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Jämförelse av plasmakoncentrationer och analgetisk effekt mellan oral transmukosal och subkutan administrering av buprenorfin hos kanin

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SUMMARY

Rabbits are becoming more popular as pet animals and surgical procedures are likewise getting more common. Hence the need for postoperative analgesic treatment in the home is increasing. Buprenorphine is a partial μ-opioid receptor agonist with long duration and is the therefore useful as a postoperative analgesic agent. It is also the most commonly used opioid in rabbits. Buprenorphine has poor bioavailability after oral dosing, therefore oral transmucosal (OTM) administration could be an option for pet owners. The aim of this study was to compare OTM with subcutaneous (SC) administration of buprenorphine by buprenorphine plasma concentrations and facial pain expression scoring.

Eighteen female New Zealand White rabbits with the mean (SD) body weight 3.90 (0.49) kg were used in a study on bone replacement, in which a full diaphysis bone segment of 20 mm was removed from the radius and replaced by either a monetite implant or autologous bone. Buprenorphine was administered postoperatively either SC (0.05 mg/kg) or OTM (0.15 mg/kg) every eighth hour. Blood samples were collected before and at 8 time points (15-540 min) after the first administration of buprenorphine. Plasma was analysed for concentrations of buprenorphine with ultra-high performance liquid chromatography – tandem mass spectrometry. Photographs were taken of the rabbits' faces prior to surgery and postoperatively before each blood sampling. The pictures were scored in a blinded fashion for pain assessment both subjectively and with the Rabbit Grimace Scale (RbtGs).

The OTM administration required large volumes and was difficult to accomplish. Buprenorphine plasma concentrations varied largely, especially after SC administration. The maximal plasma concentration (Cmax) and the area under the plasma concentration time curve (AUC) were significantly lower after OTM than after SC administration ($p < 0.05$). The median Cmax values were 0.74 ng/ml and 1.16 ng/ml, and the median AUC values were 41 ng·h/ml and 337 ng·h/ml, for OTM and SC administration respectively. Subjective pain and RbtGs scores were well correlated (OTM: $\rho = 0.94$ and SC: $\rho = 0.90$) but did not correlate with buprenorphine plasma concentrations. RbtGs pain scores for OTM administration were only significant higher ($p < 0.05$) at one time point (15 min) out of eight compared to SC administration.

In summary, neither of the administration routes were regarded as reliable as reflected by the large variation in plasma concentrations of buprenorphine. Buprenorphine given OTM was not as well absorbed as when administered SC given the plasma concentrations. More studies are needed to confirm these findings and to find other analgesic treatment alternatives that are owner-friendly.

SAMMANFATTNING

Kaniner har blivit populära som husdjur och därmed har även operativa ingrepp ökat. I och med detta ökar behovet av smärtlindring på kanin i hemmet. Buprenorfin är en partiell agonist till μ-opioidreceptorn med lång duration och är därför användbar för postoperativ smärtlindring. Dessutom är det den vanligaste opioiden som ges till kanin. Buprenorfin har dålig oral biotillgänglighet därför skulle oral transmukosal administrering kunna vara ett alternativ för behandling i hemmet utfört av djurägare. Syftet med denna studie var att jämföra två olika administrationsvägar av buprenorfin, subkutan och oral transmukosal, genom att mäta plasmahalter av buprenorfin samt utvärdera smärta genom att bedöma ansiktsuttryck.

Arton New Zealand White honkaniner med en medelkroppsvikt (SD) på 3,9 (0,49) kg användes i en experimentell studie av nybildning av benvävnad varvid 20 mm av radiusdiafysen avlägsnades och ersattes av ett monetitimplantat eller autologt ben. Buprenorfin administrerades postoperativt antingen subkutant (0,05 mg/kg) eller oralt transmukosalt (0,15 mg/kg) var åttonde timme. Blodprover togs före operation och vid 8 tidpunkter (15-540 min) efter den första buprenorfinadministrationen. Plasmahalter av buprenorfin analyserades med ultrahög-prestanda vätskekromatografi - tandem masspektrometri. Djurens ansikten fotograferades före operation samt före varje blodprovtagningstillfälle. Fotografierna bedömdes blindat för smärta både subjektivt och enligt en skala baserad på ansiktsuttryck hos kanin, Rabbit Grimace Scale (RbtGs).

Oral transmukosal administrering krävde en stor volym och var svår att utföra. Plasmakoncentrationerna av buprenorfin varierade kraftigt, särskilt efter subkutan injektion. Den maximala plasmakoncentrationen (Cmax) och arean under plasmakoncentrationskurvan (AUC) var signifikant lägre efter oral transmukosal jämfört med subkutan administration ($p < 0,05$). Medianvärden för Cmax var 0,74 ng/ml för oral transmukosal och 1,16 ng/ml för subkutan administrering. Medianvärden för AUC var 41 ng·h/ml för oral transmukosal och 337 ng·h/ml för subkutan administrering.

Smärtpoängen från RbtGs samt subjektiv bedömning var väl korrelerade efter både oral transmukosal och subkutan administration ($\rho = 0,94$ respektive $\rho=0,90$). Smärtpoängen korrelerade inte med plasmakoncentrationer av buprenorfin. Smärtpoängen från RbtGs vid oral transmukosal administrering var signifikant högre ($p < 0.05$) vid endast en tidpunkt (15 minuter) av totalt åtta jämfört med subkutan administrering.

Sammanfattningsvis kan ingen av administrationssätten anses tillförlitliga med ledning av den stora variationen i plasmakoncentrationer av buprenorfin. Buprenorfin absorberades inte like väl efter oral transmukosal som subkutan administration, givet plasmakoncentrationerna. Fler studier behövs för att konfirmera dessa fynd och för att hitta djurägarvänliga alternativ för smärtbehandling i hemmet.

CONTENT

INTRODUCTION

Rabbits are common both in medical research and as pets. In both settings, surgery is sometimes necessary, leading to postoperative pain and potential suffering. Despite the increasing knowledge about pain processes and the benefits of adequate analgesia, at present rabbits do not always receive analgesic treatment after experimental surgical procedures (Coulter *et al.,* 2011). When systemic analgesic agents are used in experimental surgery, buprenorphine is the most common agent.

Because the popularity of rabbits as pets has increased, surgical procedures in pet rabbits have become more complex, and with that the need for potent analgesic treatment has increased (as reviewed by Barter, 2011). Multimodal analgesia is generally recommended (Weaver *et al.,* 2010; Coulter *et al.,* 2011) and opioids and NSAIDs can be combined in an analgesic protocol. NSAIDs have the advantage that they can be given orally and can therefore be easily administerad by pet owners (as reviewed by Barter, 2011). Opioids and other analgesic agents than NSAIDs are usually administered parenterally. However, opioids can be administered by the oral transmucosal route (OTM), thereby avoiding the first-pass liver effect, as well as making it possible for owners to treat animals at home. This route of administration has been evaluated in e.g. cats, but only to a limited extent in rabbits (see below).

One potent opioid for treatment of postoperative pain is buprenorphine, which and can be administered by the subcutaneous (SC), intramuscular (IM) or intravenous (IV) route in rabbits (Wootton *et al.,* 1988; Shafford & Schadt., 2008; Cooper *et al.,* 2009.) The OTM administration of buprenorphine has been proved reliable regarding its analgesic effects and pharmacokinetics in cats and dogs (Robertson *et al.*, 2005; Abbo *et al.,* 2008; Giordano *et al.*, 2010), with most studies published in cats. There are only few studies published on OTM administration of buprenorphine in rabbits (Lindhardt *et al.,* 2001; Yeola *et al.,* 2014), and it seems not to be a commonly practiced administration route.

To treat pain efficiently, pain must be correctly assessed, and pain assessment in rabbits is not considered an easy task. It is most probably due to the rabbit's natural behaviour as a prey species, which is believed to have a great impact on behaviour-based pain scoring (as reviewed by Barter, 2011). Recently, facial expression scoring systems have been established in several animal species (Dalla *et al.,* 2014; Oliver *et al.,* 2014; Miller & Leach, 2015), including the rabbit (Keating *et al.,* 2012). Facial expression scoring is suggested to be both a dependable and precise method to assess acute pain in rabbits.

The aim of this study was to compare OTM with SC administration of buprenorphine for postoperative analgesia in rabbits after orthopaedic surgery. For the comparison, buprenorphine plasma levels were measured and postoperative pain assessed with the Rabbit Grimace Scale and subjective pain scoring. If OTM administration is shown to be a reliable administration route, it could be useful for treatment of postoperative pain in pet rabbits by the owners at home.

LITERATURE REVIEW

Pain Recognition in Rabbits

Pain recognition is a challenge in rabbits (as reviewed by Barter, 2011). The variation in response to pain and lack of objective parameters make assessment very difficult. Pain assessment in rabbits has mostly been performed with behaviour-based pain scoring systems, in which pain is indicated by changes in gait, posture, locomotion and group dynamics. Good knowledge of normal rabbit behaviour is important and at best the individual behaviour of the rabbit is known to the observer. The most common pain-related behaviour in rabbits is however inactivity (Leach *et al.*, 2009). Moreover, behaviour-based scoring systems have not been very successful due to the observer's impact on the rabbit (causing stress).

In 2010 Leach *et al* investigated people's ability to assess pain in rabbits. People of both sexes, different professions and experiences of rabbits scored pain in spayed rabbits from 1-minute long video episodes, using a visual analogue pain scale. With the help of eye tracking equipment, they showed that most people focus on the face of the rabbits. The authors claimed that this resulted in less accurate pain assessment because pain-related behaviour was seen on the abdomen, ears, back and hindquarters. Focusing on the whole animals was their recommendation.

However, face-focusing pain assessment scoring methods have been developed on different animal species after that. Although more research is needed, the grimace scales show promising results for pain assessment in horses (Dalla *et al*, 2014), rats (Oliver *et al*, 2014) and mice (Miller & Leach, 2015).

Rabbit Grimace Scale

The Rabbit Grimace Scale (RbtGs) is a pain assessment scale in which ear position, orbital tightening, cheek flattening, nose shape and whisker position are scored from 0-2 (0: not present, 1: moderately present, 2: obviously present). Keating *et al* (2012) evaluated the RbtGs for the capacity of assessing acute nociceptive pain. They recorded behaviour changes, heart rate, arterial blood pressure, serum cortisone concentration, facial expressions and home pen behaviours in four different groups of rabbits which underwent true or sham ear tattooing with or without prior EMLA cream application. Their results showed that the RbtGs is a useful tool for pain assessment. Prior EMLA cream treatment resulted in a significantly lower pain score at tattooing.

Pain Management in Rabbits

Coulter *et al.* (2011) surveyed 128 published scientific articles on rabbits undergoing experimental surgical procedures and compared the reported analgesic administration in 1995-1997 to 2005-2007. Unfortunately, not all rabbits received pain relief, however analgesic administration increased significantly from the first to the second time period. Buprenorphine was the most commonly used agent. Analgesia was mostly administered postoperatively and the authors recommend that analgesic treatment is given pre- and intraoperatively as well. They also suggest more frequent use of multimodal analgesia, with opioids, NSAIDs and local anaesthesia.

For analgesia, NSAIDs and opioids are most commonly used (as reviewed by Barter, 2011). Local anaesthetics, α2-agonists and NMDA-antagonists can also be used. NSAIDs are suitable for treatment of both acute and chronic pain of a mild to moderate degree. Its anti-inflammatory action and synergistic action with opioids is favourable when it comes to postoperative pain management.

Rabbits tolerate NSAIDs well and side effects like renal and hepatic dysfunction and gastric ulcers do not seem to occur in rabbits as often as in other animals (as reviewed by Barter, 2011). NSAIDs may be given by the oral route and can therefore be administered by the owner. However, in cases of moderate to severe pain the effect of NSAIDs may not fully cover the analgesic need.

Local anaesthetics act on neural transmission of antinociceptive input and lidocaine and bupivacaine are commonly used in the veterinary field (as reviewed by Barter, 2011). These can be administered topically, injected via infiltration, regional nerve block and epidurally. Many of the administration/injection techniques require sedation or general anaesthesia. In some species continuous rate infusion of lidocaine is used for analgesia and promoting gastrointestinal motility. The use of lidocaine continuous rate infusion (CRI) in rabbits has only been investigated under general anaesthesia (as reviewed by Barter, 2011).

Alpha-2-adrenergic agonists provide sedation, muscle relaxation and analgesia (as reviewed by Barter, 2011). One big advantage is the possibility to reverse their effect with α2-adrenergic antagonists. Alpha-2-agonists have a severe influence on the cardiovascular system by reduction of cardiac output and bradycardia making them unsuitable for administration by the pet owner.

Ketamine is an NMDA-receptor antagonist which is frequently used in higher doses for heavy sedation or anaesthesia in rabbits (as reviewed by Barter, 2011). The NMDA-receptor plays an important role in central sensitization of pain and continuous infusions of sub-anaesthetic doses can be used for its analgesic properties and for anaesthetic-sparing purposes. In rodent chronic pain models ketamine has been used with success to treat pain (as reviewed by Barter, 2011).

Buprenorphine

Opioids' antinociceptive properties are achieved through their binding to μ- and κ-opioid receptors which are distributed throughout the central nervous system (as reviewed by Barter, 2011). Buprenorphine is also an agonist of the nociceptin receptor ORL1. This binding inhibits ascending nociceptive activity, reduces neurotransmitter release and activates inhibitory conduits. Not only obvious parenteral administration routes can be used for opioids but also transdermal, sublingual, oral, local and epidural routes. Adverse effects include sedation, respiratory depression and decreased gastrointestinal motility. Buprenorphine, which is a partial μ-agonist, has a limited effect and is therefore recommended for mild to moderate pain. Sedation is less severe when using buprenorphine compared to full μ-agonists (as reviewed by Barter, 2011).

Buprenorphine has a high affinity and slow association/dissociation at the μ-receptor site but lower efficacy compared to full μ-agonists (as reviewed by Barter, 2011). It is a lipophilic molecule and has a high volume of distribution. It is predominately metabolised in the liver. For use in humans different sublingual and transdermal solutions of buprenorphine have been developed. The main adverse effects of buprenorphine are caused by the agonist action on the μ-opioid receptor. Thermal analgesiometric tests suggest a duration of slightly shorter than 8-10 hours in rabbits (Wootton *et al.*, 1988) The analgesic efficacy is limited; therefore increasing the dose above a certain level does not increase the degree of pain relief (as reviewed by Barter, 2011).

Studies in Rabbits

Several studies on different postoperative analgesic regimes have been performed in rabbits undergoing ovariohysterectomy. Cooper *et al.* (2009) investigated the analgesic properties of 0.03 mg/kg buprenorphine administered IM every 12 h for 2 days compared to animals receiving 0.2 mg/kg meloxicam subcutaneously SC every 24 h for 2 days, or incisional infiltration with 0.5 ml of 0.5 % bupivacaine (body weight 2.0-3.0 kg). Food intake, fecal and urine output and body temperature were measured, blood samples and rectal culture examined and clinical examination and auscultation performed. They also assessed behaviour and pain based on daily evaluation of appetite, posture, grooming and activity. There was no significant difference between the meloxicam and the buprenorphine groups. The rabbits which only received local anaesthesia showed significantly lower food consumption, fecal production and body weight than in the other two groups.

Weaver *et al.* (2010) performed a similar study on rabbits after ovariohysterectomy. Investigating the effect of 0.02 mg/kg buprenorphine SC every 12 h for 3 days in comparison with a fentanyl patch (25 μg) placed 24 hours prior to surgery, a subcutaneous injection of 1 mg/kg ketoprofen every 24 h for 3 days or no analgesia at all. There were no differences between any of the analgesic treatment groups regarding food and water consumption, fecal output, mean travel distance or remotely recorded behaviours. Like Coulter *et al.* (2011) the authors recommend the use of multimodal analgesia and consider the lack of research on efficient doses of analgesic agents to rabbits as a major limitation.

Goldschlager *et al.* (2013) showed that multimodal analgesia with buprenorphine and meloxicam has a reducing effect on the levels of fecal glucocorticoid metabolites (FCM). The dosing regimen used was 0.03 mg/kg buprenorphine SC q 12 h and 0.02 mg/kg meloxicam SC q 24 h for 3 days. The surgery performed was a minimal invasive vascular cut-down. Rabbits in the control group received an injection of 2.5 mg bupivacaine (body weight 3 kg) in the incision area. The buprenorphine-meloxicam group had unchanged levels of FCM until day 3 when the treatment was finished. The rabbits in the buprenorphine-meloxicam group gained more weight during 28 days.

Transmucosal Administration of Buprenorphine

Studies in Cats

Several studies on OTM administration have been performed in cats. In a study by Robertson *et al.* (2005) 0.02 mg/kg buprenorphine was administered IV or OTM in cats. The analgesic effect was evaluated by thermal threshold testing (53.6 ±2.1 °C for IV, 51.4 ±4.5 °C for OTM). The plasma concentrations were analysed by iodine125-labeled radio-immunoassay. Blood was sampled form the jugular vein. Plasma buprenorphine concentration over time AUC measured for both IV and OTM showed no significant difference. The peak analgesic effect occurred at 90 minutes and the onset time was 30 minutes in both groups. OTM administration of buprenorphine was therefore regarded as efficient as IV administration.

Porters *et al.* (2015) performed a similar study on the absorption of 0.02 mg/kg buprenorphine and 0.04 mg/kg dexmedetomidine given by the OTM versus the IM route in cats. The plasma concentrations were analysed with liquid chromatography-tandem spectrometry. The results showed that the time to reach Cmax was significantly longer after OTM administration and the area under concentration-time curve and the maximum plasma concentration were significantly lower. The conclusion was that absorption after OTM administration was not as good as when administered IM.

Giordano *et al.* (2010) studied the postoperative analgesic effects of 0.01 mg/kg buprenorphine administered either IV, IM, SC or OTM in cats who underwent ovariohysterectomy. To estimate pain and sedation a Simple Descriptive Scale and a Dynamic and Interactive Visual Analog Scale were used. There were no significant differences between any of the groups.

Porters *et al.* (2014) performed a study on the sedative and antinociceptive effects of 0.04 mg/kg dexmedetomidine and 0.02 mg/kg buprenorphine administered in combination by the OTM or IM routes in cats, similar to the study by Giordano *et al.* (2010). Analgesia to mechanical stimuli (pressure rate onset device and ear pinch) and sedation were assessed through an interactive analogue scale. Also used to measure pain was mechanical threshold testing. No significant differences were seen in either sedation or analgesia between the groups.

Studies in Dogs

Abbo *et al.* (2008) did a pharmacokinetic study on OTM administration of buprenorphine in dogs. Two doses were evaluated (0.02 mg/kg and 0.12 mg/kg). Liquid chromatography-electrospray ionization-tandem mass spectroscopy was used for analysing the plasma concentrations. Bioavailability was higher for the high dose (47 ± 16 % compared to 38 ± 12 %). Cmax for both doses were similar. The authors' conclusion was that OTM administration might be an alternative for pain management in dogs.

In dogs undergoing ovariohysterectomy Ko *et al.* (2011) studied plasma concentrations (using liquid chromatography-electrospray ionization-tandem mass spectroscopy) and postoperative analgesic properties (dynamic interactive pain scales) when buprenorphine was administered IV (0.02 mg/kg), OTM at a low dose (0.02 mg/kg) or OTM high dose (0.12 mg/kg). The number of dogs in need of rescue analgesia and the duration of analgesia did not differ between any of the groups. Buprenorphine at a high dose (0.12 mg/kg) given OTM prior anaesthetic induction can be an analgesic alternative was the assumption made.

Studies in Rabbits

Rabbits have been used in studies on transmucosal administration of buprenorphine to evaluate different formulations of buprenorphine but not for the purpose of treating rabbits per se (Lindhardt *et al.*, 2001; Yeola *et al.*, 2014). A study that examined transmucosal administration of buprenorphine in rabbits, sheep and humans was performed by Lindhardt *et al.* (2001). Two different types of formulations (PEG 300 and dextrose) containing 0.6 mg buprenorphine were given intranasally to rabbits with the mean weight 3.9 kg. There was not a significant difference in bioavailability between

the two formulations. The mean Cmax value (SD) after intranasal administration was 28 (11) ng/ml (PEG 300) and 27 (7) ng/ml (dextrose). Tmax in the study were 8 ± 6 min (PEG 300) and 12 ± 6 min (dextrose).

Summary of Transmucosal Administration Studies

In summary, the pharmacokinetic studies show different results when comparing OTM to other administration routes, whereas no differences in efficacy can be detected when scoring pain and sedation. The bioavailability after intranasal administration in rabbits is high.

MATERIAL AND METHODS

Animals and Housing

Twenty female New Zealand White Rabbits aged 8-9 months bred by a licensed SPF-breeder (Lidköpings kaninfarm, Lidköping, Sweden) were used in a study for evaluation of a new type of monetite dental implant. The rabbits were randomised to be housed in either in pairs in floor pens (2 m^2) or singly in cages (0.42 m^2) to examine whether housing space has an effect on bone regeneration. The rabbits were acclimatized for at least 2 weeks. They were weighed daily for one week prior to surgery. Mean body weight (SD) at the time of surgery was 3.90 (0.49) kg. They were fed with autoclaved hay and pelleted rabbit diet (Lactamin K1, Lantmännen Lantbruk, Malmö, Sweden). They had access to water *ad libitum*. The local ethics committee for animal research had approved the experiment (Dnr C107013/15).

Anaesthesia and Surgery

Preparations and Induction of Anaesthesia

On the day of surgery, the rabbits were clinically examined (estimation of general condition, auscultation of heart and lungs). Anaesthesia was induced with 0.25 mg/kg medetomidine (Sedator vet, 1 mg/ml, Dechra Veterinary Products, Bladel, Netherlands) and 15 mg/kg ketamine (Ketaminol® vet, 100 mg/ml, Intervet, Boxmeer, Netherlands) SC. Once anaesthetized, they received 5 mg/kg carprofen (Norocarp vet, 50 mg/ml, N-vet, Newry, Northern Ireland) SC, 10 mg/kg ceftiofur (Excenel® vet, 4 g, Orion Pharma Animal Health, Helsinki, Finland) IM and 0.5 mg/kg metoclopramide (Primperan®, 5 mg/ml, Sanofi, Stockholm, Sweden) SC. Ceftiofur administration was repeated after 90 min if surgery was still ongoing. The fur was clipped on the ears, the base of the tail, on one front leg and axilla (randomized side). Ropivacaine (Narop®, 10 mg/ml, AstraZeneca, Södertälje, Sweden) was injected SC at a volume of 0.9-1.5 ml at the site of the brachial plexus. One IV (22 G BD Venflon™, BD AB, Stockholm, Sweden) and one arterial catheter (20 G BD Venflon™, BD AB, Stockholm, Sweden) were placed on opposite ears. The catheters were flushed with 2 ml saline and the arterial catheter was filled with 0.14 ml heparin (Heparin LEO, 5000 IE/ml LEO Pharma, Malmö, Sweden) diluted with saline to the concentration of 100 IE/ml. The surgical site was aseptically prepared. The arterial catheters were used for measuring invasive blood pressure intraoperatively and collecting blood samples postoperatively.

Anaesthesia Maintenance and Monitoring

During surgery, anaesthesia was maintained with 0.5-1.5 % isoflurane (Attane vet, 1000 mg/g, VM Pharma, Northumberland, Great Britain) in oxygen (1.0-3.0 L/min) via a laryngeal mask (V-gel supraglottic airway device, size large, Docsinnovent ltd, London, Great Britain). Ringer acetate (Ringer-Acetat Baxter Viaflo, Baxter, Kista, Sweden) was administered at a rate of 10 ml/kg/h IV during surgery. If mean arterial pressure (MAP), measured in the auricular artery, decreased below 60 mm Hg, the rate of Ringer acetate was increased to 20 ml/kg/h. If MAP decreased below 50 mm Hg, a colloid volume expander (Voluven, Fresenius Kabi, 60 mg/ml, Uppsala, Sweden) was administered at a rate of 9 ml/kg/h. If apnea occurred, end tidal CO_2 ($ETCO_2$) increased above 7 kPa or oxygen

saturation (SpO_2) decreased below 90 %, manual ventilation was performed by squeezing the breathing bag. Every 15 minutes respiratory rate, heart rate, blood pressure, SpO_2, inspiratory concentration of isoflurane, flowrate of O_2, colour of mucous membranes and rectal temperature were noted. The surgery lasted approximately 1 hour. Total duration of anaesthesia was approximately 1.5-2 hours.

Surgery

A 20 mm long fraction of the radius diaphysis was removed, a so called critical size defect, in the sense that conservative healing was impossible. This model is described in a Standard Guide for Pre-clinical in vivo Evaluation in Critical Size Segmental Bone Defects F2721-09, (ASTM International, 2014). In place of the defect, ten rabbits received a new type of monetite implant and ten received autologous bone as control. The autologous bone consisted of the removed bone which was macerated before autotransplantation.

Postoperative Care

After termination of surgery and inhalation anaesthesia the animal was extubated and kept in a heated chamber (25 °C) and examined every 15 minutes (respiration rate, colour of mucous membranes, degree of sedation). If needed, 0.2-0.25 ml atipamezole (Antisedan® vet., 5 mg/ml, Orion Pharma Animal Health, Espoo, Finland) was administered IM.

Study Design

Pilot Study

Two weeks before the main study, a pilot study was carried out with two rabbits. One was randomised to receive a monetite implant and the other received autologous bone. One was randomised to be administered 0.05 mg/kg buprenorphine by the OTM and the other by the SC route. Data from the rabbit receiving buprenorphine SC was included in the results from the main study. The rabbit that received buprenorphine OTM, did not have detectable plasma concentrations. It was therefore decided to administer 0.15 mg/kg OTM in the main study, a dose similar to the one used in the study by Lindhardt *et al*. (2001). Additionally in the pilot study, the duration of the brachial plexus nerve block and the time to recovery from anaesthesia was examined to minimize the risk of confounding effects of ropivacaine local anaesthesia and sedation on the evaluation of the analgesic effect of buprenorphine.

Buprenorphine Administration

Rabbits were randomised to receive buprenorphine (Temgesic®, 0.3 mg/ml, RB Pharmaceuticals, Slough Berkshire, Great Britain) either SC in the neck area (0.05 mg/kg) or OTM (0.15 mg/kg). Buprenorphine was administered 3-4 hours after the brachial plexus nerve block, approximately 2-2.5 hours after end of inhalation anaesthesia. The OTM administration volume ranged from 1.65-2.5 ml and was divided between the two cheeks. The animal was either held by the scruff or wrapped in a towel for immobilization and held in a supine position to keep the buprenorphine in the mouth during administration.

Blood Sampling and Analysis

Blood samples were collected 15, 30, 60, 90, 120, 240, 360 and 540 minutes after administration of the first buprenorphine dose. Two ml of blood were collected from the arterial catheter at each time point. The catheter was flushed with 2 ml saline and filled with 0.14 ml 100 IE/ml heparin. Buprenorphine administration was repeated after 480 min.

The blood was collected in EDTA-tubes. The tubes were centrifuged and plasma was separated and frozen at -80 °C. The analyses were carried out with ultra-high performance liquid chromatography – tandem mass spectrometry by the Swedish National Veterinary Institute (SVA, Uppsala, Sweden).

Photographs and Pain Scoring

Three pictures were taken from the front and three from the side of the head before every blood sampling with a digital camera (Panasonic Lumix DMC-T27, Osaka, Japan). Two pictures of each rabbit at each time point were scored for abnormal ear position and orbital tightening (0; not present, 1: moderately present, 2: obviously present) according to the Rabbit Grimace Scale (Keating *et al*, 2012) by a person blinded to treatment. Nose pointing, whisker position and cheek bulging was not scored because no differences were detected for these facial action units. Additionally, photos were scored subjectively with a score from 0 (no pain) to 2 (obviously in pain).

Continuous Care and Medication

Once blood sampling was completed, buprenorphine was administered SC to all animals for 3 days every 8 hours at a dose of 0.03 mg/kg SC. Carprofen was administered daily for 3 days postoperatively at a dose of 5 mg/kg sc. Metoclopramide (0.5 mg/kg SC) was repeated twice at 12 hour intervals. If a rabbit was observed not eating or producing faeces after 12 h, of Ringer acetate solution (20-100 ml) was administered SC and approximately15 ml of recovery food for herbivores (Critical Care®, Oxbow Animal Health, Murdock, USA) was fed by syringe every 6 hours.

Statistical Method and Analysis

For each animal Cmax, Tmax (time to reach Cmax), t1/2 (half-life) and AUC (area under the plasma concentration curve) were calculated. AUC was further calculated for RbtGs scores and subjective pain scores. Due to the small number of animals and data not being normally distributed, Mann-Whitney Rank Sum Test was used for comparisons between groups. A-two-way-repeated-measures analysis of variance was performed on plasma concentration and RbtGs with group and time as factors. Tests were performed in SigmaPlot 11.0 (2008, Systat Software Inc, Hounslow, London, UK). A p-value of <0.05 was considered significant.

RESULTS

One rabbit died due to peracute haemorrhagic E. coli enteritis prior to the start of the study. One rabbit died shortly after intubation following a severe drop in blood pressure and apnoea. Anaesthesia maintenance was otherwise uneventful. One rabbit in the pilot study was given too low dose of buprenorphine OTM to have measurable plasma concentrations and was therefore excluded.

Two rabbits were excluded from the study because they had too few blood samples. This due to one rabbit removed the arterial catheter when the early blood samples were taken. And the other one because it developed neurological signs also early on while blood sampling most likely due to complications caused by the arterial catheter. This left seven rabbits in group SC and eight rabbits in group OTM for buprenorphine analyses. For one of the rabbits in the SC group several photographs for pain scoring were missing. This left six rabbits in the SC group and eight rabbits in the OTM group rabbits for the analyses of pain scores.

Buprenorphine Plasma Concentrations

Plasma concentrations are shown in figure 1.

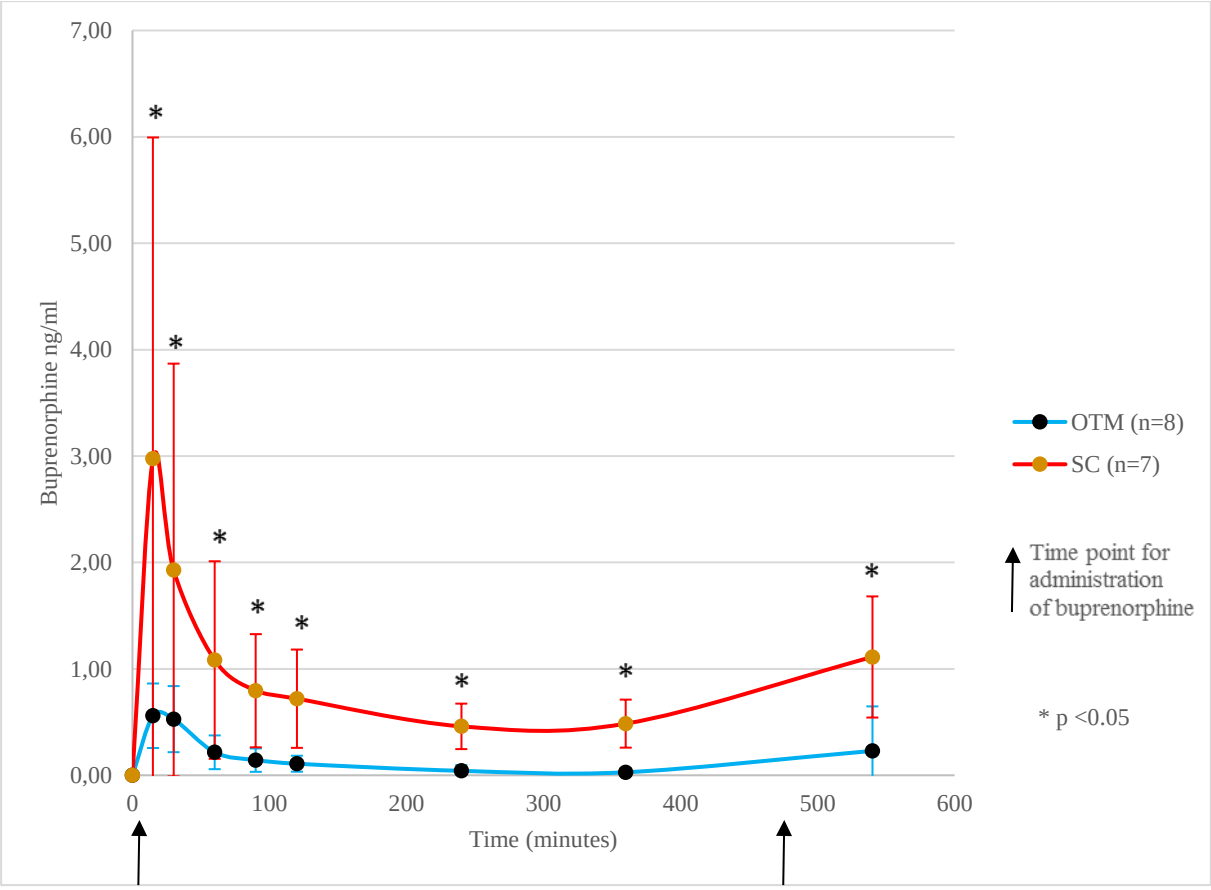

Fig. 1: Mean (SD) buprenorphine plasma concentrations over time in rabbits after administration of 0.15 mg/kg OTM (n=8) or 0.05 mg/kg SC (n=7). Concentrations were measured with ultra-high performance liquid chromatography – tandem mass spectrometry. * p<0.05 between groups (two-way analysis of variance).

Cmax and AUC were higher after SC administration. There were no differences between administration routes for t1/2 and Tmax (Table 1).

	OTM administration			**SC administration**		
	Median	**Minimum**	**Maximum**	**Median**	**Minimum**	**Maximum**
Cmax (ng/ml)	0,74*	0,15	1,05	1,16*	1,01	8,56
Tmax (min)	15	15	30	15	15	45
t1/2 (min)	32	22	139	50	25	231
AUC (ng*h/ml)	41*	5,69	159	337*	72,6	657

Table 1: Pharmacokinetic results after administration of buprenorphine in rabbits either orally transmucosally (OTM, 0.15 mg/kg, n=8) or subcutaneously (SC, 0.05 mg/kg, n=7). Median, maximum and minimum values. *p < 0.05 (Mann-Whitney Rank Sum Test).

Pain Assessment

RbtGs scores were increased in both groups at every time point compared to values before surgery (0), except at the last time point in group OTM (Fig 2). The score was higher in the OTM group at 15 min. The median AUCs did not differ between groups (RbtGs scores: 1028 for SC (n=6) and 1091 for OTM (n=8); subjective pain scores: 735 (n=6) for SC and 540 for OTM (n=8)). RbtGs scores and subjective pain scores were well correlated both after OTM and SC administration. (OTM: ρ= 0.94, SC: ρ= 0.9) (Fig 3-4). There was no correlation between buprenorphine plasma concentrations and RbtGs scores or subjective pain scores.

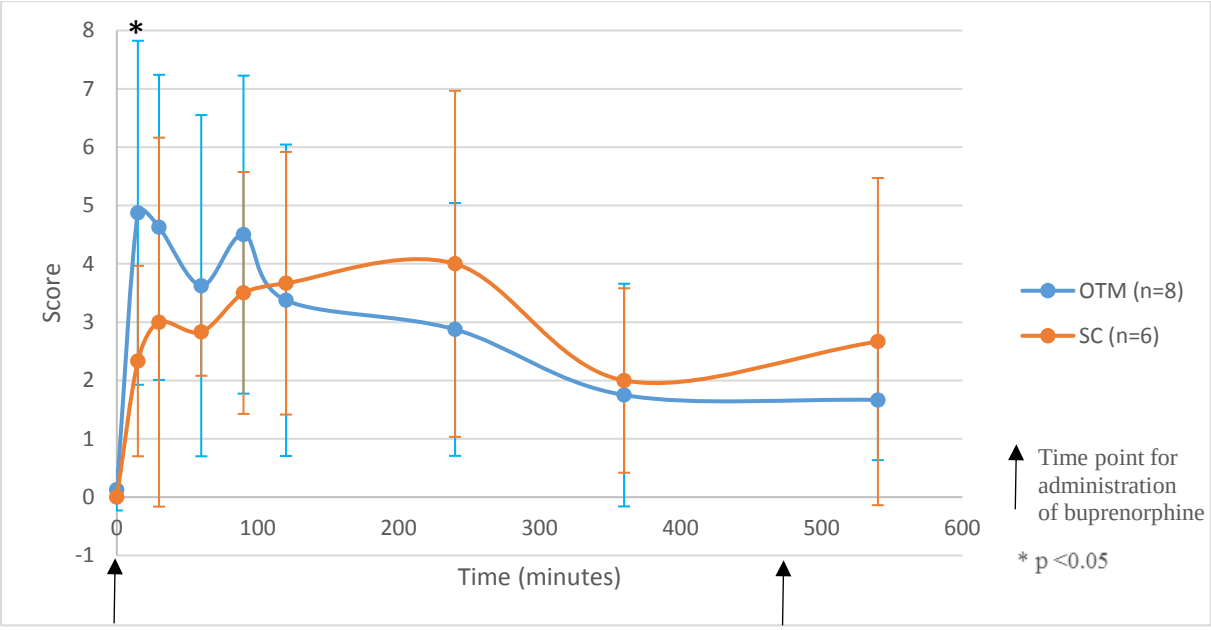

Fig 2: Mean (SD) Rabbit Grimace Scale scores over time in rabbits after administration of 0.15 mg/kg OTM (n=8) or 0.05 mg/kg SC (n=6). *p<0.05 (two way analysis of variance)

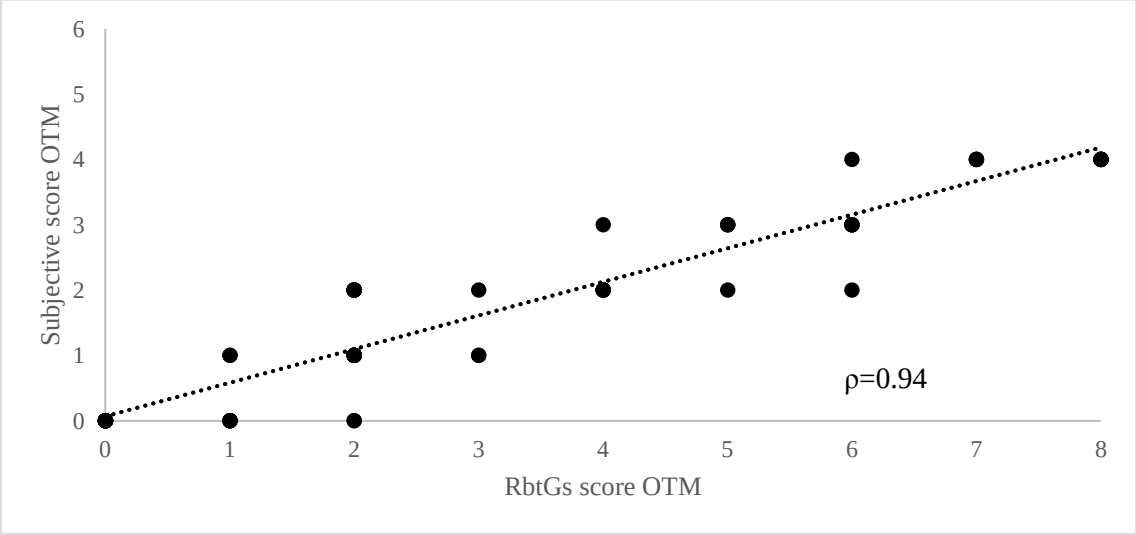

Fig. 3: Correlation between Rabbit Grimace Scale (RbtGs) scores and subjective pain scores in rabbits after administration of 0.15 mg/kg buprenorphine orally transmucosally (OTM, n=8).

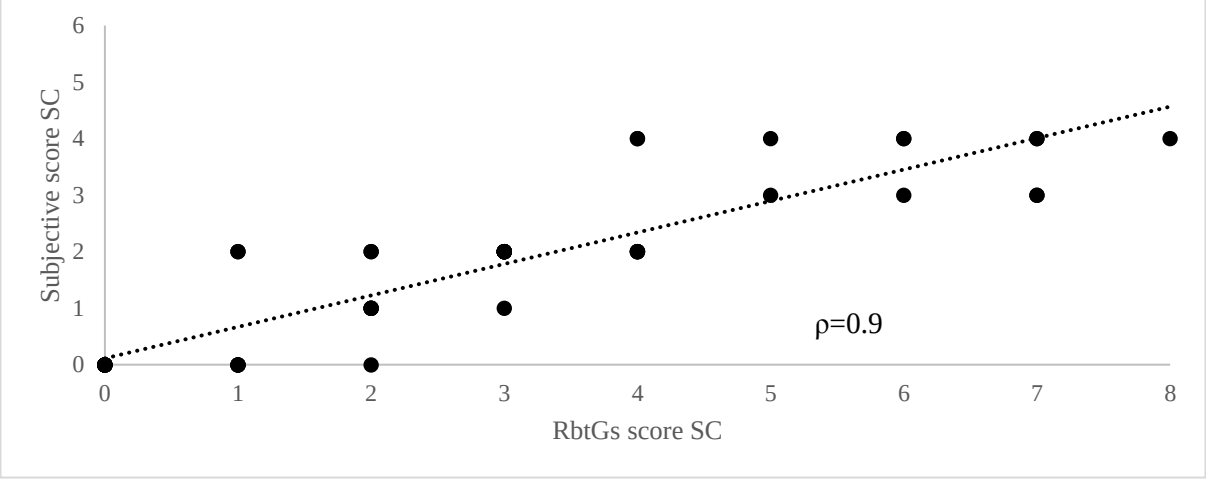

Fig. 4: Correlation between Rabbit Grimace Scale (RbtGs) scores and subjective pain scores in rabbits after administration of 0.05 mg/kg buprenorphine subcutaneously (SC, n=6).

DISCUSSION

OTM Administration

In the literature study there is mixed opinions whether OTM administration of buprenorphine is regarded as a reliable administration route when studying the pharmacokinetics. Hedges *et al.* (2014) showed that measuring drug concentrations in jugular vein blood following buccal administration may give misleading plasma concentrations. This is because the absorbed drug is drained into the jugular vein. Both Robertson *et al.* (2005) and Porters *et al.* (2015) used jugular vein catheters for collection of blood. However, despite equal OTM doses, the results differ regarding the resulting plasma concentrations of buprenorphine. In the study by Porters *et al.* (2015) the mean Cmax was 0.52 ± 0.32 ng/ml and in the study by Robertson *et al.* (2005) the median Cmax (minimum Cmax, maximum Cmax) was 12.5 ng/ml (2.55-19.4 ng/ml).

The analysis methods differed between the two studies. Robertson *et al.* (2005) used a radioimmunoassay and Porters *et al.* (2015) liquid chromatography-tandem spectrometry. In the current study ultra-high performance liquid chromatography – tandem mass spectrometry was the analysis method. Porters *et al.* used a combination of dexmedetomidine and buprenorphine which may have influenced the pharmacokinetic analysis. Robertson *et al.* also assessed pain with thermal threshold testing which gave more information than just buprenorphine plasma concentrations to conclude that OTM administration is as efficient as IV for analgesia.

One challenge in the current study was to administer buprenorphine OTM. The immobilisation of the rabbit may be difficult for owners and there is the risk of buprenorphine being swallowed or pouring out of the mouth because of the large volumes being administered. If swallowed, he drug undergoes first pass metabolism by the liver.

Buprenorphine plasma concentrations

In the current study, the variation in plasma concentrations of buprenorphine was very large after SC administration. This has also been found to be the case in cats, and SC administration was therefore discouraged (Steagall *et al.,* 2013). The variation in plasma concentrations in the current study was smaller after OTM administration. It cannot be excluded that Cmax occurred before the 15 minute time point. The AUC and Cmax were lower after OTM than SC administration, despite the fact that the OTM dose was three times higher.

The poor correlation between buprenorphine plasma concentrations and pain scores shows that plasma concentrations cannot be used to monitor analgesia. The reason is that plasma concentrations do not reflect the concentrations in the tissues where the opioid receptors are located (Ohtani *et al.,* 1994). Since buprenorphine has a slow association/dissociation at the opioid receptor site, the drug can act its antinociceptive properties for long time even if its concentration might not be measureable in plasma.

In the current study the mean (SD) Cmax after OTM administration of 0.15 mg/kg was 0.65 (0.32) ng/ml. In the study by Lindhardt *et al.* (2001) the mean Cmax value after nasal administration of a similar buprenorphine dose was 28 (11) ng/ml for the PEG 300 formulation and 27 (7) ng/ml for the dextrose formulation. Mean Tmax in the study by Lindhardt *et al* were 8 min (PEG 300) and 12 min (dextrose) whereas it was 21 min in the current study. The difference in Cmax may have been influenced by the differences in the starting point of blood sampling; Lindhardt *et al.* started blood sampling earlier, and found that Tmax occurred before 15 min. It can also be that buprenorphine is better and faster absorbed from the nasal mucosa (Lindhardt *et al.,* 2001) than the buccal mucosa, and/or that PEG increased the rate of absorption, resulting in higher plasma concentrations. The buprenorphine solution used in the current study is formulated for injection and contains, apart from

buprenorphine hydrochloride, glucose, water and hydrochloric acid. The bioavailability study by Lindhardt *et al.* showed no significant difference between the formulations.

Pain Scoring

There was no correlation between plasma concentration levels and RbtGs scores or subjective pain scores. Fifteen minutes after administration of buprenorphine a significant higher pain score was found after OTM administration compared to SC. Considering the prolonged onset of analgesia found in other studies, this is unlikely be due to the action of buprenorphine. During the first 90 min pain scores tended to be higher in the OTM group, but the variation is large and from 120 min the pattern was reversed. Since the variation in scores was very large, larger groups of rabbits need to be studied to get conclusive results about possible differences between routes of administration.

There are some weaknesses with the study. For instance pain was not scored before administration of buprenorphine (baseline pain score). There is thus the possibility that the groups differed in pain scores from the start. Further, there was no control group without buprenorphine administration. To include such a group was considered unethical, but may have given more conclusive results. Also, there was only one person that performed the pain assessment and only two of the RbtGs criteria were used. It is possible that the scoring method was too crude to detect small differences in degree of pain. It may also be that the rabbits hid their facial expressions because of stress caused by the photographer. Perhaps direct evaluation by an observer could have made it possible to assess more criteria. Some rabbits were still sedated at the time of scoring, which may have impacted pain and/or scoring. This however likely affected rabbits in both groups similarly.

It would have been valuable to have recorded other pain-associated behaviours like in other studies on buprenorphine's analgesic properties in rabbits (Cooper *et al.*, 2009; Weaver *et al.*, 2010), and to have registered physiological findings related to pain as further tools to evaluate pain additionally or in comparison to the RbtGs scores.

Arterial Catheter Use

Using arterial catheters for continuous blood sampling was a risky event. The benefit was that blood collection was easier to perform, and larger quantities could be collected faster compared to collection by venepuncture. Three rabbits developed neurological symptoms that may have been related to flushing the arterial catheter. The probable cause for the neurological symptoms were emboli of blood clots causing ischemia in the brain. This occurred despite of the catheters being flushed with saline and filled with heparinised NaCl. Some rabbits also removed the catheters themselves. In one rabbits the bleeding was substantial and probably caused the death of the rabbit despite supportive care.

CONCLUSIONS

1. OTM administration of buprenorphine was difficult to perform due to large volumes and the immobilization of the animal.
2. OTM administration of buprenorphine give significantly lower plasma concentrations than SC administration.
3. SC administration of buprenorphine leads to a large variation in plasma concentrations which question the reliable bioavailability.
4. Plasma concentrations do not correlate to estimated pain scores. Plasma concentrations are probably not a good estimate of analgesia.
5. For further clarification, pharmacokinetic studies comparing SC or OTM administration of buprenorphine with IM or IV administration are needed. As well as more dose-effect studies of buprenorphine given to rabbits and different formulations of OTM buprenorphine to improve the bioavailability.

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