

Accepted Manuscript

Title: Intra-articular administration of lidocaine plus adrenaline in dogs: pharmacokinetic profile and evaluation of toxicity in vivo and in vitro

Author: A. Di Salvo, E. Chiaradia, G. della Rocca, F. Mancini, R. Galarini, D. Giusepponi, V. De Monte, P. Cagnardi, M.L. Marenzoni, A. Bufalari

PII: S1090-0233(15)00357-3
DOI: <http://dx.doi.org/doi: 10.1016/j.tvjl.2015.09.004>
Reference: YTVJL 4615

To appear in: *The Veterinary Journal*

Accepted date: 3-9-2015

Please cite this article as: A. Di Salvo, E. Chiaradia, G. della Rocca, F. Mancini, R. Galarini, D. Giusepponi, V. De Monte, P. Cagnardi, M.L. Marenzoni, A. Bufalari, Intra-articular administration of lidocaine plus adrenaline in dogs: pharmacokinetic profile and evaluation of toxicity in vivo and in vitro, *The Veterinary Journal* (2015), <http://dx.doi.org/doi: 10.1016/j.tvjl.2015.09.004>.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



**Intra-articular administration of lidocaine plus adrenaline in dogs:
Pharmacokinetic profile and evaluation of toxicity in vivo and in vitro**

A. Di Salvo ^a, E. Chiaradia ^a, G. della Rocca ^{a,*}, F. Mancini ^a, R. Galarini ^b, D. Giusepponi ^b, V. De Monte ^a, P. Cagnardi ^c, M.L. Marenzoni ^a, A. Bufalari ^a

^a *Department of Veterinary Medicine, University of Perugia. Via S. Costanzo, 4 - 06126 Perugia, Italy.*

^b *Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Via Salvemini, 1 - 06126 - Perugia, Italy.*

^c *Department of Health, Animal Science and Food Safety, University of Milan, Via Celoria 10, - 20133 Milan, Italy.*

* Corresponding Author. Tel +39 075 5857605
E-mail address: giorgia.dellarocca@unipg.it (G. della Rocca)

Accepted Manuscript

Highlights

- The potential for cardio-, neuro- and chondro-toxicity of intra-articular administration of lidocaine plus adrenaline was evaluated in dogs undergoing arthroscopy
- The treatment was not found to cause adverse effects on the cardiovascular system.
- The plasma concentrations of lidocaine did not reach neurotoxic concentrations.
- Toxic effects of lidocaine on chondrocytes appeared to be reduced by adrenaline.

Abstract

The aim of this study was to evaluate the safety of intra-articular (IA) lidocaine plus adrenaline for improving peri-operative analgesia in anaesthetised dogs undergoing arthroscopy of the elbow. A solution of lidocaine (L) 1.98% plus adrenaline 1:100.000 was administered via the IA route and its safety evaluated in terms of cardio- neuro- and chondro-toxicity.

No bradycardia or hypotension was recorded from induction to the last observational time point. Signs of toxicity of the nervous system could have been masked by the general anaesthesia but lidocaine concentrations detected in the blood were lower than those thought to be capable of producing toxicity. The assessment of in vitro chondrotoxicity showed a dose- and time-dependent effect of lidocaine on the viability of articular cells. Adrenaline appeared to reduce the chondrotoxicity of 1% lidocaine, following an exposure of up to 30 min.

Keywords: Chondrotoxicity, Dog, Intra-articular administration, Lidocaine, Pharmacokinetics, Systemic toxicity.

Introduction

Arthroscopic surgery plays an important role in the treatment of various joint diseases in dogs (Miller and Beale, 2008; Bergenhuyzen et al., 2010) and the increasing use of the technique has highlighted the need to improve the quality of analgesia in the perioperative period. In humans undergoing arthroscopy, the relief of pain using intra-articular (IA) administration of α_2 -agonists, opioids and local anaesthetics has been highly successful (Joshi et al., 2000; Elhakim et al., 1999). In particular, there are reports in the literature that effective analgesia follows an IA administration of lidocaine (Dahl et al., 1990; Arai et al., 2005).

Lidocaine is a local anaesthetic easily adsorbed from the injection site, due to its chemical structure, has vasodilatory properties, and a pKa similar to the physiological pH (Schulman and Strichartz, 2012). Its absorption into the systemic circulation may cause cardiovascular and neurological toxic effects depending on the peak concentrations (Schulman and Strichartz, 2012). After IA administration of lidocaine 2%, Di Salvo et al. (2014) observed no signs of toxicity as regards the cardiovascular and nervous system but treated dogs were under anaesthesia, which could have masked the possible neurotoxic effects. These authors observed a rapid absorption of lidocaine from the joint and, in some dogs, the peak drug concentrations were higher than those considered by Lemo et al. (2007) to be responsible for neurotoxic effects in dogs (2.7 $\mu\text{g}/\text{mL}$). The addition of a vasoconstrictor such as adrenaline to a local anaesthetic reduces the local perfusion and generally decreases the absorption of the anaesthetic and the likelihood of reaching potentially toxic blood concentrations (Van Vynck et al., 2010).

To the best of our knowledge, no studies on the safety of the IA administration of lidocaine plus adrenaline have been carried out during arthroscopic surgery in dogs. The purpose of this research was therefore to evaluate the absorption rate of lidocaine into the bloodstream after IA administration of a solution of lidocaine plus adrenaline in dogs undergoing arthroscopic surgery, in order to assess whether plasma concentrations potentially responsible for systemic toxicity would be reached. Moreover, since the use of local anaesthetics in humans by means of IA infusion pumps has been accompanied by severe cases of chondrolysis (Noyes et al., 2012) and we are unaware of any studies on the effect of lidocaine on the cells of canine cartilage, the possible toxic effects of lidocaine and lidocaine plus adrenaline on chondrocytes were also examined.

Material and methods

The study was approved by the Bioethical Committee of the University of Perugia (number 2012-039; 5 October 2012), and the owners, who had all been appropriately informed about the aims and modalities of the study, provided their written consent.

Treatments and clinical evaluations of animals

Twelve dogs admitted to the Veterinary Teaching Hospital of the University of Perugia for an elbow arthroscopic surgery were considered as eligible subjects for the study. All animals were healthy and classified as ASA I or II. The degree of inflammation was established by clinical and radiographic orthopaedic assessment, and a scoring scale from 1 to 4 (1 = low; 2 = low/moderate; 3 = moderate/high; 4 = severe) was applied to determine the severity of the joint disease. Dogs with

significant capsular swelling related to severe and extensive synovitis (degree 4) were not included in this study. The weight, age and degree of joint inflammation of the recruited animals are summarised in Table 1.

A fresh solution of lidocaine plus adrenaline was prepared by adding 200 μ L of a solution containing adrenaline (1 mg/mL) (Adrenalina Monico Spa) to 19.8 mL of 2% lidocaine (Lidocaine Hydrochloride, SALF.). The final solution contained 1.98% lidocaine plus 1:100,000 adrenaline (pH 7.0).

As a pre-emptive analgesic treatment, dogs received 0.1 mg/kg of meloxicam for at least 3 days before surgery. Prior to arthroscopy, dogs were premedicated with 10 μ g/kg of acepromazine (Prequillan, Fatro) by the intramuscular route, then general anaesthesia was induced with 4-6 mg/kg of propofol (Proposure, Merial) and maintained by a mixture of isoflurane (Isoflo; Esteve Spa) and oxygen (50-100 mL/kg/min). Subsequently, dogs were randomly assigned to one of the following two experimental groups. Group LA (six subjects) received the solution of lidocaine plus adrenaline, and Group S (six subjects) received saline 0.9%.

In order to assure a sufficient joint distension (necessary to minimize the trauma during the establishment of the arthroscope portal), in both groups the solutions were injected until the surgeon perceived a counter pressure on the plunger of the syringe. Thus, different volumes of the lidocaine-adrenaline solution and variable doses of lidocaine were injected in each joint. A volumetric limit was established at 10 mg/kg of lidocaine, which is the maximum therapeutic dose considered safe for regional and

local anaesthesia (Lemo et al., 2007). Table 1 summarises the injected volumes and the total doses of lidocaine administered to each dog.

The arthroscopic procedure began 15 min after the injection and the joint was continuously flushed with Ringer lactate throughout the procedure. Immediately after the induction of anaesthesia, and up to recovery, clinical parameters, namely, heart rate (HR), electrocardiogram (ECG), respiratory rate (RR), non-invasive systolic (SAP), diastolic (DAP) and mean arterial blood pressure (MAP), haemoglobin oxygen saturation, end-tidal carbon dioxide partial pressure, isoflurane exhaled concentration and rectal temperature (T°) were continuously monitored by a Multi-Parameter Monitor (HB100; Foschi).

If an increase in HR, blood pressure or RR ($>20\%$ compared with baseline) was observed, one or more intravenous (IV) boluses of sufentanil ($0.1 \mu\text{g}/\text{kg}$, Sufentanil-hameln, Hospira) were administered as rescue analgesia. The value recorded at the steady state of anaesthesia just before surgical procedures was used as the baseline measurement. To assure an adequate postoperative analgesia, $0.1 \text{ mg}/\text{kg}$ of meloxicam (Metacam, Boehringer Ingelheim) was administered subcutaneously at the end of anaesthesia.

At predetermined time-points during the post-surgical period, up to discharge, ECG, HR, RR, SAP, DAP, MAP and T° were monitored and dogs were evaluated for the presence of pain by mean of the numeric rating scale of Heyller and Gaynor (1998), slightly modified. If deemed necessary, $10 \mu\text{g}/\text{kg}$ of buprenorphine were administered IV as rescue analgesia.

Blood sampling

At scheduled time points (0, before; then 5, 10, 15, 30, 60, 90, 120, 180, 240 and 360 min after the IA administration of lidocaine plus adrenaline), blood samples were taken from the dogs of the LA group in order to determine the serum concentrations of lidocaine and its active metabolite monoethylglycinexylidide (MEGX).

Analytical determination of lidocaine and MEGX

Stock solutions (1 mg/mL) of lidocaine hydrochloride monohydrate (Dr Ehrenstorfer), MEGX (Sigma Aldrich) and lidocaine HCl d10 (internal standard, CDN Isotopes) were prepared in methanol and stored at -20 °C.

Serum samples (100 µL) were added to 100 µL of internal standard and 800 µL of acetonitrile, vortexed and then centrifuged at 18,000 g for 5 min. Organic layers (100 µL) were diluted with 400 µL of acetonitrile and 10 µL were injected in the LC-system. Quantitative analyses were conducted by means of a triple quadrupole mass spectrometer (TSQ Quantum Ultra, Thermo Fisher) equipped with a LC-system (Finnigan Surveyor LC pump, Thermo Fisher) via electrospray ionization (ESI) interface. The chromatographic analytical column was a BetaBasic-18 (150 mm × 2.1 mm, 5 µm, Thermo Electron Corporation). The separation was performed in gradient mode, using as mobile phases water and acetonitrile, both containing 0.1% (V/V) formic acid; the flow rate was 0.3 mL/min. The ESI source operated in positive ion mode. The parameters were as follows: spray voltage 3.5 kV and capillary

temperature 270 °C. The concentration of lidocaine or MEGX was determined by the internal standard method.

In vitro evaluation of chondrotoxicity

Canine chondrocytes were isolated from the cartilage harvested from the knee joints of a dog which had been euthanased for reasons unrelated to the present study. Primary cultures of canine chondrocytes were prepared and used to assess the cell viability following exposure to different concentrations of lidocaine alone at 0.5, 1 and 1.98% (L) and lidocaine (same concentrations) plus adrenaline 1:100,000 (L+A). In a pilot study, the effects of chondrocyte exposure to adrenaline alone (10 µg/mL) were also tested and no significant reduction in cell viability was observed (data not shown).

Cartilage slices were rinsed in Dulbecco's phosphate-buffered saline (DPBS), minced and digested primarily with 0.25% trypsin for 10 min at 37 °C and subsequently with 2 mg/mL collagenase type IA (Sigma-Aldrich) at 37 °C for 10/12 h. The cells were cultured in a Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum, penicillin (100 U/mL) and streptomycin (100 µg/mL) in 5% CO₂ at 37 °C in a humidified atmosphere. Cell viability was assessed by measuring the conversion of tetrazolium salt (WST-8) to a water-soluble formazan dye, using a cell counting kit-8 (CCK-8; Dojindo) according to the manufacturer's instructions.

Cells were exposed for 10, 15, 30 and 60 min to L and L+A at the above reported concentrations. DPBS was used as a solvent to prepare dilution and as a

control medium. The effect of drugs on cell viability was expressed as the WTS-8 percentage reduction in treated cells compared to the controls, assuming the absorbance of control cells was 100% ($A_{(\text{treated cells})}/A_{(\text{control})} \times 100$). Data were obtained from four independent experiments, which were performed in triplicate and expressed as the means \pm standard deviation (SD).

Statistics and pharmacokinetic analysis

The homogeneity of the groups as regards age, sex, weight and degree of lesion was evaluated by the Mann-Whitney test, whereas the correlation between the maximum serum concentrations and the volume of the injected solution, the dose and the degree of lesion was performed by Spearman's rank correlation. The statistical significance of changes in chondrocyte viability was determined by a one-way analysis of variance (ANOVA), followed by Bonferroni's post hoc test. For all tests a P value <0.05 was considered to be significant. All the statistical analyses were performed by the R software¹ program.

Pharmacokinetic parameters were obtained from concentration vs. time curves, using the WinNonLin Prof 6.1 software (Pharsight Corporation), which allowed compartmental and non-compartmental analyses of the data. The Minimum Akaike Information Criterion Estimation test (MAICE; Yamaoka et al. 1978) was used to choose the model best fitting the data. All data points were weighted by the inverse square of the fitted value. The serum lidocaine curve was described not only by a standard mono-compartment model, but also by a non-compartmental analysis

¹ See: R Development Core Team (2007). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org> (accessed 3 September 2015).

(NCA), whereas the kinetics of MEGX was determined only by NCA (Gibaldi and Perrier 1982). The metabolic ratio of lidocaine to MEGX was obtained by dividing the area under the curve (AUC) of MEGX by the AUC of lidocaine.

Results

Homogeneity of experimental group

No statistical difference was observed between the control and the treated group as regards age, sex, weight and degree of the lesion ($P>0.05$).

Clinical data

No bradypnoea (reduction of 20% in RR compared to the baseline value), bradycardia (reduction of HR < 60 beats/min), hypotension (MAP < 60 mm Hg) or modifications in ECG were observed during the entire procedure, either in the LA or the S group. No neurological signs, such as muscle tremors, excitation, convulsions or depression of the CNS, were reported during or after recovery from general anaesthesia. Three dogs in the L group and five in the S group received sufentanil as rescue analgesia (Table 1).

Pharmacokinetic profile of lidocaine and MEGX

Calibration curves obtained by spiking blank serum samples showed good linearity, as attested by the correlation coefficients ($R^2 >0.999$). Detection (LOD) and quantification (LOQ) limits were equal to 5 and 10 ng/mL for lidocaine and 10 and 20 ng/mL for MEGX, respectively. At validation levels \geq LOQ, the coefficients of variation (CV%) were always < 11% and accuracy ranged between 85% and 105% for lidocaine and between 98% and 109% for MEGX.

Five minutes after the lidocaine injection, the blood concentrations of the local anaesthetic ranged from 0.013 to 0.980 $\mu\text{g/mL}$. The maximum serum concentration (C_{max}) was between 0.188 and 2.188 $\mu\text{g/mL}$ (mean \pm SD $1.27 \pm 0.81 \mu\text{g/mL}$), and the mean T_{max} was reached at 70.0 ± 84.14 min. In four subjects the serum peak was reached at 30 min after the lidocaine administration and at 60 min and 240 min in the remaining two dogs. The lidocaine active metabolite (MEGX) was detected in the systemic circulation at 5 min from the administration of lidocaine in one dog, and at 60 min in all other subjects. The C_{max} for MEGX was equal to $0.21 \pm 0.16 \mu\text{g/mL}$ (range: 0.023-0.408 $\mu\text{g/mL}$) and was reached at a T_{max} of 130 ± 67.53 min after administration. After reaching C_{max} in the blood, both lidocaine and MEGX decreased in a parallel manner, achieving a concentration of 0.283 ± 0.199 and $0.064 \pm 0.019 \mu\text{g/mL}$, respectively, at 360 min (Fig. 1). Table 2 reports the main pharmacokinetic parameters for lidocaine and MEGX. The metabolic ratio ranged between 0.08 and 0.28. The correlation coefficient between C_{max} and the dose, the severity of the lesion and the volume of injected solution was not significant ($P \geq 0.29$).

Chondrotoxicity

Fig. 2 represents the percentage of viability of the chondrocytes after exposure to different concentrations of L and L+A for different times of exposure. The exposure to 1%, 1.98% L and 1.98% L+A induced a statistically significant reduction in cell viability with respect to controls at all times tested ($P \leq 0.036$). A statistically significant cytotoxicity with respect to controls was caused by 1% L+A only after exposure for 60 min ($P = 0.001$).

The exposure of chondrocytes to 0.5% L or L+A produced no statistically significant differences in cell viability with respect to controls after any exposure time. However, it was noted that the percentage cell viability after exposure of chondrocytes to 0.5% L and L+A was 93.65 ± 5.98 and 101.93 ± 7.69 at 10 min, 89.86 ± 3.81 and 100.77 ± 4.54 at 15 min, 86.19 ± 8.50 and 99.39 ± 6.14 at 30 min, and 80.37 ± 5.65 and 92.31 ± 2.35 at 60 min, respectively, indicating a marked improvement in cell viability in the presence of adrenaline.

Discussion

In veterinary medicine, the IA administration of local anaesthetic is a common practice both for diagnostic purposes and for relieving post-surgical pain (Gurney et al., 2012; Sammarco et al., 1996, Van Vynckt et al., 2012a, 2012b).

In this study, the IA administration of a solution of 1.98% lidocaine plus 1:100,000 adrenaline (prepared in the clinic) was evaluated in dogs undergoing arthroscopy in order to assess the potential local and systemic toxicity of the local anaesthetic. The solution of lidocaine plus adrenaline was prepared extemporaneously because the commercially available formulations have a low pH resulting in a smaller percentage of the undissociated form of lidocaine and therefore a delayed onset of action (Van Vynckt et al., 2010).

Different volumes of the lidocaine plus adrenaline solution were injected into each dog's joint. This treatment option was preferred to the use of a fixed volume, as the joint capacity was dependent on the degree of inflammation. This however represents a major limitation of the study, as different volumes administered into the

joints with various degrees of inflammation resulted in a large variability both in lidocaine blood concentrations vs. time and in pharmacokinetic parameters. We must emphasise that the pharmacokinetic values obtained in this study should only be used for comparative purposes and not as absolute values.

The high variability of the obtained lidocaine C_{\max} (between 0.188 and 2.188 $\mu\text{g/mL}$) was not statistically correlated to the administered dose, the injected solution volume or the severity of the lesions ($P > 0.05$). All these variables together could have accounted for such high variability. Moreover, the different degrees of vascularization in the diseased joint and/or the different metabolic capacities of dogs may also have contributed to such variability. The AUC ratio for MEGX/Lidocaine calculated for each subject ranged from 0.08 to 0.28, indicating a large amount of variability between individuals in the production of the active metabolite. Other authors have previously observed significant variation in the capacity of individuals to metabolise IV administered lidocaine, including in dogs and humans (Conti et al., 2004; Neumann et al., 2011). It should also be noted that our study was conducted with a limited number of animals during a surgical practice and not under experimental conditions. Future studies with a larger number of more closely matched animals may reduce this variability.

The mean lidocaine C_{\max} reached in this study was equal to $1.27 \pm 0.81 \mu\text{g/mL}$ (mean \pm SD). This value is approximately half of that observed in a similar study ($2.18 \pm 0.91 \mu\text{g/mL}$; Di Salvo et al., 2014), where 2% lidocaine was administered by the IA route without the addition of adrenaline. Narchi et al. (1992) also observed a reduction in the C_{\max} to approximately half after the intraperitoneal administration of

a solution of 0.5% lidocaine plus adrenaline (1:800,000 and 1:320,000), compared to the values obtained after the administration of lidocaine alone. Conversely, Calvo et al. (1995) noticed no difference in C_{\max} values following the administration of lidocaine or lidocaine plus adrenaline in the interpleural space.

In this study, after administration of lidocaine, the T_{\max} was greater than that observed by Di Salvo et al. (2014) (70 min vs. 28.75 min). This delay agrees with data obtained by Narchi et al. (1992) and Calvo et al. (1995). Similarly, the time of the appearance of the MEGX was also delayed compared to that observed by Di Salvo et al. (2014) (130 min vs. 60 min). Moreover, the mean residence time (MRT) was prolonged if compared with previous results obtained using lidocaine alone (MRT_{0-last}: 155.43 min vs. 91 min for lidocaine and 163.21 min vs. 115.40 min for MEGX).

Conversely, the mean AUC of lidocaine was comparable to that obtained in the previous study (224.76 $\mu\text{g}\cdot\text{min}/\text{mL}$ vs. 228.64 $\mu\text{g}\cdot\text{min}/\text{mL}$). As the administered doses in the two studies were similar (6.40 - 9.43 mg/kg, vs. 5.26-10 mg/kg), these data indicate that the addition of adrenaline to lidocaine did not reduce the total amount of the drug which reached the systemic circulation, but only slowed its absorption.

Signs of cardiotoxicity, such as bradycardia, transient lengthening of AV node conduction, a decrease in myocardial contractility and vasodilation, have been described by Bruelle et al. (1996) following the administration of lidocaine (16 mg/kg, IV) in dogs under general anaesthesia. In our study, no modifications in ECG, hypotension or bradycardia were observed during the entire procedure, demonstrating

a reliable safety margin of IA lidocaine towards the cardiovascular system at the applied dose rates.

Muscle tremors are considered the first sign of lidocaine toxicity in dogs (Skarda and Tranquilli, 2007). Lemo et al. (2007) noted this occurred when the lidocaine blood concentration reached $2.7 \pm 1.1 \mu\text{g/mL}$. Since in the present study any neurotoxicity of lidocaine would have been masked by general anaesthesia, the possibility that potentially neurotoxic blood concentrations might occur following IA administration of the solution of lidocaine plus adrenaline was investigated. The results showed that the maximum concentration of lidocaine reached was $2.188 \mu\text{g/mL}$, which was lower than that indicated by Lemo et al. (2007) as responsible for the appearance of neurotoxic effects. In the study conducted by Di Salvo et al. (2014) with lidocaine alone concentrations $> 2.7 \pm 1.1 \mu\text{g/mL}$ were reached. As that study was conducted using the same protocol as the present study (the only difference was the presence or the absence of adrenaline) it is possible to postulate that the addition of adrenaline, which slows the absorption of lidocaine, does not allow blood concentrations high enough to cause neurological adverse effects.

In the *in vitro* evaluation of local toxicity, lidocaine appears to be chondrotoxic in a time- and concentration-dependent manner. These results agree with the observations of Karpie and Chu (2007) who tested lidocaine chondrotoxicity on bovine cells. However, after exposure to lidocaine plus adrenaline, the reduction in chondrocyte viability was less pronounced than after lidocaine alone (Fig. 2). That is particularly evident after cells exposure to 1% L or L+A: in the first case viability was statistically reduced with respect to controls at all observation times, whereas in the

second case the viability was significantly reduced only 1 h after exposure. Similar results were obtained by Jacobs et al., (2011) following the exposure of human chondrocytes to 1% L and 1% L + A. These authors speculated that adrenaline may exert a protective effect on chondrocytes but the data presented in the literature are equivocal. Dragoo et al. (2008) reported an increased toxicity of local anaesthetics plus adrenaline, but in that study cells were exposed to the mixture for more than 24 h. Moreover, in a following study, Dragoo et al. (2010) hypothesized that increased cellular necrosis may have been associated with the low pH and preservative substances contained in the local anaesthetic plus adrenaline solution. The use of a solution made up in-house in the present study may have further contributed to the different results obtained. Consistent with the results of the study by Lo et al. (2009), no effect of adrenaline alone on chondrocyte viability was observed.

The observations carried out following in vitro studies do not necessarily reflect what occurs in vivo. The effects of lidocaine on chondrocytes could be reduced by the dilution of the anaesthetic, its absorption into the bloodstream and/or the washing during the arthroscopic procedure. Conversely, the delayed absorption of lidocaine in the presence of adrenaline could prolong the contact of the local anaesthetic with the cells and thus lead to a higher potential for chondrotoxicity. In vivo studies aimed at further elucidating the adverse effects of lidocaine on articular structures are warranted.

Conclusions

The IA administration of 1.98% lidocaine plus adrenaline in dogs which have undergone arthroscopic surgery did not appear to produce any adverse effects on the

cardiovascular system. Following the absorption of the anaesthetic, the peak plasma concentrations did not reach those considered to be responsible for side effects at the central nervous system level. Although lidocaine plus adrenaline was found to have a lesser impact on cell viability compared with lidocaine alone, some chondrotoxicity was demonstrated in vitro.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

References

- Arai, Y.-C.P., Ikeuchi, M., Fukunaga, K., Ueda, W., Kimura, T., Komatsu, T., 2005. Intra-articular injection of warmed lidocaine improves intraoperative anaesthetic and postoperative analgesic conditions. *British Journal of Anaesthesia* 96, 259–261.
- Bergenhuyzen, A.L.R., Vermote, K.A.G., van Bree, H., Van Ryssen, B., 2010. Long-term follow-up after arthroscopic tenotomy for partial rupture of the biceps brachii tendon. *Veterinary and Comparative Orthopaedics and Traumatology* 23, 51–55.
- Bruelle, P., Lefrant, J.Y., De La Coussaye, J.E., Peray, P.A., Desch, G., Sassine, A., Eledjam, J.J., 1996. Comparative electrophysiologic and hemodynamic effects of several amide local anesthetic drugs in anesthetized dogs. *Anesthesia and Analgesia* 82, 648–656.
- Calvo, B., Pedraz, J.L., Gascón, A.R., Hernández, R.M., García-Ortega, E., Vara, F., Muriel, C., Domínguez-Gil, A., 1995. The influence of adrenalin on the pharmacokinetics of interpleurally administered lidocaine in patients with pancreatic Neoplasia. *Journal of Clinical Pharmacology* 35, 426–431.
- Conti, F., Dousseta, B., Cherruauc, B., Guérind, C., Soubranea, O., Houssina, D., Calmus, Y., 2004. Use of lidocaine metabolism to test liver function during the long-term follow-up of liver transplant recipients. *Clinical Transplantation* 18, 235–241.

- Dahl, M.R., Dasta, J.F., Zuelzer, W., Mcsweeney, T.D., 1990. Lidocaine local anesthesia for arthroscopic knee surgery. *Anesthesia and Analgesia* 71, 670-674.
- Di Salvo, A., Bufalari, A., De Monte, V., Cagnardi, P., Marenzoni, M.L., Catanzaro, A., Vigorito, V., della Rocca, G., 2014. Intra-articular administration of lidocaine in anaesthetized dogs: pharmacokinetic profile and safety on cardiovascular and nervous systems. *Journal of Veterinary Pharmacology and Therapeutics*, Doi: 10.1111/jvp.12187.
- Dragoo, J.L., Korotkova, T., Kanwar, R., Wood, B., 2008. The effect of local anesthetics administered via pain pump on chondrocyte viability. *American Journal of Sports Medicine* 36, 1484–1488.
- Dragoo, J.L., Korotkova, T., Kim, H.J., Jagadish, A., 2010. Chondrotoxicity of low pH, epinephrine, and preservatives found in local anesthetics containing epinephrine. *American Journal of Sports Medicine* 38, 1154–1159.
- Gurney, M.A., Rysnik, M., Comerford, E. J., Cripps, P. J., Iff, I., 2012. Intra-articular morphine, bupivacaine or no treatment for postoperative analgesia following unilateral elbow joint arthroscopy. *Journal of Small Animal Practice* 53, 387–392.
- Hellyer, P.W., Gaynor, J.S., 1998. Acute post-surgical pain in dogs and cats. *Compendium of Continuing Education for the Practicing Veterinarian (Small Animals)* 20, 140–153.
- Hube, R., Tröger, M., Rickerl, F., Muench, E.O., von Eisenhart-Rothe, R., Hein, W., Mayr, H.O., 2009. Pre-emptive intra-articular administration of local anaesthetics/opiates versus postoperative local anaesthetics/opiates or local anaesthetics in arthroscopic surgery of the knee joint: a prospective randomized trial. *Archives of Orthopaedic and Trauma Surgery* 129, 343–348.
- Jacobs, T.F., Vansintjan, P.S., Roels, N., Herregods, S.S., Verbruggen, G., Herregods, L.L., Almqvist, K.F., 2011. The effect of lidocaine on the viability of cultivated mature human cartilage cells: an in vitro study. *Knee Surgery, Sports Traumatology, Arthroscopy* 19, 1206-1213.
- Joshi, W., Reuben, S.S., Kilaru, P.R., Sklar, J., Maciolek, H., 2000. Postoperative analgesia for outpatient arthroscopic knee surgery with intraarticular clonidine and/or morphine. *Anesthesia and Analgesia* 90, 1102–1106.
- Karpie, J.C., Chu, C.R., 2007. Lidocaine exhibits dose- and time-dependent cytotoxic effects on bovine articular chondrocytes in vitro. *The American Journal of Sports Medicine* 35, 1621-1627.
- Krohm, P., Levionnois, O., Ganster, M., Zilberstein, L., Spadavecchia, C., 2011. Antinociceptive activity of pre- versus post-operative intra-articular

- bupivacaine in goats undergoing stifle arthrotomy. *Veterinary Anaesthesia and Analgesia* 38, 363–373.
- Lemo, N., Vnuk, D., Radisic, B., Skender, L., Karacic, V., Brcic, I., 2007. Determination of the toxic dose of lidocaine in dogs and its corresponding serum concentration. *Veterinary Record* 160, 374-375.
- Lo, I.K., Sciore, P., Chung, M., Liang, S., Boorman, R.B., Thornton, G.M., Rattner, J.B., Muldrew, K., 2009. Local anesthetics induce chondrocyte death in bovine articular cartilage disks in a dose and duration-dependent manner. *Arthroscopy*, 25, 707–715.
- Miller, J., Beale, B., 2008. Tibiotarsal arthroscopy. Applications and long-term outcome in dogs. *Veterinary and Comparative Orthopaedics and Traumatology* 21, 159-165.
- Narchi, P., Benhamou, D., Bouaziz, H., Fernandez, H., Mazoit, J.X., 1992. Serum concentrations of local anaesthetics following intraperitoneal administration during laparoscopy. *European Journal of Clinical Pharmacology* 42, 223–225.
- Neumann, S., Frenz, M., Streit, F., Oellerich, M., 2011. Formation of monoethylglycinexylidide (MEGX) in clinically healthy dogs. *The Canadian Journal of Veterinary Research* 75, 317–320.
- Noyes, F.R., Fleckenstein, C.M., Barber-Westin, S.D., 2012. The development of postoperative knee chondrolysis after intra-articular pain pump infusion of an anesthetic medication: a series of twenty-one cases. *The Journal of bone and joint surgery. American volume* 94, 16, 1448-1457.
- Sammarco, J.L., Conzemius, M.G., Perkowski, S.Z., Weinstein, M.J., Gregor, T.P., Smith, G.K., 1996. Postoperative analgesia for stifle surgery: a comparison of intra-articular bupivacaine, morphine, or saline. *Veterinary Surgery* 25, 59-69.
- Schulman, J.M., Strichartz, G.R., 2012. Local anesthetics pharmacology. In: *Principles of pharmacology*, 3th Ed., Walters Kluwer, Baltimore, Maryland, USA, pp. 147-160.
- Skarda, R.T., Tranquilli, W.J., 2007. Local anesthetics. In: *Lumb and Jones' Veterinary anesthesia and analgesia*, 4th Ed., Blackwell Publishing, Ames, Iowa, USA, pp 395-418.
- Van Vynckt, D., Polis, I., Verschooten, F., Van Ryssen, B., 2010. A review of the human and veterinary literature on local anaesthetics and their intraarticular use. *Veterinary and Comparative Orthopaedics and Traumatology* 23, 225–230.

Van Vynckt, D., Samoy, Y., Mosselmans, L., Verhoeven, G., Verschooten, F., Van Ryssen, B., 2012a. The use of intra-articular anesthesia as a diagnostic tool in canine lameness. *Vlaams Diergeneeskundig Tijdschrift* 81, 290-297.

Van Vynckt, D., Verhoeven, G., Saunders, J., Polis, I., Samoy, Y., Verschooten, F., Van Ryssen, B., 2012b. Diagnostic intra-articular anaesthesia of the elbow in dogs with medial coronoid disease. *Veterinary and Comparative Orthopaedics and Traumatology* 25, 307–313.

Woolf, C.J., Chong, M.S., 1993. Preemptive analgesia-treating postoperative pain by preventing the establishment of central sensitization. *Anesthesia and Analgesia* 77, 362-379.

Figure legend

Fig. 1. Semi logarithm plot of average lidocaine (solid line, triangle) (-▲-) and MEGX (dotted line, square) (-■-) concentrations vs. time after lidocaine IA administration in dog ($n=6$). Bars represent the standard deviation.

Fig. 2. Cell viability % (mean + standard deviation) after exposure to different concentrations of lidocaine and lidocaine plus adrenaline for 10, 15, 30 and 60 min compared with saline (CTR).

Table 1. Age, weights, sex, articular pathology, degree of inflammation of recruited animals and pharmacological treatments.

Age (months)	Weight (kg)	Sex	Disease	Score attributed to lesion	Treatment	mL admin.	Dose (mg/kg)	n. Sufentanil boluses *
10	31.5	M	FCP	3	Lidocaine + adrenaline	15	9,43	0
84	37.1	M	DJD	1	Lidocaine + adrenaline	12	6,40	3
24	56.4	M	ED	2	Lidocaine + adrenaline	20	7,02	1
12	31.7	M	FCP	2	Lidocaine + adrenaline	12	7,50	0
9	29.7	F	FCP	2	Lidocaine + adrenaline	13	8,67	0
18	28.7	M	MCD	2	Lidocaine + adrenaline	12	8,28	1
6	27	M	OCD	2	saline	15		1 + CRI
11	33	M	OCD	1	saline	15		3
12	38	F	FCP	3	saline	18		0
24	57	M	OCD	2	saline	20		1
84	37.5	M	FCP	1	saline	9		4
30	34	M	FCP	3	saline	10		1

* 0,1 µg/kg as rescue analgesia

FCP, Fragmented coronoid process; DJD, Degenerative joint disease; ED, Elbow dysplasia; MCD, Medial Compartment Disease; OCD, Osteochondritis dissecans; 1,low; 2, moderate; 3, high; F, Female; M, Male; CRI, Continuous rate infusion(0.5 µg/kg).

Table 2. Pharmacokinetic parameters of lidocaine and MEGX obtained by mono-compartmental and non-compartmental model following lidocaine IA administration in dog ($n=6$).

Parameter	Unit	Mean	SD.
Lidocaine			
Mono-compartmental analyses			
AUC	$\mu\text{g}\cdot\text{min}/\text{mL}$	418.83	348.80
$t_{1/2\text{ abs}}$	min	22.94	15.58
$t_{1/2\text{ elim}}$	min	158.29*	88.42*
T_{max}	min	78.74	44.59
C_{max}	$\mu\text{g}/\text{mL}$	1.05	0.73
Non-compartmental analyses			
$t_{1/2\lambda z}$	min	149.18*	105.84*
$T_{\text{max obs}}$	min	70.00	84.14
$C_{\text{max obs}}$	$\mu\text{g}/\text{mL}$	1.27	0.81
AUC _{0-last}	$\mu\text{g}\cdot\text{min}/\text{mL}$	224.76	132.90
MRT _{0-last}	min	155.43	23.93
MEGX			
$t_{\lambda z}$	min	118.25*	56.02*
$T_{\text{max obs}}$	min	130.00	67.53
$C_{\text{max obs}}$	$\mu\text{g}/\text{mL}$	0.21	0.16
AUC _{0-last}	$\mu\text{g}\cdot\text{min}/\text{mL}$	44.89	30.55
MRT _{0-last}	min	163.21	18.82
* harmonic mean \pm pseudo SE; AUC: area under serum concentration-time curve; $t_{1/2\text{ abs}}$: absorption half-life; $t_{1/2\text{ elim}}$: elimination half-time; MRT _{0-last} : mean residence time; $t_{1/2\lambda z}$: terminal half-time; T_{max} = time of peak concentration; $T_{\text{max obs}}$ = Time of maximum concentration observed C_{max} = Maximum concentration; $C_{\text{max obs}}$ = Maximum concentration observed; .			

Accepted Manuscript