Acupuncture and pharmacopuncture are as effective as morphine or carprofen for postoperative analgesia in bitches undergoing ovariohysterectomy

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ABSTRACT

PURPOSE: To investigate the analgesic effect of acupuncture (AP) or micro-dose pharmacopuncture (PA), using carprofen or morphine, in bitches undergoing ovariohysterectomy (OHE).

METHODS: Thirty five dogs were randomly assigned to five groups after sedation with acepromazine IM: AP, 0.5 mg.kg⁻¹ of morphine subcutaneously (SC), 4 mg.kg⁻¹ of carprofen SC, and PA with 0.05 mg.kg⁻¹ of morphine or 0.4 mg.kg⁻¹ of carprofen. Anaesthesia was induced with propofol and maintained with isoflurane. Pain was assessed after OHE by a blind observer for 24h, by dynamic visual analogue scale (DIVAS), Glasgow (CMPS-SF), Melbourne (UMPS) and Colorado University pain scale (CSU). Animals reaching 33% of the UMPs score received rescue analgesia with morphine IM. Non parametric data were analysed by Kruskal-Wallis or Friedman tests where applicable, followed by Dunn’s test. Parametric data were analysed by two way ANOVA, followed by Tukey test.

RESULTS: There were no differences among groups in number of rescue analgesia. Except for the DIVAS score where animals treated with morphine had the lowest score compared with AP and carprofen, at 1h after surgery, there were no other differences among groups.

CONCLUSION: Acupuncture or pharmacopuncture were equally effective as morphine or carprofen to control postoperative pain in bitches undergoing ovariohysterectomy.

Key words: Hysterectomy. Pain Management. Analgesics, Opioid. Anti-Inflammatory Agents, Non-Steroidal. Dogs.
Introduction

Non-steroid anti-inflammatory drugs (NSAIDs) and opioids have been extensively used for postoperative analgesia in dogs1-3. Adverse effects of opioids may include vomiting, dose-dependent respiratory depression, urinary retention, excitement/dysphoria and histamine release4. NSAIDs should not be used in patients suffering hepatic, renal, gastrointestinal and coagulation dysfunctions5.

AP may be an alternative to avoid the adverse effects of opioids and NSAIDs in acute and chronic pain management. This technique has been successfully used in animals to treat neurological syndromes and musculoskeletal diseases which culminate in pain5 and several studies and reviews have addressed the analgesic and antinociceptive effects of AP and PA related techniques in acute pain in man6 and dogs7-11. Postoperative analgesia of AP is comparable or even better than opioids or anti-inflammatory drugs8-11, showing that AP has a clinical promising effect to treat postoperative pain in dogs.

Considering that AP produces antinociception and analgesia and that in some cases NSAIDs and opioids may produce adverse effects or may be contraindicated, the replacement of analgesic drugs in pre-anesthetic medication prior to painful procedures is a potential and attractive use of AP. This would eliminate the adverse effects of conventional analgesics and reduce treatment costs. Other beneficial effects of AP beyond analgesia, which would be relevant in the perioperative period, are reduction of postoperative nausea, vomiting and sympathoadrenal responses in man7. Such features make AP ideal for a great range of patients, particularly those likely to develop adverse drug reactions or when adverse effects of drugs might be risky.

Although dry needle AP or electroacupunture (EA) appears to be as effective as NSAIDs and opioid analgesics for postoperative analgesia in bitches undergoing OHE8-11, maintenance of needles prior to anaesthesia might be cumbersome, particularly in agitated animals. In order to overcome this disadvantage, other techniques might be used for stimulation of acupoints. The most usually used techniques for stimulation of acupoints are application of heat (moxibustion) and low power laser AP, however both are time consuming and the last one requires specific equipment. A possible alternative would be the injection of substances or micro-clinical doses of drugs into acupoints, which is known as pharmacopuncture (PA). This method combines the traditional acupuncture stimulation with the local delivery of a pharmacological agent and it is supposed to potentiate and prolong the drug effect12. Pharmacopuncture has been successfully used to sedate horses13 and dogs14.

The hypothesis of this study was that AP or micro-dose PA would be as effective as the conventional doses of NSAID or morphine to treat postoperative pain in dogs. In order to test this hypothesis this study investigated the analgesic effect of the preventive administration of AP or micro-dose PA, using carprofen or morphine, in bitches undergoing OHE. This was assessed by comparing these treatments against two positive control groups (conventional dose of carprofen or morphine).

Methods

This study was conducted under the owners’ consent and after the Institutional Animal Research Ethical Committee approval. The study was prospective, randomized, and blind for pain assessment.

Thirty-five healthy bitches from several breeds were used. They aged 24±7 months and weighed 10.1±1.7 kg. One day before surgery all animals were evaluated and maintained in individual kennels. Selection criteria included docile, unpregnant and healthy bitches based on normal clinical and laboratorial assessment (haematocrit, total protein, alanine aminotransferase, creatinine and alkaline phosphatase). Animals were fasted for 12 h and water was removed for two hours prior to surgery.

All animals received acepromazine (Acepran, Univet - Vetnil, São Paulo, Brazil) 0.05 mg.kg⁻¹ IM. Ten minutes later, they were randomly assigned to one of five groups. Animals from group A (acupuncture) were treated for 20 minutes with manual bilateral AP in the acupoints Gall Bladder 34 (GB34 - Yang Ling Quan), Spleen 6 (SP6 - San-yin-jiao), Stomach 36 (ST36 - Zu San Li) and Liver 3 (LIV3 - Tai Chong), using 0.25 x 30 mm needles (Dongban, Chungnam, Korea, Republic of South Korea). Location of the acupoints was described elsewhere. Group M animals received 0.5 mg.kg⁻¹ of morphine SC (Dimerorf, Cristalia, Itapira-SP, Brazil) and group C animals received 4 mg.kg⁻¹ of carprofen SC (Rimadyl, Pfizer Animal Health, Veircore Limited, Dundee, Scotland). Both were diluted in 0.9% NaCl (Cloreto de Sódio, Halexstar, Goiana-GO, Brazil) to reach a volume of 0.8 mL. Group AM and AC were treated by bilateral PA with 0.05 mg.kg⁻¹ of morphine SC (Dimorf, Cristalia, Itapira-SP, Brazil) or 0.25 x 30 mm needles (Dongban, Chungnam, Korea, Republic of South Korea) to reach a volume of 0.8 mL. The total volume was subdivided in eight equal amounts of 0.1 mL and each amount was injected bilaterally with a 25 gauge needle at the acupoints previously described for group A.

The cephalic vein was cannulated twenty minutes after all treatments, for administration of anaesthetics and 10 mL.kg⁻¹.h⁻¹ of Lactated Ringer (Ringer com Lactato, Segmenta, Univet - Vetnil, São Paulo, Brazil). Adverse effects of opioids may include vomiting, dose-dependent respiratory depression, urinary retention, excitement/dysphoria and histamine release4. NSAIDs should not be used in patients suffering hepatic, renal, gastrointestinal and coagulation dysfunctions5.

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Acupuncture and pharmacopuncture are as effective as morphine or carprofen for postoperative analgesia in bitches undergoing ovariohysterectomy (Glicolabor, Ribeirão Preto-SP, Brazil). Anaesthesia was induced with 4 to 6 mg/kg$^{-1}$ of propofol IV (Propovan, Cristalia, Itapira-SP, Brazil). After endotracheal intubation, animals were connected to a circle anaesthetic system (HB Galant, HB Hospitalar, São Paulo-SP, Brazil), to deliver a mixture of isoflurane (Isoforine, Cristalia, Itapira-SP, Brazil) in oxygen (15 mL.kg$^{-1}$.min$^{-1}$). The vaporizer setting was adjusted to maintain a surgical plane of anaesthesia judged by eye position, ocular reflexes, jaw tone, blood pressure changes, heart and respiratory rates in response to surgical stimuli. Dogs breathed spontaneously unless hypopnoea, hypercapnia or signs of inadequate anaesthetic depth occurred. Under these circumstances animals were manually ventilated.

Anaesthesia monitoring included heart rate, respiratory rate, pulse oximetry, end tidal CO$_2$ and inspired and expired isoflurane concentration (Capnomac Ultima, Datex Engstrom, Helsinki, Finland), indirect systolic arterial blood pressure (Doppler Ultrasonic Flow Detector, Model 811-B, Parks Medical Electronics, Aloha, Oregon, USA), with the sensor placed on the metacarpal artery and rectal temperature using a standard digital thermometer. All measurements were recorded every five minutes only as control variables, therefore data are not presented in results.

After surgery the animals were moved to their kennels for recovery from anaesthesia, where the observer, blind to the treatment and experienced in pain assessment for dogs, assessed postoperative pain.

Pain measurements were performed at 1, 2, 4, 8, 12 and 24 h after surgery, by dynamic visual analogue scale (DIVAS), short form Glasgow composite pain scale (CMPS-SF)$^{16}$, University of Melbourne pain scale (UMPS)$^{17}$ and Colorado State University canine acute pain scale (CSU). DIVAS measured pain intensity by a 100 mm line with zero corresponding to no pain and 100 to the maximum pain.

Animals reaching 33% of the UMPS score (nine points) received rescue analgesia with 0.5 mg/kg$^{-1}$ of morphine IM. In these cases animals continued to be assessed and were not excluded from the statistical analysis. Seven days after surgery all bitches were clinically assessed, weighed and surgical suture was removed.

**Statistical analysis**

Non parametric data (UMPS, CMPS-SF, CSU scores and number of rescue analgesic treatment) were analysed by Kruskal-Wallis test followed by multiple comparisons performed by Dunn’s test. Differences over time within each group were evaluated by repeated measures using Friedman test, followed by multiple comparisons using Dunn’s test. Results were presented as medians and semi-ranges. Parametric data, as DIVAS score and transoperative cardiorespiratory data were analysed by repeated measure two way analysis of variance (ANOVA), with multiple comparisons and Tukey test. Animals’ age and weight were analysed by one way ANOVA. Significance level was fixed at p<0.05. Tests were performed by Sigma-Stat 3.5 software.

**Results**

There were no statistical differences between groups for age, body weight and propofol induction dose. Surgery and anaesthesia duration were 9±3 and 18±6 minutes (mean and standard deviation) respectively in all groups. Heart rate was greater in AC compared to the other groups at some time points, but values were within the normal range for the species. There were no other differences in the cardiorespiratory variables, including end tidal isoflurane concentration, during anaesthesia among groups.

Rescue analgesia was performed in 12 out of 35 bitches (34%) (Table 1). There were no differences among groups in number of animals receiving rescue analgesia or number of times rescue analgesia was performed.

**TABLE 1 -** Number and time of analgesic rescues in the postoperative period of bitches undergoing ovariohysterectomy previously treated with acupuncture (A: n=7); carprofen (C: n=7); morphine (M: n=7); pharmacopuncture with carprofen (AC: n=7) or pharmacopuncture with morphine (AM: n=7).

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<thead>
<tr>
<th>Group</th>
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Pain scores by UMPS (Figure 1), CSU and CMPS-SF were not different between groups (p>0.05). Maximum pain scores before rescue analgesia was never above 11 in any subject (41% of maximum score) according to UMPS and 11 (46% of maximum score) according to CMPS-SF, but achieved 2.5 (63% of maximum score) in CSU and 70 (70% of maximum score) in DIVAS. The only difference in pain assessment between groups was at one hour after OHE, when DIVAS score was lower for
Pain scores evaluated by CMPS-SF, CSU and DIVAS were significantly greater during the early postoperative hours when compared to 24 h after surgery in all groups. UMPS was significantly greater at 1 h, compared to 4 and 24 h after surgery in group C only (Figure 1).

**FIGURE 1** - Medians and semi-amplitudes of the University of Melbourne pain scale in bitches undergoing ovariohysterectomy. Acupuncture (A: n=7); carprofen (C: n=7); morphine (M: n=7); pharmacopuncture with carprofen (AC: n=7) and pharmacopuncture with morphine (AM: n=7). Capital letters expresses differences in time in the animals treated with carprofen, being A>B; maximum possible value of the scale = 27

**FIGURE 2** - Means and standard deviations of the dynamic and interactive visual analogue scale in bitches undergoing ovariohysterectomy. Acupuncture (A: n=7); carprofen (C: n=7); morphine (M: n=7); pharmacopuncture with carprofen (AC: n=7) and pharmacopuncture with morphine (AM: n=7). Small letters indicate differences between groups at 1 h after surgery, being a>b; maximum possible value = 100. Asterisks indicate differences between 24 h and other moments in each group.

**Discussion**

The study was designed to standardize the control variables that might interfere in the results. The surgical technique was performed by the same experienced surgeon; mean surgical time was less than 10 minutes; a minimal invasive surgical technique was performed to reduce the intensity of tissue trauma; the same anaesthetist, as well the same International Veterinary Acupuncture Society (IVAS) certified acupuncturist treated all patients; and the same experienced evaluator accomplished pain assessments.

Both AP and PA provided the same analgesic effect compared to either carprofen or morphine, confirming that AP and PA may be clinically used to minimize pain following OHE in dogs. Previous studies had already reported similar preemptive analgesic effects of conventional systemic doses of NSAIDs and opioids for postoperative pain in dogs as observed here. However it is generally accepted that preventive rather than preemptive analgesia should be performed in order to avoid pain in the pre, trans and postoperative period. Differences of these two analgesic modalities have been well discussed previously and further research would be necessary to compare the pre-versus postoperative use of acupuncture and pharmacopuncture in controlling postoperative pain.

Pain should be assessed as a complex and multidimensional phenomenon. According to that it has been generally accepted that a multimodal treatment should be used in order to accomplish all mechanisms of pain. None of the treatments were completely effective for postoperative analgesia in bitches undergoing OHE. Rescue analgesia was necessary in all groups probably because only a single agent and dose was used preoperatively for postoperative analgesia. This might be a reflex of the unimodal analgesic treatment used in this study.

Pain response to surgery and sensitivity to analgesic therapy are unique to each individual, including genetic variation of the number, distribution and morphology of opioid receptors. In general, experience of pain is highly variable across individuals, even when identical stimuli are applied under identical environmental conditions.

Acupuncture has been successfully used to treat pain originating from a variety of disorders and a substantial part of the studies designed to explain the physiological basis of AP have been involved with pain assessment. The proposed mechanisms explaining the AP analgesic effect may be found elsewhere. The selection of acupoints for this study was based on previously published studies where acupuncture successfully
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To our knowledge there are no reports investigating the injection of micro-doses of opioids or NSAIDs into acupoints. It could be postulated that the injection of a solution into an acupoint would produce a local pressure, prolonging the mechanical stimulus of AP. Pharmacopuncture might enhance the effects of the injected substances combining the traditional acupuncture stimulation with the local drug delivery and drug action. This technique has been successfully used to sedate horses, as efficiently as the conventional dose of acepromazine. Other substances have also been used for PA in traditional Eastern medicine.

Teleantagonism, a phenomenon based on the pharmacodynamic property of the primary nociceptive neurons, is another postulated mechanism to explain the analgesic effect of micro-doses of carprofen and morphine injected into acupoint. This can be explained by the fact that mechanical hypernociception elicited by intraplantar injection of NMDA, glutamate and PGE, was inhibited by intraplantar injection of morphine and metamizole. This shows that hypernociception includes the whole neuron and there is antagonism between two substances when administered distant from one another. Under these circumstances it might be postulated that the peripheral injection of both opioid and NSAID at a distal end of the nerve fiber would produce antinociception, by modification of the primary nociceptive neuron sensitivity in response to sensory stimulation. This effect is not systemically mediated, but by diffusion of the drugs along the primary nociceptive neurons, as labelled naloxone was detected in the sciatic nerve, dorsal root ganglion and spinal cord, but not in the paw or blood after intraplantar injection.

The peripheral analgesic effect of opioids and NSAIDs in previously inflamed tissue, when the primary nociceptive neurons are formerly sensitized, has also been described and could account for the analgesic effect of peripheral administration of micro-doses of morphine and carprofen in this study. These effects are mediated by enhancement of the nitrous oxide related pathway.

Another possible limitation of the study was that sedation may increase the pain scores in dogs and might contribute to the use of rescue analgesia. This was probably not the case in this study, as although most of the rescue analgesia was performed at 1 h after the end of anaesthesia, the UMPS has been previously validated and specially developed to be used postoperatively. According to that the possible effect of sedation in increasing the pain score is less pronounced in the UMPS, when compared to CMPS, and by this time dogs were responsive to stimulation due to the short period of anaesthesia and surgery.

Ovariohysterectomy is considered a moderate painful procedure, modifying postoperative behaviour in dogs for up to 20 h. Except for the animals submitted to rescue analgesia, prolonged analgesia (up to 24 h) was observed in most animals from all groups, showing that all protocols produced sufficient analgesia in the majority of the animals.

Four different pain scales for measuring postoperative pain associated with surgery were used in this study, to improve the quality of pain assessment. Different assessors assessing pain in different animals, at different time points might produce conflicting results. On these grounds, a single expert assessor was chosen to simplify and uniform pain evaluation. Pain scores with UMPS, CSU and CMPS-SF did not show divergences among groups and animals, thus confirming a comparable sensitivity of each scale.

Possible limitations may be attributed to this study. The surgeon was skilled and surgery was performed in a short period, which could have mask major differences among analgesic treatments. There was no placebo group or introduction of needles in false acupoints. Placebo has not been used any more in recent studies due to obvious ethical issues and by the fact that it is well accepted that OHE is a moderate painful procedure which requires postoperative rescue analgesia, when animals do not receive postoperative pain treatment. All treatments were preventive as it has been currently recognized that the administration of analgesics before surgery in man and dogs decreases central sensitization and postoperative analgesic requirement, therefore the effect of AP and PA performed postoperatively warrants further investigation.

A group of animals submitted to false AP points has also not been included because it has been reported that the analgesic effect of false AP is either absent or of low intensity when compared to the use of real points.

The UMPS was used to determine the use of rescue analgesia, based on the fact that the evaluator was more familiarized in using the scale, as this was the scale adopted in the Veterinary Hospital routine and therefore accuracy would be improved. Another possible limitation of the study was that sedation may increase the pain scores in dogs and might contribute to the use of rescue analgesia. This was probably not the case in this study, as although most of the rescue analgesia was performed at 1 h after the end of anaesthesia, the UMPS has been previously validated and specially developed to be used postoperatively. According to that the possible effect of sedation in increasing the pain score is less pronounced in the UMPS, when compared to CMPS, and by this time dogs were responsive to stimulation due to the short period of anaesthesia and surgery.
Considering that there is no information regarding the intervention point to use rescue analgesia based on the UMPs, it was decided to use 30% from the total score because this is considered the transition between light and moderate pain. This is also close to intervention score reported for the CMPS, which is 25% from the total score, i.e. 6.4.

Probably the main limitation of this study was the relatively small sample size in each group. It was difficult to increase the sample size as this was a clinical study and several experimental groups were studied. The number of rescue analgesia was either two or three in all groups, showing that the analgesic effect of all protocols was similar.

Dogs receiving rescue analgesia were not removed from the data, to minimize type 2 error and to investigate duration and efficacy of rescue analgesia in treating pain. Another approach like continuing to use the same value of the pain score measured at the time point rescue analgesia was firstly administered would increase type 1 error, as well as data would not be genuine.

A strong motivation for withholding systemic analgesics in animals is the concern for their potential toxicity or adverse reactions. Pharmacopuncture might avoid the well-known adverse effects of standard systemic doses of opioids and NSAIDs. Further clinical studies using PA and dry AP, as a promising alternative to pharmacological pain treatments, are encouraged, especially under conditions where the use of NSAIDs and/or opioids are contra-indicated.

Conclusions

Pharmacopuncture was as effective as acupuncture, and both were equally effective as either morphine or carprofen to control postoperative pain in bitches undergoing ovariohysterectomy. The clinical relevance is that PA might produce less adverse effects when compared to the use of the conventional doses of drugs. The advantage of PA when compared to dry needle AP is that the injections may be performed quickly without maintaining the needles into acupoints before surgery.

References

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