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Clinical Studies

Analgesic Effect of Acupuncture in Dogs Undergoing Tibial Tuberosity Advancement Surgery: A Double-blind, Randomized, Controlled Pilot Study

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ABSTRACT

Tibial tuberosity advancement (TTA) surgery restores joint function in dogs with cruciate ligament failure. This study investigated the efficacy of reducing perioperative systemic opioid/non-opioid analgesics (i.e. fentanyl, ketamine), with acupuncture for TTA surgery. Twenty-one dogs admitted for surgery were divided into Controls (inhalation anesthesia, opioid/non-opioid analgesics), Electro-acupuncture (EAP) Group (inhalation anesthesia, EAP), or Dry-needle Acupuncture (DNAP) Group (inhalation anesthesia, DNAP). Intraoperative nociception, boluses of fentanyl, anesthetic recovery, post-extubation pain, duration until first postanesthetic actions, biomarker levels, and isoflurane end-tidal concentration, were recorded. Findings were analyzed for percent change from control/baseline values and statistical significance ($p<0.05$). Results for percent change from control/baseline included: mean fentanyl boluses during surgery: Control: 3.6±2.0, EAP: 3.6±1.5, DNAP: 2.7±1.2 (25% lower than control); mean recovery score: Control: 2.1±1.2, EAP: 1.5±0.8 (30% better), DNAP: 1.0±0.0 (50% better); median post-extubation pain score: Control: 5.21±4.3, EAP: 4.38±2.1 (16% lower), DNAP: 3.75±1.6 (28% lower), and maximum pain score: Control: 10.83±7.0, EAP: 8.80±3.5 (19% lower), DNAP: 7.92±3.7 (27% lower). DNAP had the greatest increase of serotonin (twice controls) and smallest increase of substance P (half controls) during the 24-hour post-extubation period. Statistically significant findings included: DNAP had shortened duration to postanesthetic micturition; and higher isoflurane end-tidal concentration in acupuncture groups. Under the experimental conditions of this pilot study, perioperative acupuncture treatment may reduce the need for additional opioid/non-opioid analgesics during TTA surgery, along with providing better anesthetic recovery.

Keywords: acupuncture, analgesia, anesthesia, balance method acupuncture, dry needle, electro-acupuncture, integrative veterinary medicine, tibial tuberosity advancement surgery

ABBREVIATIONS: ACL: anterior cruciate ligament; AP: acupuncture; BMA: Balance Method Acupuncture; C-0: initial skin incision/cutting time; C-f: skin closure/cutting finish; DAP: diastolic arterial pressure; Dial'Iso: dial position isoflurane vaporizer; DNAP: dry-needle acupuncture; EAP: electro-acupuncture; Et'CO_2: end tidal CO_2 level; Et'Iso: end tidal isoflurane level; HR: heart rate; IM: intramuscular administration; IV: intravenous administration; MAP: mean arterial pressure; NFB: number of fentanyl boluses; P_{max}: pain maximum; P_{med}: pain median; PIP: peak inspiratory pressure; RPM: respirations per minute; RR: respiratory rate; SAP: systolic arterial pressure; $Sero_{max}$: serotonin maximum; $Sero_{med}$: serotonin median; SP: substance P; SP_{max}: substance P maximum; SP_{med}: substance P median; Sp'O_2: arterial oxygen saturation; T-0: anesthesia induction but before acupuncture start; T-1: arrival at hospital; TCVM: traditional Chinese veterinary medicine; TTA: tibial tuberosity advancement

The term "anterior cruciate ligament (ACL) disease" is used to encompass a range of conditions that compromise this structure, and it is an important cause of hind limb lameness in dogs.[1] The pathologies covered by this term include traumatic ligament avulsion of the tibial or femoral junction, acute traumatic rupture, as well as chronic progressive degeneration that may culminate in partial or total rupture of the ligament.[1] One of the most frequently used surgical techniques for correction of joint instability created by ACL failure is advancement of the tibial tuberosity (TTA).[1] It is a relatively simple surgical technique that is able to modify the angle of the patellar tendon by altering its effective point of insertion.[1] Even though TTA is a surgical technique that presents very few complications, it is a painful procedure (arthrotomy, osteotomy, bone plate placement) with intense postoperative pain that may be difficult to resolve.[2-5]

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To augment perioperative analgesia in balanced anesthetic protocols of patients undergoing TTA, common options to choose from include: 1) opioids (e.g. morphine, fentanyl); 2) non-opioids such as ketamine (dissociative anesthetic), lidocaine, non-steroidal anti-inflammatory drugs (NSAIDs); and 3) regional anesthesia such as epidural administration of local anesthetics, or local ultrasound guided nerve blocks (e.g. sciatic, saphenous, obturator, femoral nerves).[5-9] Postoperative secondary effects which may include vomiting, delayed postoperative recovery, urinary retention, decreased level of consciousness are numerous and can be severe; prompting investigation into therapeutic alternatives.[9-14]

Perioperative acupuncture has been introduced in both human and veterinary surgeries as having potential to augment analgesia and improve patient outcome following surgery.[15-17] It has been shown that it can reduce consumption and complications of anesthetics.[18-21] Guidelines from the American Animal Hospital Association (AAHA) specifically list acupuncture as an appropriate intervention for pain management in soft tissue, orthopedic and dental surgeries.[16] This ancient technique, associated with traditional Chinese medicine (TCM), has demonstrated remarkable analgesic properties, and has received intense scrutiny by investigators to define mechanisms of action. Of particular interest is its ability to modulate the pain response at multiple levels of the nervous system. Needle insertion at an acupoint creates: 1) local pain relief (e.g. fascial changes, endogenous opioid production, release of neuropeptides, prostaglandin inhibition); 2) segmental (spinal cord) pain modulation (e.g. gating of nerve fibers in dorsal horn, decreased N-methyl-D-aspartate (NMDA) receptor activity, increased serotonin/endogenous opioids, substance P inhibition, visceral analgesia); and 3) suprasegmental (brain) effects that function to decrease nociceptive signaling and pain perception.[22,23] A combination of different acupoints can recruit local, segmental, and suprasegmental responses from the nervous system offering pain control at multiple levels of the body.[23]

A variety of techniques are used for stimulation of needles at acupuncture points with 2 of the most common techniques including dry-needle acupuncture (DNAP) and electro-acupuncture (EAP). Each technique offers slightly different benefits enabling a clinician to tailor treatment effects. The EAP procedure, by applying electrical stimulation to needles at different frequencies, produces important changes in the nervous system and is a common adjunct to acupuncture treatment to enhance the outcome and prolong the benefit of acupuncture (AP) treatment.[24-26] When set at low-frequency (2-20 Hz), needle stimulation leads to a release of beta-endorphin, enkephalin, and nociceptin/orphanin. At higher frequencies (50-100 Hz), the primary opioid released is dynorphin. At even higher frequencies (>200 Hz), the most prominent neurochemical mediator released is serotonin.[26-28] When integrated into a general anesthesia procedure, EAP can effectively complement the analgesic component, as demonstrated in surgeries such as limb amputation/replanting, and in muscle/bone fracture reconstruction.[29-32] In contrast to EAP, there is some evidence that DNAP may be most effective at mediating analgesic effects through C fibers, while EAP primarily mediates analgesic effects through Aβ and Aδ fibers.[26-28] There is also rationale for combining modes of acupuncture (DNAP, EAP) and different frequencies of EAP to produce a greater analgesic effect than would be possible with only one modality or one frequency.

There are numerous investigational and clinical studies with laboratory animals and humans, respectively, that have demonstrated AP benefit such as improved local vasodilation, increased immune response, release of natural endorphins and analgesic segmental effects on the spinal cord.[6-8] In veterinary clinical medicine, EAP treatments are commonly used for postoperative or musculoskeletal pain in dogs, but there is a lack of studies that have examined the effects of such treatments intraoperatively, especially orthopedic surgeries such as TTA. The objective of this study was to determine if applying acupuncture before, during and after TTA surgery would create sufficient analgesia to reduce the use of adjunct systemic analgesia (both opioid and non-opioid based). The primary hypothesis of the study was that canine TTA surgery patients receiving acupuncture integrated into their general anesthesia protocol in place of additional systemic analgesics, would present similar perioperative analgesia as the control patients receiving conventional systemic analgesia. Secondary endpoints compared quality of recovery, biomarker levels and inhalation anesthetic requirements for surgery patients receiving AP in place of systemic analgesics.

MATERIALS AND METHODS

Study Design, Subject Population

The experimental design was a prospective, double-blind, randomized and controlled clinical trial. Dogs recruited for study enrollment were patients admitted for TTA surgery at the Alfonso X El Sabio University (HCV-UAX) veterinary hospital, Madrid, Spain. Inclusion criteria included dogs of any age, sex, breed or body weight with cruciate ligament disease, a complete medical history, and determined to be healthy on physical examination and complete bloodwork (hematology and biochemistry) prior to surgery. Exclusion from the study included patients that were 1) considered aggressive animals and/or, 2) diagnosed with a systemic disease detected during the physical examination or laboratory tests of the preanesthetic evaluation. Enrolled dogs that had surgical or anesthetic complications during the surgical procedure were withdrawn from the study and data collected from these dogs excluded from statistical evaluation. The reason(s) for study withdrawal (including intraoperative nociception) were documented and included in the study findings. The owners of all patients included in the trial signed an informed consent document that included information about the study and authorized their animals to be enrolled.

Qualified subjects were randomly assigned to one of the following treatment groups: Control Group (inhalation anesthesia with additional systemic opioid/non-opioid analgesics), EAP Group (inhalation anesthesia with EAP pre-, intra-, and post-operative) and DNAP Group (inhalation anesthesia with DNAP pre-, intra-, and post-operative). The method of randomization was based on the use of a list of random numbers obtained by using a commercial statistical software program[a].

Premedication and Inhalation Anesthesia – All Groups

On the day of surgery, all patients upon arrival had an initial exam conducted by a veterinarian blinded to treatment groups. This person assigned baseline scores using previously validated scoring systems for behavior/attitude (Boscan and Wennogle scale) and pain (Glasgow Composite Pain scale), (Tables 1 and 2).[8,9,33] Subsequently, the assigned anesthetist (not blinded to treatment), performed a brief preanesthetic physical and if no abnormalities were found, the patient received intramuscular (IM) administration (dorsal lumbar musculature) of methadone[b] (0.1 mg/kg), midazolam[c] (0.2 mg/kg), and acepromazine[d] (20 mcg/kg).

After anesthetic premedication was administered, all animals were allowed to rest in the anesthetic recovery area with lights off to minimize stimulation. After 20 minutes, the animals were brought to the anesthetic induction room where they received pre-induction oxygenation by administering 100% flow-by oxygen (5 L/min for 5 minutes), followed by general anesthesia induced by intravenous (IV) administration of midazolam[c] (0.2 mg/kg) and dose-effect propofol[e] administered with a gradually increased dose until its effect was observed (dose varied between 2-6 mg/kg among subjects).

The trachea of the patient was intubated and the animal was connected to the anesthetic machine through a semi-closed circular respiratory system with isoflurane[f] at 1% vaporized in 100% oxygen at a flow of 1-2 L/min. All patients were connected to a mechanical ventilator with initial peak inspiratory pressure (PIP) settings of 10 mmHg and respiratory rate of 10 respirations per minute (rpm). These parameters were adjusted and recorded throughout the anesthetic procedure to maintain normocapnia (Et'CO_2 of 35-45 mmHg). All patients received IV fluid[g] therapy (balanced electrolyte-glucose). Once the instrumentation and monitoring of the patient was completed and their stability had been confirmed, aseptic shaving and preparation of the surgical limb proceeded.

At this point, a record of all previously recorded parameters was made, establishing the baseline measurements (Time 0/T-0). Likewise, meloxicam[h] dosed at 0.2 mg/kg IV, and cefazolin[i] at 22 mg/kg IV were administered. Induction with acupuncture (30-minute length) was started with insertion of acupuncture needles if a patient was in either the EAP Group or DNAP Group.

Table 1: Boscan and Wennogle grading scale used to assign attitude and anesthesia recovery score to study dogs[8]

Attitude/ Recovery Grade	Quality of Recovery	Description
1	Good	Dog does not seem agitated, raises its head calmly
2	Acceptable	Dog transiently panting and/or lightly pedaling with forelimbs just after extubation; then calms down
3	Poor	Dog panting or crying, occasionally pedaling, or showing uncoordinated movements, shows exaggerated sensitivity to sound but appears "absent". This behavior continues and gets worse over time.
4	Unsatisfactory	The dog is very agitated, tries to bite, squirms or tries to jump off the table with uncoordinated movements; appears to be "absent"

Table 2: Glasgow pain score scale[33]

Behavior Category	Score	Definition
Overall/Posture	0	Comfortable
	1	Unsettled
	2	Restless
	3	Hunched/tense
	4	Rigid
Appearance in Kennel "Call Name"	0	Happy/content/bouncy
	1	Quiet
	2	Nervous/anxious/fearful
	3	Depressed/disinterested
	4	Aggressive
Vocalization	0	Quiet, no vocalizing
	1	Whimpering/crying
	2	Groaning
	3	Screaming
Wound/Surgical Site	0	Ignoring
	1	Looking at surgery site
	2	Licking/rubbing surgery site
	3	Chewing at surgery site
Touch at Surgery Site	0	Do nothing
	1	Looks around
	2	Flinch
	3	Growl/guard site
	4	Snap
	5	Cry/scream
Mobility	0	Normal
	1	Lame
	2	Slow/reluctant to rise/sit
	3	Stiff
	4	Refuses to move

Systemic Analgesia Protocol – Control Group

After a patient was intubated, connected to a rebreathing circuit and maintenance inhalation anesthesia with isoflurane was established, the control subjects received a bolus of fentany[j] (3 mcg/kg) IV followed by a constant rate infusion of fentanyl[j] (0.3 mcg/kg/min), as well as a bolus of ketamine[k] (0.25 mg/kg) IV, followed by a constant rate infusion of ketamine[k] (10 mcg/kg/min) IV. To maintain blindness to study group, the controls had tape applied over acupuncture points along with cable and EAP machine hook up, identical to the acupuncture groups. After 30 minutes they were transferred to the surgical suite.

Acupuncture Analgesia Protocol – AP Groups

After a patient was intubated, connected to a rebreathing circuit and maintenance inhalation anesthesia with isoflurane was established, dogs in the acupuncture groups (EAP or DNAP) then received 30 minutes of acupuncture treatment while still in the induction room. For both the EAP and DNAP groups, this period of acupuncture prior to the surgical procedure, established the induction period for acupuncture intervention. This period was required to be completed prior to authorizing transfer to the surgery suite and start of the surgical procedure. The dogs in the acupuncture groups did not receive a bolus or constant infusion of ketamine and fentanyl during this AP induction period.

When the acupuncture needles were placed by the author (L.LL), they were twisted clockwise at the moment of insertion only. They were not touched again or monitored during the surgical procedure. An electrical stimulator device/acupunctoscope[l] was connected to the acupuncture needles[m] by EAP specific cables. The length of the needles used depended on the patient size and acupoint location, and measured 28-gauge×2.0 inch, 28-gauge×1.5 inch, 28-gauge×1.0 inch, and 27-gauge×0.5 inch.

All groups (including controls) had identical acupuncture needle placement (all needles taped to obscure type of treatment), along with cables and attachment to the acupunctoscope to maintain group blindness of the staff conducting the study (Figure 1). The only difference between controls and acupuncture groups was the needles were not inserted into the acupoints of the controls. Acupuncture points used for surgery analgesia consisted of 1) distal acupoints which provided general endorphin-release and 2) acupoints that provided local analgesia to the surgery site. Due to the necessity of maintaining maximum sterility for orthopedic surgery, local paraincisional acupoints were not used. Instead, acupoints that mirror this area were selected using Balance Acupuncture Methodology.[34]

Distal acupoints used on all dogs included: LI-4, LIV-3, GB-41, ST-43. In the EAP Group, electrical stimulation applied to distal points included LI-4+LI-4 (right to left front leg), and LIV-3+GB-41 (surgery leg only). The acupoints used to mirror paraincisional points and received electrical stimulation in the EAP group included: LI-11+HT-3 (each front leg), ST-36+GB-34 (unilateral, opposite leg to surgery leg), 1 needle crossing SP-6 through GB-39 (unilateral, opposite leg to surgery leg, electric cable hooked to each side of the needle), 1 needle crossing GB-33 through LIV-8 (unilateral, opposite leg to surgery leg, cable on each side of needle). Two classical acupoints, *Bai-hui* and *Tian-ping*, had a dry needle placed in each acupoint and were not attached to the acupunctoscope or had cables attached to the acupoints (Tables 3 and 4).[35]

A dog undergoing TTA surgery on the left hindleg in the EAP Group, for example, would have an acupuncture point stimulation pattern consisting of: LI-4 connected to LI-4 (right to left front legs, EAP), LI-11+HT-3 (right front leg, EAP), LI-11+HT-3 (left front leg, EAP), LIV-3 +GB-41 (surgery leg, left hindleg, EAP), ST-43 (surgery leg, left hindleg, DNAP), LIV-3, GB-41, ST-43 (right hindleg, DNAP), ST-36+GB-34 (right hindleg, EAP), one needle inserted through SP-6 and GB-39 (right hindleg, EAP), one needle inserted through LIV-8 and GB-33 (right hindleg, EAP); *Bai-hui*, *Tian-ping* (classical points on back, DNAP). The DNAP Group had identical acupoints used, however, cables were not connected to the needles, therefore, no electrical stimulation was administered.

Patients in the EAP group began 30 minutes of induction with EAP. They had needles stimulated with electricity (F1=20 Hz and F2=0 Hz) at distal acupoints. Acupoints that mirrored the incision site were stimulated with higher frequency (F1=100 Hz, F2=200 Hz). The amplitude started at 3 mA and gradually increased 1 mA every 5 minutes up to a maximum of 6-8 mA during the intervention. The DNAP Group patients, had identical placement of acupuncture needles, however, cables were not connected to the needles, therefore, no electrical stimulation was administered. The acupuncture needles and cables were covered by surgical drapes to maintain blindness of the staff during the surgical procedure (Figure 2).

Figure 1: Picture of the right hindleg of a study dog prepared for TTA surgery on the left hindleg, demonstrating tape applied over study acupuncture points and cables: SP-6 and GB-39 (black cable), ST-36 and GB-34 (blue cable), LIV-8 and GB-33 (red cable).

Table 3: Acupuncture point stimulation protocol for study dogs in the DNAP (dry-needle AP) and EAP (electro-AP) treatment groups*

Distal Acupoints	Local Acupoints** (Unit Set F1=100 Hz, F2=200 Hz)	Classical Acupoints (No Electrical Stimulation)
LIV-3+GB-41 Unilateral – surgery leg (Unit set F1=20 Hz, F2=0 Hz for EAP)	ST-36+GB-34# SP-6 through GB-39#^ GB-33 through LIV-8#^	*Tian-ping* Dry needle stimulation both groups
LIV-3#, GB-41# ST-43 (both hindlegs) Dry needle stimulation both groups	LI-11+HT-3 (right front leg) LI-11+HT-3 (left front leg)	*Bai-hui* Dry needle stimulation both groups
LI-4+LI-4 Right front leg connected to left front leg (Unit set F1=20 Hz, F2=0 Hz for EAP)	—	—

*=All acupuncture needles and cables covered with tape to maintain blindness of study personnel; cables from acupunctoscope hooked to needles for EAP Group and not connected to needles for DNAP Group; **=to maintain sterility of the surgical site, the paraincisional (local) points were replaced by acupoints selected by Balance Method; #=unilateral placement in opposite leg to surgery leg; ^=one needle placed through adjacent acupoints and electric cable placed on each side of needle

Figure 2: Blinding of treatment groups during surgery; note the sterile drapes obscuring view of needles and covering a raised area over the body to prevent the drapes from disturbing the acupuncture needles. The acupunctoscope is visible on a surgical tray. The machine was always on (note green light) but only the EAP Group had electrical stimulation, as controls didn't have needles inserted, and DNAP Group did not have cables hooked to inserted needles.

Table 4: Anatomic location of acupuncture points used to provide analgesia pre-, intra-, and postoperatively to study dogs undergoing TTA surgery[35]

Acupuncture Point	Location
LI-4 (*He-gu*)	On the medial side of the thoracic limb between the second and third metacarpal bones at the midpoint of the third metacarpal bone
LI-11 (*Qu-chi*)	On the lateral side of the thoracic limb at the lateral end of the cubital crease, halfway between the lateral epicondyle of the humerus and the biceps tendon with the elbow flexed
ST-36 (*Hou-san-li*)	On the craniolateral aspect of the pelvic limb, 3 *cun* distal to ST-35, 0.5 *cun* lateral to the cranial aspect of the tibial crest, in the belly of the cranial tibialis muscle; this is a long linear point.
ST-43 (*Xian-gu*)	Just about proximal to the metatarsophalangeal joint between the third and fourth metatarsals of the pelvic limb
SP-6 (*San-yin-jiao*)	On the medial side of the pelvic limb 3 *cun* proximal to the tip of the medial malleolus in a small depression on the caudal border of the tibia (opposite GB-39 on the lateral side)
HT-3 (*Shao-hai*)	On the medial side of the elbow just cranial to the medial epicondyle (between the end of the cubital crease and the medial epicondyle of the humerus)
GB-33 (*Xi-yang-guan*)	On the lateral side of the pelvic limb at the stifle, in the large depression just proximal to the lateral epicondyle of the femur between the insertion of the biceps femoris tendon and the femur
GB-34 (*Yang-ling-quan*)	On the lateral side of the pelvic limb at the stifle, in a small depression cranial and distal to the head of the fibula
GB-39 (*Xuan-zhong*)	On the lateral side of the pelvic limb distal to the stifle, 3 *cun* proximal to the tip of the lateral malleolus of the fibula in a depression on the caudal border of the fibula (near where the lateral saphenous vein crosses)
GB-41 (*Zu-lin-qi*)	On the lateral side of the pelvic limb distal to the hock, on the dorsum of the foot proximal to the metatarsophalangeal joint, just distal to the junction of the fourth and fifth metatarsal bones
LIV-3 (*Tai-chong*)	On the medial side of the pelvic limb, proximal to the metatarsophalangeal joint between the second and third metatarsal bones
LIV-8 (*Qu-quan*)	On the medial stifle, proximal to the medial end of the popliteal crease, in a depression between the medial femoral condyle and the insertion of the semimembranosus and semitendinosus muscles, proximal and cranial to KID-10
Bai-hui	On the dorsal midline between L7 and S1 vertebrae
Tian-ping	On the dorsal midline in the depression between the dorsal spinous processes of the T13-L1 vertebrae

Once both acupuncture groups started their respective type of AP stimulation during the 30-minute induction period, they continued to receive intraoperative AP treatment, as well as post-surgical acupuncture during the recovery period (additional 30-minute length).

Surgical Procedure and Postoperative Assessments – All Groups

To ensure double blindness in the operating room, all subjects were connected to two infusion pumps and an EAP device unit. Animals from all three groups were warmed through the application of a forced warm air blanket[n] programmed at the highest temperature setting (43°C). Before starting the surgery, a blinded observer was admitted to the operating room and was responsible for keeping a record every five minutes of the following data: heart rate (HR), respiratory rate (RR), body temperature, systolic arterial pressure (SAP), mean arterial pressure (MAP), diastolic arterial pressure (DAP), arterial oxygen saturation (Sp'O_2), end tidal CO_2 level (Et'CO_2), end-tidal isoflurane level (Et'Iso) dial position of the isoflurane vaporizer (Dial'Iso), as well as presence/absence of intraoperative nociception.

The presence of intraoperative nociception was defined as a response from the patient to surgical stimulation with movement, or the patient's HR, MAP, or RR increased by 20% over their base values. The baseline values were taken at Time 0 (T-0) after induction, but before AP was started. If any of these situations occurred, a bolus of fentanyl (2 mcg/kg IV) was administered, and a positive nociception event was recorded by the blinded evaluator. If none of these changes resolved after the administration of 3 boluses of fentanyl within a period of 5 minutes, the setting of the vaporizer was increased by 0.25%, the reservoir bag was emptied, and the oxygen flow was increased to 2-3 L/min for 10 minutes, after which it returned to 1-2 L/min. If this was repeated more than three times for a dog in either of the acupuncture groups, the acupuncture patient then received continuous infusions of fentanyl and ketamine at the same doses and speeds as the control patients. This patient was then removed from the study with reason for removal documented.

Throughout the surgical procedure, the following values were also recorded: number of episodes of hypotension (defined as MAP<60 mmHg), number of fentanyl boluses administered, cumulative concentration of Et'Iso (mean of all records taken every 5 minutes, from the start of surgery until the end), and nociception in the five minutes following surgical milestones (skin incision, arthrotomy, osteotomy, skin closure). Subject recovery quality at extubation was assessed through a previously validated recovery scale (Table 1).[8] Additionally, once the subject's body temperature reached a minimum of 36.7°C, the assessor evaluated sedation with the Boscan and Wennogle scale (Table 1) and pain with the Glasgow scale (Table 2) every 60 minutes, starting from the moment of extubation during the first 4 hours. Subsequently, the assessment was performed every 4th hour until the 24th hour post-extubation.[8,9,33]

For all subjects, the surgical procedure was consistent and carried out by the same surgeon. Once the surgery was completed, the patients remained anesthetized for 30 minutes in the operating room. During this period, the Control Group subjects had continuous infusions of fentanyl and ketamine stopped per regular procedure for TTAs. For EAP/DNAP subjects, acupuncture continued for 30 minutes after TTA surgery completion. Isoflurane was then stopped, and once animals recovered their swallow reflex, extubation was performed.

The serotonin and substance P (SP) levels were measured at 13 different time points. One milliliter of blood was collected and preserved in EDTA tubes. The EDTA tubes were kept on ice until centrifuged. The plasma obtained was stored at -80°C. Subsequently, serotonin levels were measured according to the methodology previously described as well as SP levels, following the methodology previously described, both by ELISA technique.[36,37]

Outcome Data and Statistical Analysis

Physiologic parameters measured included HR, RR, MAP, DAP, and body temperature beginning at patient arrival at the hospital before anesthesia (T-1), after induction of anesthesia but before initiation of AP (T-0), at initiation of the surgical procedure (cutting time, C-0), every 5 minutes during the surgical procedure, at the end of surgery (cutting finish, C-f), and 30 minutes, 1, 2, 3, 4, 8, 12, 16, 24 hours after extubation. Anesthetic related parameters measured included Sp'O_2, Et'CO_2, Et'Iso, Dial'Iso at T-0, C-0, C-f, 30 minutes after extubation and at 1, 2, 3, 4, 8, 12, 16, 20, 24 hours after extubation.

Statistical assessment of outcome data measurements included number of fentanyl boluses (NFB) administered during surgery due to intraoperative nociception, MAP during surgery/episodes of hypotension, nociceptive positive response to surgical milestones (skin incision, arthrotomy, osteotomy, skin closure), recovery score, post-extubation pain scores, serotonin levels, SP levels, time needed until first postanesthetic actions (ambulation, micturition, defecation, liquid intake, solid intake), and cumulative Et'Iso from C-0 to C-f. The pain scores were calculated from each subject and used for group comparison. The group comparisons of serotonin and SP levels were conducted with respect to the median ($Sero_{med}$, SP_{med}) and maximal change ($Sero_{max}$, SP_{max}) over the 10 post-extubation times compared to the pre-surgery sample.

As these outcome measurements were numerical response variables, for the comparison among three treatment groups, one-way ANOVA or Kruskal-Wallis test was applied, depending on the outcome of the normality test (Shapiro-Wilk Normality test). When the null hypothesis (H_0: all group means are equal) was rejected, Tukey multiple comparisons test was used to determine which groups were different from each other. Significance level for all tests was 0.05. When planning the study, it was determined that a minimum of 15 patients in each treatment group would be required to ensure an 80% power to reject the null hypothesis with a 0.05 significance level when a group difference at least 25% above the overall standard deviation among data was to be detected.

RESULTS

A total of 23 dogs admitted for TTA surgery qualified for inclusion in the study. The randomization procedure resulted in 8 subjects being assigned to the Control Group, 8 in the EAP Group, and 7 in the DNAP Group. Two subjects, one in the Control Group and the other in the DNAP Group, did not complete the trial. Their withdrawal from the study was due to the presence of intraoperative nociception. These two patients responded to surgical stimulation with movement and their HR, MAP, and RR increased by 20% over their base values such that rescue analgesia had to be applied three times or more. Hence, data from 21 subjects (7 Control, 8 EAP, 6 DNAP) were included in the analysis.

Signalment

Subject signalment data was compared among groups. The mean±SD age was 8.4±1.7, 5.4±1.8, 6.5±4.3 years old in the Control, EAP, and DNAP groups, respectively. There was a significant difference between the Control and EAP groups (p=0.031). Body weight (mean±SD) in the Control Group was 19.9±14.6 kg, 36.4±16.6 kg in the EAP Group, and 34.1±7.1 kg in DNAP Group (p=0.169). There were 28.6% (2/7) females in the Control Group; 75% (6/8) of the subjects in the EAP Group were females, and all DNAP subjects (100%) were females. Comparisons concluded that there existed a significant group difference (p=0.022) in subject sex proportions.

Surgery

➢ Intraoperative Nociception

The percentage of dogs where observations of intraoperative nociception were observed, with respect to four different procedures, skin incision, arthrotomy incision, osteotomy incision, skin closure; were recorded and analyzed (Table 5). Acupuncture (either EAP or DNAP) had the lowest percent of nociception observed for 3 out of the 4 intraoperative procedures. Electro-acupuncture was associated with the lowest nociception for skin incision and skin closure, while DNAP had the least percent of dogs with nociception associated with the osteotomy incision. Controls had the least nociception observed for the arthrotomy incision. Due to the small sample size, statistical conclusions of these differences could not be made.

➢ Boluses of Fentanyl Administered

The number of fentanyl boluses (NFB) administered during surgery to address intraoperative nociception was analyzed. The Control and EAP groups (mean±SD) were similar with 3.6±2.0 and 3.6±1.5 NFB, respectively. There was a 25% reduction of fentanyl bolus rescue (2.7±1.2, p=0.424) in the DNAP Group compared to the controls, (Table 6).

➢ Recovery Score

The recovery score for the Control Group was 2.1±1.2 (mean±SD). It decreased (improved) to 1.5±0.8 in the EAP Group and 1.0±0.0 in the DNAP Group (p=0.084). Compared to the Control Group, the EAP Group and DNAP Group were 30% and 50% better, respectively (Table 6).

➢ Isoflurane End-tidal Concentration and Mean Arterial Pressure

During intra-surgical monitoring, it was observed that the acupuncture groups had significantly greater mean Et'Iso values (in mL) than the Control Group which had a mean of 1.01±0.07. The EAP Group had the greatest mean Et'Iso (1.39±0.38), which was a 37.6% increase compared to control; followed by DNAP (1.38±0.42), which was a 36.6% increase compared to control (p=0.041, Table 7).

It was also found that the acupuncture groups had greater mean MAP values (in mmHg) than the Control Group (mean±SD, 72.6±9.7). The EAP Group had the largest value at 78.5±9.4 (8.1% increase compared to control), followed by the DNAP with a mean of 86.0±10.1 (18.5% increase compared to control); the difference was close to significance (p=0.053).

Table 5: Observations of intraoperative nociception (% of presence) within each group

	Control	DNAP	EAP
Skin incision	3/7=42.9%	3/6=50.0%	2/8=25.0%
Arthrotomy incision	2/7=28.6%	4/6=66.7%	7/8=87.5%
Osteotomy incision	2/7=28.6%	1/6=16.7%	3/8=37.5%
Skin closure	3/7=42.9%	1/6=16.7%	1/8=12.5%

Table 6: Summary of mean additional boluses of fentanyl administered during surgery to address pain and mean recovery score of study dogs from anesthesia

	Control	EAP	DNAP
Boluses of Fentanyl	3.6±2.0	3.6±1.5	2.7±1.2 (25% reduction)
Recovery Score	2.1±1.2	1.5±0.8 (30% better)	1.0±0.0 (50% better)

% in parentheses=reduction compared to the Control Group

Table 7: Summary table of isoflurane end-tidal concentration (Et'Iso) values (in milliliters), and mean arterial pressure (MAP) during intra-surgical monitoring with group comparison p-values

Groups	Isoflurane End-tidal Concentration (milliliters)	p-value	Mean Arterial Pressure (mmHg)	p-value
Control	1.01±0.07	0.041*	72.6±9.7	0.053
EAP	1.39±0.38 (+37.6%)		78.5±9.4 (+8.1%)	
DNAP	1.38±0.42 (+36.6%)		86.0±10.1 (+18.5%)	

During intra-surgical monitoring, the Control Group had smaller Et'Iso values than the acupuncture groups, and the difference was statistically significant (p=0.041). The DNAP Group had greater median MAP values than the other two groups; % in parentheses=compared to the Control Group; *=statistical significance p<0.05

Post-extubation Pain Scores

For each subject, pain scores were assessed at the time of admission to the hospital (pre-anesthesia) and 10 post-extubation times (0.5, 1, 2, 3, 4, 8, 12, 16, 20, and 24 hours). The Control Group had the highest mean pain scores during the first 4 hours post-extubation compared to the acupuncture groups (Figure 3). The EAP Group had the lowest mean pain scores during the first hour after extubation (~ 32% lower than controls). This was followed by the DNAP Group having the greatest pain reduction at 2 hours (58.3% better), 3 hours (34.8% better), and 4 hours (35.8% better) when compared to controls (Table 8). Statistical significance (p>0.05) was not attained for group difference for the 10 post-extubation times in this small study.

Using summary statistics over time, group comparisons were conducted with respect to (1) median pain score (P_{med}) and (2) maximal pain score (P_{max}). In both pain statistics, the DNAP Group had the smallest values (i.e. scores are better) among the three groups, followed by the EAP Group; and with the Control Group having the largest pain scores. For the mean P_{med}, the DNAP and EAP groups were 28% and 16%, respectively, lower than the Control Group. The mean P_{max}, had reductions of 27% and 19% for the DNAP and EAP groups, respectively, when compared to the Control Group. Group difference for each pain statistic, however, did not reach statistical significance (p>0.05).

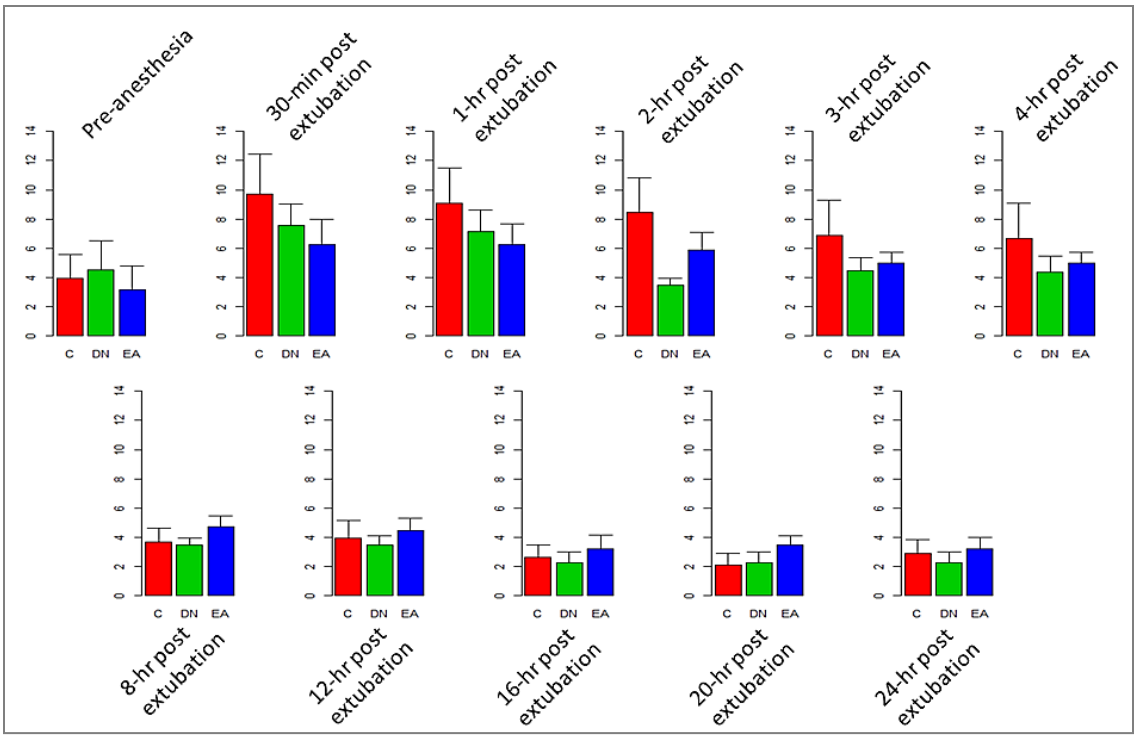

Figure 3: Mean value (with standard error bar) of post-extubation pain score within each treatment group assessed at 10 time points (0.5, 1, 2, 3, 4, 8, 12, 16, 20, and 24 hours); red=Control Group, green=DNAP Group, blue=EAP Group

Table 8: Post-extubation pain score (mean±SD) during the first 4 hours, with median and maximum values over the 10 post-extubation pain scores

	30-min	1-hr	2-hr	3-hr	4-hr
Control	9.7±7.8	9.1±6.9	8.4±6.7	6.9±6.8	6.7±6.9
DNAP	7.6±3.9 (21.6%)	7.1±3.9 (22.0%)	3.5±1.2 (58.3%)	4.5±2.3 (34.8%)	4.3±2.9 (35.8%)
EAP	6.2±4.8 (36.1%)	6.2±4.0 (31.9%)	5.9±3.4 (29.8%)	5.0±2.0 (27.5%)	5.0±2.0 (25.4%)

	Control	EAP	DNAP
Median Pain Score	5.21±4.3	4.38±2.1 (16% lower)	3.75±1.6 (28% lower)
Maximum Pain Score	10.83±7.0	8.80±3.5 (19% lower)	7.92±3.7 (27% lower)

% in parentheses=reduction compared to the Control Group

Table 9: Summary statistics (mean±SD) within each group for duration (in hours) to a first post-extubation action

	Control	DNAP	EAP	*p*-value
Ambulation	3.4±2.3	3.2±1.0 (-5.9%)	4.0±3.0 (+17.6%)	0.977
Micturition	14.3±6.1	7.9±2.5 (-44.8%)	14.8±5.1 (+3.5%)	0.042*
Defecation	18.0±6.1	17.9±8.1 (-0.6%)	18.2±9.4 (+1.1%)	0.797
Liquid intake	9.2±2.3	14.1±11.5 (+53.3%)	9.6±4.4 (+4.3%)	0.999
Solid intake	8.1±2.4	7.8±3.5 (-3.7%)	9.9±5.1 (+22.2%)	0.791

% in parentheses=compared to the Control Group; *= statistical significance $p<0.05$

Serotonin and Substance P

Serotonin levels were assessed at 13 time points. The $Sero_{med}$ level for the Control Group had the largest reduction compared to the pre-surgery value measured on arrival at the hospital (-6.2±7.3), whereas the EAP Group had the smallest change (-0.9±1.7). The mean reduction in the Control Group was about 6 times that in the EAP Group and 3 times the DNAP Group. When considering $Sero_{max}$ level, the DNAP Group had the largest increase (15.9±23.0), more than 2 times the Control Group (6.9±12.9). The group difference for each serotonin statistic did not reach statistical significance (all $p>0.05$).

When looking at the SP values, all 3 groups demonstrated increases during surgery. At 30-minutes post-extubation, SP decreased for all 3 groups. The DNAP Group remained below baseline value with decreases for 24 hours (study termination); while at 16 hours post-extubation, the Control and EAP groups increased above baseline. The group difference for each SP statistic was not statistically significant (all $p>0.05$).

Postanesthetic Ambulation, Micturition, Defecation, Liquid Intake, Solid Intake

Groups were compared with respect to the time needed until first postanesthetic actions: ambulation, micturition, defecation, liquid intake, and solid intake. The time to ambulation had a mean±SD in the Control Group of 3.4±2.3 hrs, EAP Group 4.0±3.0 hrs, and shortest time of 3.2±1.0 in the DNAP Group ($p=0.977$). On the time to micturition, the DNAP Group had a much smaller mean value (7.9±2.5 hrs) than the other two groups. It was 45% shorter than the Control's 14.3±6.1 hrs, and 47% shorter than EAP's 14.8±5.1 hrs, which was statistically significant ($p=0.042$). All three groups had similar mean time duration between extubation to the first defecation with the DNAP Group again having slightly shorter time (18.0±6.1, 17.9±8.1, and 18.2±9.4 hrs) for the Control, DNAP, and EAP groups, respectively ($p=0.797$). First liquid intake was shortest for the controls (9.2±2.3 hrs) and longest for the DNAP Group (14.1±11.5 hrs), with the EAP Group (9.6±4.4 hrs) similar to Control ($p=0.999$). Solid intake was shortest for the DNAP Group (7.8±3.5 hrs), followed by controls (8.1±2.4 hrs) and longest for EAP subjects (9.9±5.1 hrs). In summary, for post-extubation important actions, the DNAP Group had the shortest times for ambulation, defecation, solid intake, and micturition; with micturition being statistically significant (Table 9).

DISCUSSION

This double-blind, randomized, controlled pilot study investigated the efficacy of reducing the use of additional opioid/non-opioid systemic analgesics with acupuncture in TTA surgeries. Large doses of these drugs are associated with adverse events such as vomiting, nausea, bradycardia, urinary retention, and poor anesthetic recovery in dogs. All three groups (Control, DNAP, EAP) received the same anesthetic premedication, followed by inhalation anesthesia (isoflurane). The controls additionally received a bolus and constant rate infusion of fentanyl and ketamine. The acupuncture groups (DNAP, EAP) replaced the bolus/constant rate drug infusion with either dry-needle acupuncture or electro-acupuncture before, during, and after the surgical procedure. Study findings compared between the 3 groups included: intraoperative nociception of surgical milestones, additional fentanyl boluses to address intraoperative nociception, anesthesia recovery quality, post-extubation pain, serotonin levels, substance P levels, mean arterial pressure, isoflurane end-tidal concentration, and first postanesthetic actions (micturition, ambulation, defecation, liquid, and solid intake). Findings were evaluated for percent change from control or baseline values to identify trends in this pilot study, along with calculating statistical significance ($p<0.05$). Study findings highlighted by percent change demonstrated a number of trends in addition to 2 statistically significant findings.

Within the field of veterinary medicine, an integrative approach of combining acupuncture analgesia with systemic analgesia has demonstrated benefit, such as decreased narcotic adverse effects, improved cardiovascular/respiratory function, and better quality anesthetic recovery.[38,39] In the current study, similar to literature, the quality of anesthetic recovery (recovery score) was better in both acupuncture groups (50% better DNAP, 30% EAP) when compared to controls. Additionally, mean arterial pressure value assessed for patients in the study demonstrated higher MAP value for the acupuncture groups when compared to controls that was close to statistical significance ($p=0.053$). Normal MAP for dogs is 85-120 mmHg and 70-100 mmHg during surgery. Isoflurane is a potent vasodilator with MAP values below 60 mmHg during surgery considered a medical emergency due to critical loss of perfusion of the heart, brain, and kidneys. The AP groups in this study maintained blood pressure levels in the center of normal surgery range versus controls that were close to the range minimum (i.e. 70 mmHg). This study is similar to other studies that have demonstrated improved perioperative hemodynamic stability associated with acupuncture treatment during surgery.[38,40,41]

The DNAP and EAP treatment groups demonstrated good analgesia when compared to the controls. Acupuncture groups (either EAP or DNAP), had the lowest percent of nociception observed for 3 out of the 4 intraoperative milestone procedures. Electro-acupuncture was associated with the lowest nociception for skin incision and skin closure, while DNAP had the least nociception observed for the osteotomy incision. Control and EAP had similar numbers of intraoperative fentanyl boluses (3.6±2.0 and 3.6±1.5, respectively) to address nociception associated with intraoperative procedures, while there was a 25% reduction (2.7±1.2) of rescue fentanyl boluses in the DNAP treated group. Post-extubation pain scores also demonstrated a positive trend for superior pain reduction by the AP groups. During the first 4 postoperative hours, the controls demonstrated the highest pain scores, including both their median and maximum scores. When compared to control findings, the AP groups had anywhere from a 21.6% to 58.3% improved pain reduction over this period.

Analgesia is known to involve opioid peptides, adrenergic receptors, serotonin, and dopamine receptors.[42] With both DNAP and EAP, the spinal and brain concentrations of serotonin, as well as serotonin receptor activation, are increased in rat models.[43] Substance P, a neurotransmitter, is associated with neuroinflammation, microvascular permeability and pain transmission, particularly in inflammatory diseases of the respiratory, gastrointestinal and musculoskeletal systems.[44,45] Changes in serotonin levels (increase) and SP levels (decrease) may be a valuable explanation for beneficial acupuncture mechanisms in painful and pro-inflammatory conditions, therefore, serotonin and SP were assessed during the study.[46,47] The median serotonin levels for controls in this study had a large reduction compared to the pre-surgery value (-6.2±7.3), whereas the EAP Group had the smallest changes (-0.9±1.7). The mean reduction in the Control Group was about 6 times that in the EAP Group and 3 times the DNAP Group. When evaluating plasma SP levels, all 3 groups demonstrated increases during surgery. At extubation, SP decreased for all 3 groups. This decrease continued in the DNAP Group for 24 hours (study termination), while at 16 hours the other 2 groups had SP levels that had risen above baseline again.

There were two statistically significant findings in the study. The first was significantly smaller isoflurane end-tidal concentration (Et'Iso) values during surgery in the controls when compared to the two acupuncture groups (p=0.041). During surgery, Et'Iso values are generally maintained between 1.0 and 1.3% according to individual needs of the patient, balancing depth of anesthesia with the potent adverse effect of isoflurane associated vasodilation.[48-50] In the EAP and DNAP groups, the Et'Iso values were in the upper part of the average range (1.3% acupuncture versus 1.0% controls). It has been reported that multimodal analgesia similar to that received by controls is associated with lowered Et'Iso values.[51]

Postoperative urinary retention has been recognized as a side-effect of opioid use. Similar to a recent study in dogs demonstrating less urinary retention in the 24 hours following surgery associated with reduced opioid consumption; post-anesthetic micturition in this study demonstrated a statistically significant shortened duration (7.9±2.5 versus 14.3±6.1 hours, p=0.042), for DNAP patients compared to controls.[9] Although not statistically significant, DNAP patients additionally had the shortest times for first ambulation, defecation and solid intake. The controls and EAP had similar times for first postanesthetic actions.

Selection of acupoints for a surgical procedure will depend on the type of surgery performed and will include both distal and paraincisional acupoints (i.e. local acupoints).[39] In the present study, the selection of local acupoints were problematic, as the orthopedic surgery site needed to maintain very strict sterility due to surgical exposure of the bone. The authors, therefore, investigated the use of Balance Method Acupuncture (BMA) to find acupoints that would provide analgesia for the actual surgery site but would not create contamination of the site.

The BMA system is a special acupuncture modality derived from the *Yi Jing* (I Ching) in Book of Changes, initially written during the Western Zhou Period (750-100 BC) and is the oldest teachings of *Yin* and *Yang*.[34] This method uses distal acupoints (below the elbows and stifles) located on the 12 regular Channels and is based on the concept of correcting disease in the body by balancing the Channels (i.e. Meridians) coursing through the affected the area.[52,53]

There are 2 commonly used methods of selecting acupoints in this system [Mirror/Imaging concept (Dr Tan's Six Systems) and I-Ching *Ba Gua*s]. The mirror/imaging approach (which was used for the present study) recognizes that there are multiple tissue similarities in the body. The mirror concept treats blockage of one appendicular Channel (i.e. surgery incision site) by other appendicular acupoints (balancing Channel) in a mirror fashion. The benefits for this system include minimal needle use, strong acupoint stimulation and rapid clinical effects.[34,54-56] In the present study, elbows (LI-11+HT-3) mirrored the stifles and the opposite stifle (from the surgery stifle) used GB-33 through LIV-8 (1 needle) and ST-36+GB-34 to mirror the affected Channels at the surgery site. One investigational study determined that opposing and same side electro-acupuncture generates similar analgesic effects on pain after knee surgery, similar to the present study where the use of opposite stifle acupuncture points to mirror the surgery stifle was successful.[57]

The technique of 1-needle inserted through adjacent acupoints used in this study, was based on inserting needles at the depth of tissue to be treated. For example: treat at superficial depth for skin conditions, treat at muscle depth if muscle is involved, treat tendon if tendon is involved or bone if bone is involved.[58,59] There was need for analgesia of skin, muscle, tendon and bone; thus, the use of 1-needle technique which included all 4 areas. Finally for the EAP procedure, the frequency settings chosen for distal acupoints (LIV-3, GB-41, ST-43, LI-4) were based on studies showing that low frequencies (50 Hz or less) release beta endorphins resulting in analgesia.[52,60] The higher frequencies (100-200 Hz) setting was used for stimulation at both opposite and balancing acupoints (LI-11, HT-3, GB-33, LIV-8, ST-36, GB-34, SP-6, GB-39) as "mirroring" acupoints. Stimulation of these would serve as quick, potent local analgesia due to the release of dynorphins, serotonin and dopamine at the higher frequencies.[61,62]

A significant unexpected finding in the current study was the superior results in a number of study outcomes for DNAP when compared to EAP. Review of literature generally agrees that EAP is superior to DNAP in terms of analgesia, not only intraoperatively but also postoperatively.[63-65] The authors believe the answer for this discrepancy is based on the current study's choice of unique paraincisional acupoints based on Dr. Tan's Balance Method Acupuncture (BMA).[34] The fast and effective analgesic effect of these acupoints included increased levels of serotonin and decreased SP levels which decreased intraoperative nociception study findings. The skin incision/skin closure pain was effectively covered by these BMA-mirrored paraincisional acupoints. This is a very interesting effect and needs to be investigated in larger studies with better powered statistics. If substantiated in larger studies, it would eliminate the need for surgery site paraincisional acupoints leaving the surgery site free of needles and cables.

One of the principal limitations of this study was the small number of animals per group (i.e. less than minimum statistical sample size estimation of 15/group), which is a common challenge for small pilot studies. When evaluating findings in this type study, the small patient sample may not be sufficiently powered to detect a statistical difference between groups (type II error). With this in mind, percent change from baseline/control was also calculated in this study to identify trends (positive or negative); which then could be investigated further in a larger adequately powered study. Additionally, due to the statistically significant difference existing regarding age and sex among groups, further studies with larger sample sizes would again be recommended. According to some literature, it can be considered that both age and sex may play an interesting role in different outcomes for acupuncture analgesia, as there can be individual variation to acupuncture.[39,50]

In conclusion, findings of this study demonstrated the addition of acupuncture to inhalant anesthesia had similar perioperative analgesia when compared to control patients receiving conventional systemic analgesia; with a combination of the statistically significant finding which included shortened duration until postanesthetic micturition; and nonsignificant findings of less intraoperative rescue fentanyl boluses, improved intraoperative cardiovascular stability, improved quality of anesthetic recovery and decreased post-extubation pain scores in one or both acupuncture groups. These positive trends support the study hypothesis and suggest that successful use of acupuncture to reduce/replace added opioid/non-opioid systemic analgesics associated with TTA surgery should be considered. In addition, when dry needle technique was compared to electro-acupuncture technique, study findings suggest, under the experimental conditions of this study, that the dry needle technique associated with BMA acupoint selection was superior. Based on the results of this pilot study, a larger, adequately powered study which is randomized, blinded, and controlled is recommended to further explore the early findings from this study.

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FOOTNOTES

- [a] Stata 13.0, StataCorp LLC, College Station, Texas, USA, http://www.randomization.com
- [b] Semfortan (methadone), Dechra Veterinary Products SLU, Barcelona, Spain
- [c] Midazolam Normon, Laboratories Normon S.A., Tres Cantos, Madrid, Spain
- [d] Equipromacine (acepromazine), Fatro Ibérica S.L., Barcelona, Spain
- [e] Propofol Lipuro, BBraun Vetcare SA, Barcelona, Spain
- [f] Isovet, BBraun Vetcare S.A., Barcelona, Spain
- [g] Isofundin (balanced crystalloid solution), BBraun Vetcare S.A., Rubí, Spain
- [h] Meloxidolor, Fatro Iberica S.L., Barcelona, Spain
- [i] Cefazolin, generic, supplier used unknown
- [j] Fentadon, Dechra Veterinary Products SLU, Barcelona, Spain
- [k] Ketamidor, Laboratorios Karizoo S.A., Caldes de Montbui, Barcelona, Spain
- [l] Acupunctoscope, Model JM2A, Wuxi JiaJian Medical Instrument Inc., Wuxi, China
- [m] Acupuncture needles (stainless steel), EnerQi, Novasan, Madrid, Spain
- [n] Bair Hugger, model 505, Arizant Healthcare, Minnesota, USA

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