randomly allocated to 1 of 2 treatment groups. Dogs in group 1 received EA treatment immediately after closed reduction and application of modified Robert-Jones bandage with splints[6]; those in group 2 were submitted to fracture reduction as described above and received meloxicam (0,1 mg/kg, q 24 h, for 3 days), instead of EA stimulation. The owners of dogs from group 1 were advised to medicate their dogs with meloxicam if there were signs of pain.

All dogs were submitted to anesthetic protocol of the Anesthesia Service (SVM/USP): sedation with intramuscular acepromazine (0,05 mg/kg) and tramadol (2 mg/kg), followed by intravenous propofol (5mg/kg) as general anesthesia at the moment of manual and closed reduction of the bone fragments. The limitation of external immobilization was the impossibility of complete coaptation of the bone fragments. The objective was to approximate the fragments as much was possible and maintain the alignment of the limb by observing its anatomy during the procedure and extending the member. This procedure would allow a secondary bone healing.

The modified Robert-Jones bandage was made after the closed reduction. An assistant kept the limb in extension and with the correct alignment during the procedure. An adhesive tape was first placed on the cranial and caudal surface of middle radius and ulna until the foot. Orthopedic rolled cotton was wrapped around the limb, making sure of a mild compression. Elastic gauze was wrapped over the cotton to compress it; at this moment the free end portion of the adhesive tape was turned over the extremity of the gauze. This procedure helped to avoid posterior slip of the bandage. The limb was then recovered by plaster associated by two wood splints, at medial and lateral faces, which helped to maintain the limb alignment. The bandage was made from the carpal region to the elbow, taking care to maintain exposition of the areas of the acupuncture points.

It was recorded the time (in days) when the dog had complete use of the affected limb at the moment of ambulation. Clinical evaluation also included the stability of the fracture site through the palpation of the limb, pain and increase of local soft tissues. Those signs were evaluated when the bandage was changed at 30 and 45 days of treatment.

The animals were submitted to radiographic and optical densitometry evaluation at three moments: at the first day of clinical evaluation at the hospital (M1), at 30 days (M30) and 45 days (M45) of evolution. Assessment of fracture healing was done through radiographic scoring system adapted from Silva[25] and Souza[26] (Table 1). The experienced observer that evaluates the radiographic scores was blinded to the treatment procedure. Bone mineral density of a portion of the proximal fractured fragment immediately above the fracture line of the radius was evaluated by radiographic optical densitometry as methodology described by Muramoto, Sterman and Fonseca Pinto[23]. The measure of BMD (mmAl) was done through software,

Table 1 - Radiographic scoring system for assessment of fracture healing, São Paulo - 2007

Scores	Radiographic signs
0	presence of recent fracture with no bone formation
1	irregularity at fragment lines of fracture site
2	initial/discrete periosteal proliferation
3	exuberant/organized periosteal proliferation
4	osseous callus in evolution with presence of periosteal proliferation
5	exuberant osseous callus in evolution and discrete radiolucent line at the gap between the fracture fragments
6	exuberant osseous callus and absence of radiolucent line

ImageLab (Softium®, Computer System). The radiographies were done at medial-lateral position with the individual technique due to different sizes and weight of the dogs. The same radiographic technique was used at the three moments of evaluation for the same dog. The first radiography was taken before the bandage was applied (M1). The bandages of all dogs were removed before the radiographies were taken at the two other moments (M2, M3). They were restored after each procedure. When the dogs presented clinical union, the bandages were removed at all.

Acupuncture points used in group 1 were: LI (Large Intestine) 4, LI11, BL (Bladder) 11, BL 23, KI (Kidney) 3 transfixed with BL60, GB (Gallbladder) 39 transfixed with SP (Spleen) 6 (Figure 1). The localization and selection of acupuncture points were done according to the literature[27,28] and the author's clinical experience. Percutaneous EA was performed with electronic device (Sikuro®, DS100CB model) and sterile acupuncture needles (Cloud Dragon®, 0,25X25mm dimension). Two acupuncture points were connected with an electrode to form a set, at frequency of 5 Hertz (Hz) alternated with 200 Hz, at three-second of intervals, during 15 minutes. The sets were LI4 and LI11, at the same side; BL11; BL23; KI3/BL60 and GB39/SP6 at the same side. Dogs were positioned at lateral recumbency and contained by owner's help. The frequency of application was twice a week during 4 weeks.

The major hypothesis was that EA would improve the time of return of ambulation and bone mineral density of the fractured limb. Mean values of age, bodyweight and time of clinical evolution were compared between the two groups using Student T test. The radiographic scores obtained from serial evaluations were compared between group 1 and group 2 at the same moment through Student T test. Within the same group it was performed also Student T test comparing M30 between M45. Mean time of complete weight bearing of the affected limb was compared between the two groups with Student T test. Mean values of BMD (mmAl) were analyzed with ANOVA. Values of $P<0.05$ were considered significant.

Results

Group 1 (with EA) consisted of 5 dogs, 3 females and 2 males, breed distribution: 3 Poodles, 1 Mongrel and 1 Pinscher. Data obtained was (mean±SD): age (41.8±39.53 months); bodyweight (4.8±2.2 kg); duration of clinical signs which represents mean time between the trauma and presentation at the Hospital (2.8±1.09 days). Site distribution of complete transverse radius-ulna fracture was: 3 at distal third and 2 at middle third. Group 2 (without EA) consisted of 5 dogs (one dog with bilateral radius-ulna fracture), 1 female and 4 males, breed distribution: 4 Poodles and 1 Yorkshire. Data obtained was (mean±SD): age (26.8±39.59months); bodyweight (4.2±2.1kg); duration of clinical signs (2±1.54 days). Site distribution of complete radius-ulna fracture was: 4 at distal third and

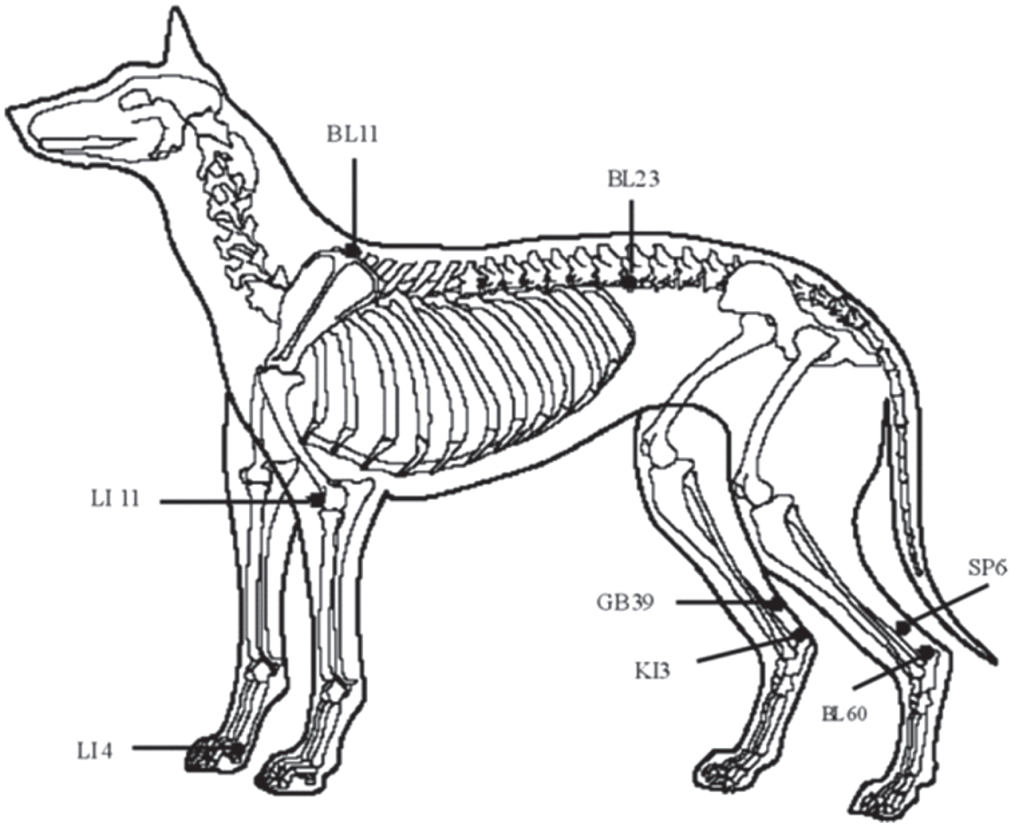

Figure 1 - Acupuncture points: LI4 – between first and second metacarpal bones, LI11- at the lateral end of the cubital crease, BL 11 – midpoint between caudal border of spinous process of first thoracic vertebra and the medial border of scapula, BL23-lateral to the caudal border of the spinous process of the second lumbar vertebra, SP6-3/16 of the distance from medial malleolus of the tibia to the stifle joint and caudal to tibial bone, KI3-between the malleolus and the talus, BL60- opposite from KI3, GB39- opposite from SP6

oblique, 2 at distal third and transverse.

Clinical variables between the groups did not differ significantly according to age (P=0.56), bodyweight (P=0.67) and duration of clinical signs (P=0.35). Mean±SD time of complete weight bearing of the limb in group 1 (15±3.8 days) was higher than group 2 (10.2±5.58 days), although there was not significant difference (P=0.15). Mean±SD BMD values (mmAl) between group 1 and group 2 did not differ significantly (P=0.82) at all time points, respectively M1 values (4.94±0.94; 4.3±1.14), M30 (5.19±1,24; 4,91±1.45) and M45 (5.16±1.12; 5.31±1.71).

Mean±SD radiographic scores of group 1 at M30 was significantly different (P=0.003) from M45. Although, mean±SD radiographic scores of group 2 at M30 was not significantly different (P=0.30) from M45. Comparisons of mean±SD radiographic scores between group 1 and 2 showed no difference at M30 (P=0.07) and M45 (P=0.19) (Table 2).

Table 2 - Mean ± SD radiographic score values for evaluation of bone healing of group 1 (with Electroacupuncture treatment – EA) and group 2 (without EA) at 30 days (M30) and 45 days (M45) after treatment, São Paulo – 2007

Time points	Group 1	Group 2
M30	2.8 ± 0.83^{aA}	4.4 ± 1.51^{aA}
M45	4.6 ± 0.54^{aB}	5.16 ± 0.75^{aA}

[a-b]Within a row, values with different superscript small letters are significantly (P<0.05) different; [A-B]Within a column, values with different superscript capital letters are significantly (P<0.05) different

Discussion

Several conditions can influence bone healing after fracture, because it is realized by cells. These cells can be modified by almost all endogenous and exogenous factors that are related to cell metabolism. Bone healing can be promoted by some factors like growth hormone, thyroid hormones, calcitonin, insulin, vitamin A and D, anabolic steroids, condroitin sulfate, hyaluronidase, anticoagulants, electric currents, oxygen and physical exercise.[29] Growth factors like bone morphogenetic protein and platelet-derived growth factor are also important in accelerating bone healing.[30]

Acupuncture was related to growth hormone release in patients with chronic pain and it was regulated by endogenous opioid peptides.[31] The thyroid hormones were correlated with electroacupuncture effect in fracture healing[16]. All these hormones are related to promote bone healing and can explain the good results with acupuncture and bone healing described by Sharifi and Bakhtiari[18].

Some neuropeptides like calcitonin gene-related peptide (CGRP), substance P (SP), vasoactive intestinal peptide (VIP), neuropeptide Y (NPY) are supposedly involved in bone growth, fracture repair and bone remodeling. The initial angiogenesis seems to be impossible without the influence and transmission of peptidergic fibers innervation.[32] CGRP and VIP are both efficient vasodilators and also modulate the adhesive properties of endothelial cells. CGRP is also mitogenic to endothelial cells.[11] The sprouting and reinnervation associated with the healing of rat tibial fracture suggest that CGRP may play an important regulatory role during the inflammatory, reparative and remodeling phases of injured bone tissues, and not only the possible origin of pain in bone injuries.[9]

The immunohistochemical demonstration of nerve fibers in the vicinity of bone tissue raises the possibility that neuropeptides may directly or indirectly modulate the activity of bone cells in physiological and pathological conditions, in line with the view of neuroendocrine and neuroimmune interactions. Some nerve fibers where positive for SP, CGRP, VIP, NPY, tyrosine hydroxilase (TH) and interleukin 1 (IL1).[12] However, neuropeptides in the sensory and autonomic nervous systems are synthesized in dorsal root or local sympathetic/parasympathetic ganglia, from where they are transported to their site of storage. Non-stimulated nerves do not seem to release their peptides to any great content, although release from the nerve terminals may lead to significant local concentrations upon stimulation.[11]

Two studies demonstrated that sensory stimulation (acupuncture) increased the CGRP and VIP release in the saliva of xerostomia sufferers and this fact was related to beneficial effects on salivary flow.[14,33] These two neuropeptides could be produced after acupuncture stimulation and released to a target organ. Although there is the possibility of acupuncture and CGRP interaction on bone repair, this fact must be more investigated.

In the present study, EA did not lead to an increase in bone healing when compared to the group 2 when evaluating the radiographic scores. Also there were no changes in ROD values of the proximal fragment of the fractured radius. It seems that acupuncture could be related to be positive in initial vascular regrowth that is seen radiographically as demineralization. If there was an increase of this vascular regrowth with acupuncture, it could explain the lower scores in group 1 obtained at M30 when compared with group 2, even though it was not significant. The radiographic scores might not be sensible enough to detect any beneficial effect. This fact must be evaluated with another method such as bone biopsy, usually at experimental studies. Also the neuropeptides involvement in these cases after acupuncture must be investigated with immunohistochemical studies or serum detection and bone metabolism markers.

The analgesic effects of acupuncture

are better well understood than its ability to improved impaired function[34]. Although it was not the aim of this study, EA did not exclude analgesic treatment because one dog of group 1 needed to be medicated by meloxicam. Another group of animals receiving analgesic treatment combined with acupuncture could lead to better results.

The aim of acupuncture and Traditional Chinese Medicine (TCM) is to reach homeostasis by using the own physiological effects of the animal or person. In this way, both pathological and physiological process can be balanced. The basic concept is represented by the terms *Yin* and *Yang*, opposite energies but also complementary. This can be briefly represented by anabolism (*Yin*) and catabolism (*Yang*) and parasympathetic and sympathetic influence.[35]

The TCM explores the association of bone to the energetic organ – kidney, so that stimulating the last one could accelerate fracture healing. So the acupoints BL23 and KI3 influence kidney function.[36] The acupoint BL11 influences bone; GB39 influences medulla and bone and it is also related to a stimulant action of parafolicular cells of thyroid. The maintenance of bone mass is the result of dynamic balance between *Yin* and *Yang*. This can be represented by balance of osteoblast (bone formation) and osteoclast (bone remodeling) activities, respectively *Yin* and *Yang*.[37]

The lack of comprehension of TCM language has restricted its application. The acupuncture research is a very important way to contribute for its better acceptance and approval by translating this ancient knowledge.[13]

Another clinical study with higher number of animals and/or other group treatment must be done to elucidate the influence of acupuncture on vascular regrowth and bone mineral content in fracture healing. The use of another immobilization technique with open reduction will possibly bring different results by eliminating the problems of secondary bone healing and partial coaptation of bone fragments. They can occur with manual closed reduction and could have influenced the values of the present study. Other frequencies of EA and acupuncture points must be used in order to observe the response of bone metabolism that could be modified.

Conclusions

The results of the present study demonstrated that EA treatment and external immobilization with closed reduction did not accelerate bone healing and did not enhance bone mineral density in canine radius-ulna fracture during the 45 days of follow-up.

Avaliação da eletroacupuntura na consolidação óssea em cães com fratura de rádio-ulna

Resumo

Acupuntura promove estimulação sensorial periférica e liberação de neuropeptídeos, podendo influenciar a consolidação óssea. O objetivo deste estudo clínico prospectivo foi avaliar os efeitos da eletroacupuntura (EA) na consolidação óssea e densidade mineral óssea em cães com fratura de rádio-ulna (FRU) após redução fechada e imobilização externa. Dez cães com FRU foram aleatoriamente destinados em dois grupos. Grupo 1 (n=5) recebeu EA percutânea imediatamente após a redução fechada e imobilização externa; após este primeiro momento, foram feitas aplicações duas vezes por semana durante 4 semanas. Grupo 2 (n=5) não recebeu EA. Um sistema de escore radiográfico (ER) e densitometria óptica radiográfica (DOR)

Palavras-chave:
Eletroacupuntura.
Consolidação óssea.
Fratura.
Densitometria óptica radiográfica.
Cães.

foram utilizados para avaliar a consolidação da fratura no primeiro dia de avaliação clínica no hospital (M1), 30 dias (M30) e 45 dias (M45) após tratamento. O nível de significância adotado foi de 5%. Valores (média ±DP) de DOR (mmAl) entre os grupos 1 e 2 não tiveram diferença significativa (P=0.15) em todos os momentos, respectivamente valores de M1 (4.94±0.94; 4.3±1.14), M30 (5.19±1,24; 4,91±1.45) e M45 (5.16±1.12; 5.31±1.71). Média±DP de ER do grupo 1 no M30 (2.8±0.83) foi significativamente diferente (P=0.003) do M45 (4.6±0.54). Entretanto, média±DP de ER do grupo 2 no M30 (4.4±1.51) não teve diferença significativa (P=0.30) do M45 (5.16±0.75). Comparações dos valores de ER entre os grupos 1 e 2 não tiveram diferença significativa no M30 (P=0.07) e M45 (P=0.19). Conclui-se que a EA não acelerou a consolidação óssea e não aumentou a densidade mineral óssea em cães com FRU durante os 45 dias avaliados após o tratamento.

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