Carprofen neither reduces postoperative facial expression scores in rabbits treated with buprenorphine nor alters long term bone formation after maxillary sinus grafting.

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Abstract

In connection with bilateral maxillary sinus augmentation, the acute effects of the nonsteroidal anti-inflammatory drug carprofen on facial expression and long-term effects on bone formation were evaluated in 18 male New Zealand White rabbits. A 10 x 10-mm bone window was drilled in the maxilla, the sinus membrane elevated and a titanium mini-implant inserted. One of two test materials was randomly inserted unilaterally and bovine bone chips (control) on the contralateral side in the created space. Rabbits were randomly allocated to receive buprenorphine plus carprofen (n=9) or buprenorphine plus saline (n=9) postoperatively. Buprenorphine was administered subcutaneously every 6 h for 3 d in a tapered dose (0.05-0.01 mg/kg) and carprofen (5 mg/kg) or saline administered subcutaneously 1 h before, and daily for 4 d postoperatively. To assess pain, clinical examination, body weight recording and scoring of facial expression from photos taken before, and 6-13 h after surgery was performed. Twelve weeks after surgery the rabbits were euthanized and sections of maxillary bones and sinuses were analysed with histomorphometry and by qualitative histology.

Carprofen had no effect on mean facial expression scores, which increased from 0.0 to 3.6 (carprofen) and 4.3 (saline), of a maximum of 8.0. Neither did carprofen have an effect on bone formation or implant incorporation, whereas the test materials had.

In conclusion, treatment with 5 mg/kg carprofen once daily for 5 d did not reduce facial expression scores after maxillary sinus augmentation in buprenorphine treated rabbits and did not affect long term bone formation.

Introduction

Orthopaedic procedures are considered among the most painful in experimental animal research and still only 50 % of orthopaedic studies in rabbits report the use of any analgesia (Coulter et al., 2011). One reason for withholding analgesics may be that pain is difficult to assess in rabbits. Animals of prey are believed to mask pain-related behaviour to increase chances of survival, which may lead to the assumption that they do not experience pain (Johnston and Avian, 2005). Rabbits are for example known to remain motionless in the face of stress and pain. According to the European directive 63/2010, analgesic drugs must be provided to experimental animals in painful procedures (*Directive 2010/63/EU*, n.d.), and to effectively treat pain, it needs to be assessed correctly.

Lately, evaluating facial expression has been shown to be useful for pain assessment in several species such as mice (Langford et al., 2010), rats (Sotocinal et al., 2011), horses (Dalla Costa et al., 2014; Gleerup et al., 2014), and rabbits (Keating et al., 2012). For assessment of pain in rabbits, facial expression scoring was validated for intense nociceptive stimulation (Keating et al., 2012). To our knowledge, there are no reports on the use of facial expression for the assessment of postoperative pain in rabbits.

Another reason for withholding analgesics to rabbits in orthopaedic research is the fear of interaction with bone healing (Coulter et al., 2011). The effect of non-steroidal anti-inflammatory drugs (NSAIDs) on bone healing has long been debated (Dodwell et al., 2010; Pountos et al., 2012) and the recommendation for their use in human beings varies between orthopaedic clinics. According to a recent meta analysis of bone healing in humans there is little evidence of an increased risk of non-union by the use of NSAIDs (Dodwell et al., 2010). Other studies report conflicting reults of NSAID on bone formation in humans (see Konstantinidis et al., 2013). Some authors recommend not using NSAIDs in animals in which delayed bone healing can be suspected (Richardson, 2011), but there is insufficient

evidence to generally support the withholding of NSAIDs after orthopaedic surgery (Dimmen et al., 2009; Krischak et al., 2007). There is evidence from experimental studies in rabbits and rodents, that most NSAIDs have the potential to inhibit bone healing in the short-term perspective, depending on the timing, dose and duration (Endo et al., 2005; Gerstenfeld et al., 2003; Simon et al., 2002). The inhibitory effects seem to be the greatest during the early phase of bone healing, but once administration is discontinued, compensatory mechanisms lead to normal healing (Gerstenfeld et al., 2007; Nyangoga et al., 2010).

The rabbit is the most commonly used animal species in orthopaedic research (as reviewed by Pearce et al., 2007; Stübinger and Dard, 2013), with advantages of low cost, ease of handling and early skeletal maturity. Rabbits are suitable for screening of material implants in bone before further evaluation is undertaken in other large animals (International standards, ISO 10993-2, 2006). Another area of research is the development of new materials for the replacement of bone. In humans with maxillary atrophy a widely used technique in connection with dental replacement is maxillary sinus augmentation (Stübinger and Dard, 2013). With this technique, a new compartment is created between the floor of the maxillary sinus and the elevated sinus membrane, and new bone is allowed to be formed (Sohn et al., 2010). This is needed for fixation of titan implants in the maxilla when bone is scarce. In rabbits, sinus augmentation can be modelled for the evaluation of new materials that can replace autologous bone (Kim et al., 2012).

The aim of the present study was to evaluate if the perioperative treatment with the NSAID carprofen 1) reduces facial expression scores in the immediate post -operative phase in buprenorphine treated rabbits and 2) interferes with long term formation of new bone in a model of maxillary sinus augmentation. The hypotheses were that carprofen would reduce facial expression scores and not interfere with long term bone formation.

For ethical reasons, all rabbits received buprenorphine for treatment of postoperative pain. In order to enclose a complete bone remodelling cycle, bone formation was evaluated after 12 weeks (Bodde et al., 2007).

Materials and methods:

The experiment was approved by the local ethics committee for animal experiments in Uppsala (C 70/13).

Experimental design

The rabbits were randomized to receive either buprenorphine and carprofen (Group Bup+Carp, n=9) or buprenorphine and saline (Group Bup+Sal, n=9). Carprofen, at a dose of 5 mg/kg (Norocarp vet, 50 mg/ml, N-Vet, Uppsala, Sweden), or the equivalent volume of saline, was administered subcutaneously (s.c.) 40 min before surgery and daily for 4 d after surgery. All rabbits received buprenorphine (Temgesic®, 0.3 mg/ml, RB Pharmaceuticals, Slough, Berkshire, UK) after completion of surgery at a dose of 0.03 mg/kg intravenously (i.v) and 0.02 mg/kg s.c and repeated s.c. at 0.05 mg/kg after 6 h, at 0.03 mg/kg between 12 and 24 h, at 0.02 mg/kg between 36 and 48 h, and at 0.01 mg/kg between 60 and 72 h postoperatively. The first dose of buprenorphine was partly administered i.v. to antagonise the effects of the anaestesic agent sufentanil, and thereby increase the rate of recovery.

The rabbits were randomized to receive one of two bone augmentation materials (A and B) within each analgesia group for their potential as substitute for autologous bone and their bone induction properties. One material was a new granular composition of calcium phosphates (OssDsign, Uppsala, Sweden; Engstrand et al., 2014) and the other material a bisphosphonate linked hyaluronic acid hydrogel (BHA; Hulsart-Billström et al., 2013). The materials (n=9+9) were placed in the maxillary sinus on a random side, and on the contralateral side standard bovine-derived hydroxyapatite chips (Bio-Oss®, Geistlich Pharma

AG, Wolhusen, Switzerland; Mordenfeld et al., 2010) were placed as control (n=18). See table 1.

Animals and housing

Eighteen male New Zealand White (NZW) rabbits from a specific-pathogen-free colony (Lidköpings Kaninfarm, Lidköping, Sweden) were used. The breeding colony was free from known rabbit pathogens, as according to health monitoring reports (Mähler Convenor et al., 2014). At the time of surgery, the rabbits were 36 ± 4 weeks old and weighed 3.7 ± 0.2 kg (mean ± SD). The rabbits were housed individually in cages with a floor area of 0.42 m^2, and were equipped with a shelf and a covered area. Standard pelleted rabbit diet (Lactamin K3, Lantmännen, Stockholm, Sweden) and autoclaved hay was fed to the rabbits, which had access to autoclaved straw for bedding and water in bottles. The light – dark cycle was 12:12 h with lights on at 07:00. Room temperature was 18 ± 3° C and humidity 55 + 10 %.
The animals were acclimatized for two weeks and accustomed to handling. On the day before surgery, the rabbits were clinically examined (gereral appearance and behaviour, heart and lung auscultation, body orifices inspected) and 2 ml blood was collected from the ear artery after topical treatment with a local anaesthetic cream (EMLA®, AstraZeneca, Södertälje, Sweden). The acute phase protein serum amyloid A (SAA) was analysed by enzyme-linked immunosorbent assay (Tridelta Development Ltd, Maynooth, Ireland) as a marker of inflammation. The rabbits were not fasted at any time point.

Preparations for surgery

On the day of surgery, the rabbits were administered 2 mg/kg of medetomidine (Domitor® Orion Pharma Animal Health, Sollentuna, Sweden) s.c. and local anaesthetic cream (EMLA®) on the skin over the ear veins and arteries. After 30 min, the rabbits were moved to the surgery preparation room and administered 5 mg/kg of ceftiofur (Exenel®, Orion Pharma AB,

Animal Health, Sollentuna, Sweden) s.c. Catheters (Venflon™, BD AB Stockholm, Sweden) were placed in one ear artery (20 G) and vein (22 G). Anaesthesia was induced by continuous infusion of 4.5 µg/kg/h sufentanil (Sufenta®, Jansen-Cilag, Sollentuna, Sweden) and 0.9 mg/kg/h midazolam (Midazolam Actavis, Actavis AB, Stockholm, Sweden) and maintained with continuous intravenous infusion of sufentanil and midazolam according to Hedenqvist et al. (2013). After induction, a larynx mask (V-gel, large, Docsinnovent, London, UK) was introduced into the oral cavity, and connected to the anaesthesia machine (Anmedic Q-Circle System; Anmedic AB, Stockholm, Sweden) via a paediatric breathing system (Intersurgical Ltd, Wokingham, UK). Oxygen was administered at a rate of 1.5 L/min. To maintain an end tidal CO_2 of 5–7 kPa, the lungs were ventilated with a respiratory frequency of 10–20/min, a maximum peak inspiratory pressure of 20 cm H_2O and an inspiration: expiration ratio of 1:3 with a ventilator (Anmedic vent JB1; Anmedic AB, Stockholm, Sweden). After skin preparation of the incision areas, 4.5 mg (0.9 ml) of prilocain (Citanest®, 5 mg/ml, AstraZeneca, Sweden) was administered s.c. per side.

Surgical procedure

Surgery was performed by a skilled, experienced maxillofacial surgeon (ATh). A bilateral maxillary sinus augmentation was performed by incising the skin over the maxilla a few mm above the ventral border of the incisive bone and the maxilla. The muscles and periosteum were exposed and elevated. A bone window (10 x 10 mm) was created by piezo-electric surgery (Mectron s.p.a., Carasco, Italy), the sinus membrane elevated and a miniature dental titanium implant (2.2 x 6 mm, custom made, Enge Mikroteknik, Vittsjö, Sweden) inserted in the edentulous alveolar ridge. The newly formed sinus space in the bone was augmented with test or control material. A 25 x 25 mm collagen membrane (Geistlich Bio-Gide®, Geistlich Pharma AG, Wohlhausen, Switzerland) was applied over the grafted site to keep the material in place. The soft tissue was relocated and sutured with Monocryl sutures 5-0 (Ethicon,

Johnsons & Johnson AB, Sollentuna, Sweden) in two layers (muscles and skin). The surgery lasted for 60-90 min.

Monitoring and postoperative care

During anaesthesia end-tidal CO_2, heart rate, respiratory rate, rectal temperature and hemoglobin oxygen saturation (SpO_2, probe on tail) were monitored continuously (CS/ 3; Datex-Engstrom, Datex Ohmeda, Bromma, Sweden). After completion of surgery, the anaesthesia infusion was discontinued and atipamezole, 1 mg/kg s.c., (Antisedan® vet., Orion Pharma Animal Health, Sollentuna, Sweden) and flumazenil, 0.1 mg i.v. (Flumazenil Actavis, Stockholm, Sweden), were administered. Postoperatively, body temperature, respiration rate, heart rate and SpO_2 were recorded every 15 minutes for 3 h, after which rabbits were returned to their home cage. Ceftiofur (5 mg/kg) was administered s.c.once daily for 4 d postoperatively. Rabbits were weighed daily for one week after surgery and thereafter weekly.

Pain assessment

For facial expression scoring, photographs (Panasonic Lumix DMC-T27, Osaka, Japan) were taken of the rabbit's face from the front and from the side in the home cage, before surgery and at 6, 7, 12 and 13 h postoperatively. The 6 and 12 h photographs were taken immediately before administration of buprenorphine. A total of 180 pictures were taken, randomly numbered, and scored by five observers which were blinded to treatment and time point. The observers were two of the authors (ATr, MJW), two veterinary students (GH, MK) and one non-veterinary student (LJW), which were instructed by reading descriptions and viewing examples pictures from the publication on the Rabbit Grimace Scale by Keating et al. (2012). The scale comprises 5 so called Facial Action Units (FAUs); orbital tightening, ear poition, cheek flattening, pointed nose and whisker change, each evaluated on a scale from 0-2 (0=no change, 1= moderate change, 2=obvious change from normal). Additionally, pictures were

scored subjectively for the presence of pain, ranging from 0 (no pain) to 3 (severe pain).

Postmortem sampling

Three months after surgery, the rabbits were euthanized i.v. with pentobarbital (Allfatal® vet., Omnidea AB, Stockholm, Sweden). The heads were immediately removed and examined by cone beam computer tomography (3D Accuitomo 170 J.Morita, Irvine, CA, USA), to guide the exact excision of the maxillary bone blocks (Fig 2). The parts of the maxilla containing the implants were then removed using a band saw (KT-460, Klaukkala, Finland) .

Sample processing

In brief, the bone tissue samples were immersed in 10 % neutral-buffered formalin fixative followed by rinsing in tap water. The samples were dehydrated in increasing concentrations (70% - 100%) of ethanol, preinfiltrated in diluted resins prior to infiltration in pure resin and finally embedded in pure resin and allowed to polymerize under UV light. Undecalcified cut and ground sections were prepared according to the method described by (Donath and Breuner, 1982) using the ExaktR equipment (Exakt Apparatebau, Norderstedt, Germany). The cured samples were divided in the water-cooled band-saw and the surface of each bloc was ground parallel prior to be glued onto a supporting plexi-glass. An initial thick section was prepared which then was ground (using SiC wet grinding papers starting with 800 grit up to 1200 grit papers) in a water-cooled grinding equipment. No final polishing was used. The sections were prepared to have a final thickness of about 15µm and finally the sections were routinely stained in a mixture of toluidine blue and pyronin G (Johansson and Morberg, 1995)

Histology evaluation

Qualitative histology was examined by light microscopy (Nikon eclipse E600) by two veterinary pathologists (CL, SE) who were blinded to treatments (material A/B/BHA and

carprofen/saline). After scoring individually, the two pathologists agreed on a consensus scoring. One section per animal from each side (material A/B/BHA) was examined for degree of implant incorporation in bone (good, satisfactory or poor) and test material association with bone (yes or no) (Table 2). The degree of implant incorporation was evaluated according to the following criteria: amount of bone surrounding the implant, amount of grooves containing bone and amount of direct contact between bone and implant. If all three parameters were fulfilled; i.e. the implant was surrounded by moderate or abundant bone, and moderately-extensively incorporated in regard to numbers of grooves filled with bone and presence of direct bone-implant contact, the incorporation was scored as good and if only one or two of the criteria were adequate the incorporation was satisfactory, otherwise poor. When the test material showed a moderate or major association with bone it was graded yes (Table 2). No association with bone or a minor focal area of bone adjacent to the test material was considered no.

The sections were also designed as adequate when: all components could be assessed or where artefacts were present but bone formation still could be assessed.

For quantitative analysis histomorphometry was performed (Nikon E600 microscope, Nikon DXM 1200 digital camera, software: NIS-elements Basic Research, Nikon, Tokyo, Japan) The new bone within the grafted material and the implant incorporation were measured by one of the authors (ATr) who was blinded to treatment with carprofen but not to the materials. The new bone was differentiated from the existing "old" bone in the specimens by slightly lighter coloration in staining. The old bone had a more matured apparence (sharper defined lamellae) and contained less osteocytes. Because of the fact that the sinus membrane was elevated by the micro-implant, old and mature bone could only be expected to be found in the cervical part of the implant where the mechanical retention of the implant primarily took place, meaning bone at the first two-three implant threads. New bone attached to the bovine

bone particles could also easily be detected in a similar way as seen in Fig 3. For evaluation, two regions of interest (ROI) were defined: 1) A free size region of the observed test or control material area in 1 x magnification ("free ROI") and 2) A defined rectangular sized area of interest (3.5 x 2.5 mm), with augmented material in close approximation to the implant apex, in 4 x magnification ("rectangular ROI"). The implant incorporation was evaluated by measuring the bone formation in an area between six implant threads located marginally and on both sides of the implant ("implant ROI"). The amount of new bone was marked, measured and expressed as a percentage of the total ROI area. For example areas see Fig 3A-C.

Statistical analyses

Data were analysed for normality and homogeneity of variance. Body weight, subjective pain scores and facial expression scores were compared with two-way repeated measures ANOVA, with group and time point as factors. For comparison between time points, a posthoc Bonferroni t-test was performed (all pair wise multiple comparison).

For the facial expression and subjective pain evaluation respectively, scores were calculated for each animal, time point and observer, by adding the scores from two pictures. From the scores from all observers means and standard deviations were calculated for the ANOVA.

SAA levels were compared with repeated measures ANOVA on ranks within each group and with rank sum test between groups at all three time points. The area under the curve (AUC) for facial expression scores over time were compared with t-test between analgesia groups.

Qualitative histology scoring was evaluated using Fisher's exact test on the distribution of scores in Group Bup+Carp and Group Bup+Sal in the sinus treated with BHA (control).

Morphometric results of bone formation in the sinus treated with BHA were compared by independent t-tests between groups with and without carprofen. The effect of carprofen on bone formation on the side with test material was evaluated by a two-way ANOVA, with

material A/B and carprofen/saline as factors. The new bone amount measured in the ROIs were compared between materials A and B by t-test, and between the materials and the controls by paired t-test. A p-value <0.05 was considered significant.

Results

Clinical parameters

Anaesthesia, surgery and recovery from the procedure were uneventful. Mean duration of anaesthesia was 118 ± 14 min and time to recovery of the righting reflex after discontinuation of anaesthesia was 15 ± 6 min. Mean doses of sufentanil and midazolam during surgery were 3.8 µg/kg/h and 0.76 mg/kg/h, respectively.

Preoperative SAA values were below detection level (<4.7 ng/ml) in all but two rabbits (6.4 and 7.6 ng/ml). Three days postoperatively, the median levels were increased to 37 ng/ml (range 12-182 ng/ml) in Group Bup+Carp, and 51 ng/ml (range 23-199 ng/ml) in Group Bup+Sal, with no difference between groups. By d 21 the levels were similar to preoperative levels (Fig 4).

All rabbits resumed eating within 12 h without handfeeding. One of the first rabbits that underwent surgery was reluctant to drink from the water bottle, and therefore water bowls were placed in all cages. Mean body weight loss was 0.28 ± 0.13 kg (8%) in Group Bup+Carp and 0.24 ± 0.26 kg (7%) in Group Bup+Sal and had returned to baseline on postoperative day 21. Body weights did not differ between groups on any day (Fig 5).

Facial expression and subjective pain scores.

Of the FAUs, nose pointing or cheek flattening were not detected at any time point in the present study and whisker position was found difficult to assess, thus these FAUs were excluded from the statistical evaluation. Orbital tightening and ear position (eye and ear

scores) were easy to assess and thus, the maximum score for each image was 4 and at each time point 8. For examples of FAU scores see Fig 1. The maximum subjective score for each animal at each time point was 6.

Eye and ear scores as well as subjective pain scores were increased in both groups 6 and 7 h after surgery, and in Group Bup+Carp the scores were still increased after 12 and 13 h. Fig 6 shows eye and ear scores. The AUC of the eye and ear score over time did not differ between groups (31 ± 8 and 31 ± 15). Mean subjective scores increased from 0.0 to 2.6 ± 0.6 in Group Bup+Sal and to 2.3 ± 0.2 in Group Bup+Carp.

There was no difference between groups at any time point, or between scores before and after buprenorphine administration in either group. The same results were found when ear position and orbital tightening were evaluated individually (data not shown).

Bone formation

There was neither an effect of carprofen on new bone formation on the side treated with the control material BHA (Table 2 and Fig 7A), nor on the side treated with test material A/B (Fig 7B). There was however a difference for all three ROIs in the amount of new bone formation between material A and B, but not between the materials and their controls (Fig 7C).

Discussion

The use of NSAID in orthopaedic research is controversial. The current study was conducted to evaluate the potential of carprofen to provide additional postoperative analgesia in buprenorphine treated rabbits as well as its effect on bone healing in a sinus lift model. Facial expression scoring (Keating et al., 2012) revealed that the FAU scores for orbital tightening and ear position were increased, suggesting that they can be useful for assessing postoperative pain in rabbits. There was however no difference beween rabbits that received carprofen and

those that did not, suggesting that either the scoring method was not very sensitive, that the carprofen could not add any additional analgesia, or that the dose was too low. Further there was no difference between scores before and 1h after buprenorphine administration, indicating that either the dose of buprenorphine was inadequate, the time point for evaluation too soon, or that there was residual analgesia from the previous administration of buprenorphine. Buprenorphine is slowly concentrated in deep tissue department (Andaluz et al., 2009) and the peak analgesic effect in our rabbits may not have been reached after 1 h. Analgesiometric tests in NZW rabbits have shown that the peak effect after s.c. injection of 0.03 mg/kg occurs after 3-4 h (Flecknell and Liles, 1990). The duration of action is known to be long, due to the high affinity to the opioid μ-receptor and slow dissociation (Roughan and Flecknell, 2002). In rabbits, re-administration is recommended after 8-12 h (Carpenter, 2005), yet in the current study, administration was repeated every 6 h. A control group without analgesic treatment would be helpful in determining the efficacy of treatment, but is considered unethical. Another way would be to examine if there is a dose-effect realationship. However, buprenorphine has been shown to have an analgesic effect in rabbits in doses lower than that used in the present study (Flecknell and Liles, 1990; Wootton et al., 1988) and likewise carprofen after ovariohysterectomy in rabbits in the same dose as in the present study (Flecknell et al., 2004). The dose of carprofen in the present study was higher than the recommended dose interval: 2-4 mg/kg (Carpenter, 2005; Ramsey, 2008; Varga, 2014). With a group of rabbits receiving only carprofen, a comparison of the efficacy between carprofen and buprenorphine would be possible. At 12 and 13 hours after surgery, the ear and eye scores were still increased only in Group Bup+Carp. This could be explained by an opioid induced hypersensitivity, which however is typically regarded as a hyperalgesic state and not necessarily an increase in spontaneous pain (Wala and Holtman, 2011).

Subjective scoring was as effective as facial expression scoring in the current study, which may be due to the fact that they were performed simultaneously and thus liable to bias by the observer. Another possibility could be that the observers were experienced in observing animals in painful conditions. Scoring postoperative pain by evaluating facial expression has been described in other species. In mice, facial expression scores were reduced by meloxicam treatment after vasectomy (Leach et al., 2012). In rabbits on the other hand, it has been suggested that it may be more effective not looking at the face for postoperative pain assessment, although that is what people tend to do (Leach et al., 2011). In a study of pain related behaviour in rabbits it was further suggested that the increased duration of orbital tightening after abdominal surgery was due to sedation by residual isoflurane anaesthesia, and it was therefore disregarded as a sign of pain (Farnworth et al., 2011). In the present study, the rabbits were anesthetised by intravenous infusion, and antidotes were administered for rapid recovery. From a pilot study it was further estimated that anaesthesia was completely worn off after 6 h, the first time point for facial expression scoring. The benefit of using facial expression for assessment seems to be that it is not affected by opioid treatment *per se*, which has been shown in naïve mice (Langford et al., 2010) and rabbits (Keating et al., 2012). Moreover, pain facial expression seems to be related to the affectionate part of pain, which is indicated in studies in mice by the destruction of the cingulate cortex (CC), indicated to be the site of the affective part of pain. After destruction of the CC in mice with inflammatory conditions, facial expression scores are reduced whereas behaviours such as writhing are unaffected (Langford et al., 2010).

A previously developed behaviour based pain scoring system for assessing postoperative pain in rabbits, which does not take FAUs in account, is much more time consuming and therefore of little practical use (Leach et al., 2009). Moreover it uses behaviours that are specific to abdominal surgery, restricting its use even further. Facial expression scoring on the other

hand, can be performed quickly and easily, and may be useful for pain assessment after different types of surgical procedures. Though simple to perfom, facial expression scoring also needs to be sensitive enough to distinguish different degrees of pain to be of value.

In the present study, scoring was performed by assessing only two FAUs; orbital tightening and ear position. These are easily and rapidly evaluated even by inexperienced persons.

The other FAUs described by Keating et al. (2012); flattening of the cheeks, pointy nose and a change in whisker position, were not distinguishable in the present study. This could be due to inhibition of grimacing by the localization of the pain or because these FAUs are not expressed with postoperative pain which is of another character and connected with activation of c-fibers unlike acute physiological pain that is elicited by activation in A-delta-fibers (National Research Council, 2009).

The intensity of pain was not expected to be severe, since sinus lift is a rather small surgical procedure which only causes mild pain in humans (Block and Kent, 1989; Pal et al., 2012). With the exception of tonsillectomy, ear-nose-throat surgery, comparable to the surgery in the present study, is known to cause relatively little pain in humans (Gerbershagen et al., 2013). Moreover, the surgeries in the present study were performed by a skilled surgeon (ATh), causing a minimum of trauma. The perioperative analgesic treatment consisted of local anaesthesia infiltration, opioid-based anaesthesia and immediate postoperative buprenorphine administration which may have prevented the development of excessive hyperalgesia and facilitated the control of postoperative pain. The highest mean score reached was 4.0 out of a maximum of 8.0, which could be interpreted as moderate level of pain. Considering the multimodal analgesic approach, the scores are relatively high and the possibiltiy that the pain was undermanaged cannot be excluded.

The levels of the acute phase protein SAA, which is a valuable inflammatory marker, did not differ between groups. This may be an indication that the dose of carprofen was suboptimal.

In a study of piglets undergoing castration, SAA levels were lower in piglets that received meloxicam in comparison to controls (Hansson et al., 2011). The levels in the current study were similar to those in a study on NZW rabbits undergoing knee cartilage surgery (Aulin et al., 2013).

The evaluation of carprofen treatment on bone formation showed that there was no difference compared to controls after three months. All installed micro-implants integrated well into the bone. The incorporation of implants into bone as well as the amount of new bone created in proximity to the materials were found to be equal regardless of treatment with carprofen. The mechanism of inhibition on bone formation by NSAIDs has not been fully explained, but the predominant theory is that the reduced production of prostaglandins causes interference with cell signalling during the inflammatory phase of bone healing (as reviewed by Barry, 2010). Studies in knock-out mice have shown that cyclooxygenase (COX)-2 is essential for endochondral and intramembranous bone formation (Zhang et al., 2002). Carprofen is believed to be a weak peripheral inhibitor of COX iso-enzymes (McKellar et al., 1994) and other mechanisms by which carprofen can act anti-inflammatory have been identified *in vitro*, such as modification of interleukin (IL)-1 and IL-6 release and inhibition of reactive oxygen species (Armstrong and Lees, 2002).

In summary, obital tightening and ear position scores were increased after maxillary sinus surgery in rabbits. Perioperative analgesic treatment with 5 mg/kg carprofen for 5 d, in addition to buprenorphine treatment, did not reduce the scores and did not interfere with long term bone formation in this sinus lift model.

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Table 1: Exprerimental design

Eighteen male NZW rabbits were randomised to analgesia Group Bup+Carp or Group Bup+Sal. Within each analgesia treatment group, the rabbits were randomised to sinus augmentation subgroups with either test material A (granular composition of calcium phosphates) or B (hyaluronic acid hydrogel) on one side of the skull. All rabbits were augmented with standard bovine-derived hydroxyapatite chips (BHA) as control on the contra lateral side.

Table 2: Subjective evaluation of bone formation

Qualitative evaluation of the maxillary sinus augmented with standard bovine-derived hydroxyapatite chips for control (BHA). Fisher's exact test was performed between analgesia Group Bup+Carp and Group Bup+Sal. For the comparison, the sections that were scored good or intermediate for incorporation of implant in bone were pooled.

Fig 1. Examples of pictures with different scores for facial action units ear position (0-2), and orbital tightening (0-2). A: Photo taken before maxillary sinus surgery and augmentation (ear position 0, orbital tightening 0). B-D: Photos taken after surgery (note the location of the incision). B: ear position 2, orbital tightening 2. C: ear 1, eye 2. D: ear 2, eye 0.

Fig 2. Scans of a rabbit head with cone beam computer tomography showing the location of the titanium mini-implants (black arrow) and the augmentation material (white arrow) in the maxillary sinus. A: Lateral exposure. B: ventral exposure.

Fig 3. Photographs of three region of interests that were defined for the objective evaluation of new bone formation (red colour) after sinus lift and augmentation in NZW rabbits. The figures all show the control side with bovine bone chips (purple colour) in the area of the titanium implant (black colour). Old bone surrounds the upper part of the implant. The size of the implant is 2.2 x 6 mm. Note how new bone has been created around the bovine bone chips (B). A: a free region of interest placed in the area of material deposit. B: a defined rectangular region of interest (3.5 x 2.5 mm) in 4 x enlargement placed in the area of material deposit. C. Implant region of interest in the area of the 6 most marginal located threads on both sides of the implant.

Fig 4. Serum levels of amyloid A in NZW rabbits before and 3, 21 and 94 d after maxillary sinus lift and augmentation in Group Buprenorhine + Carprofen and Group Buprenorphine + Saline. Whiskers represent minimum and maximum. Repeated measures ANOVA on ranks, *$p<0.05$.

Fig 5. Mean (SD) body weight in NZW rabbits before and after maxillary sinus surgery and augmentation. A two-way repeated measures ANOVA (posthoc Bonferroni t-test) showed that there were no differences between Group Buprenorhine + Carprofen and Group Buprenorphine + Saline at any time point. Rabbits in both groups lost weight during the first week: *$p<0.05$ in comparison with body weight before surgery.

Fig 6. Mean (SD) facial expression scores based on ear position (0-2) and orbital tightening (0-2) in NZW Rabbits before and after maxillary sinus lift and augmentation. Rabbits received either Group Buprenorhine + Carprofen and Group Buprenorphine + Saline. Buprenorphine was administered immediately after the 6 h and 7 h time points. * $P<0.05$, † $P<0.05$ for comparison over time within each group. Two-way repeated measures ANOVA, posthoc Bonferroni t-test.

Fig 7. Mean amount of new bone measured with morphometry and expressed as a percentage of the total region of interest in NZW Rabbits after maxillary sinus lift and augmentation. Comparison between groups with Mann-Whitney Rank Sum Test (*$p<0.05$, *** $p<0.001$). 7A: No difference between Group Buprenorphine + Carprofen and Group Buprenorphine + Saline in bone amount on the control side, which was augmented with bovine bone chips (BHA).

7B: No difference between between Group Buprenorphine + Carprofen and Group Buprenorphine + Saline in bone amount on the side that was augmented with material A or B.

7C: Difference in bone amount between material A and B in all three measurements. T-test, (*$p<0.05$, *** $p<0.001$).

Fig. 1A

Fig. 1B

Fig. 1C

Fig. 1D

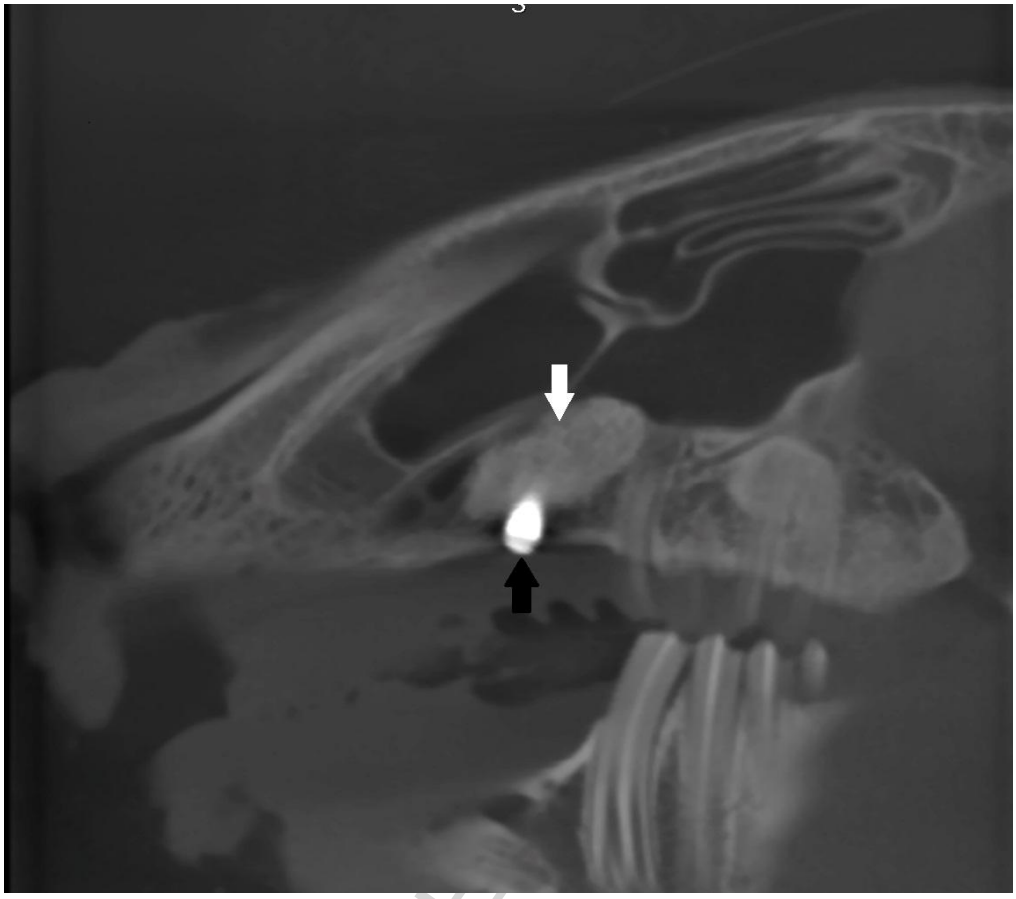


Fig. 2A

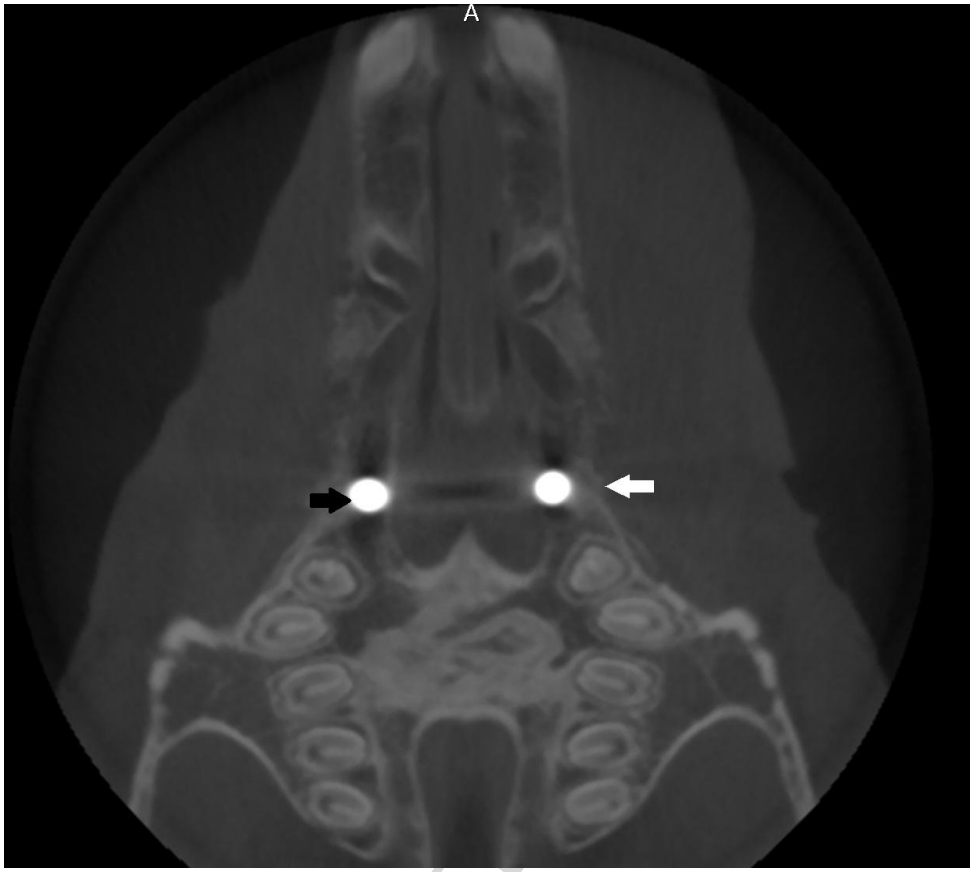


Fig. 2B

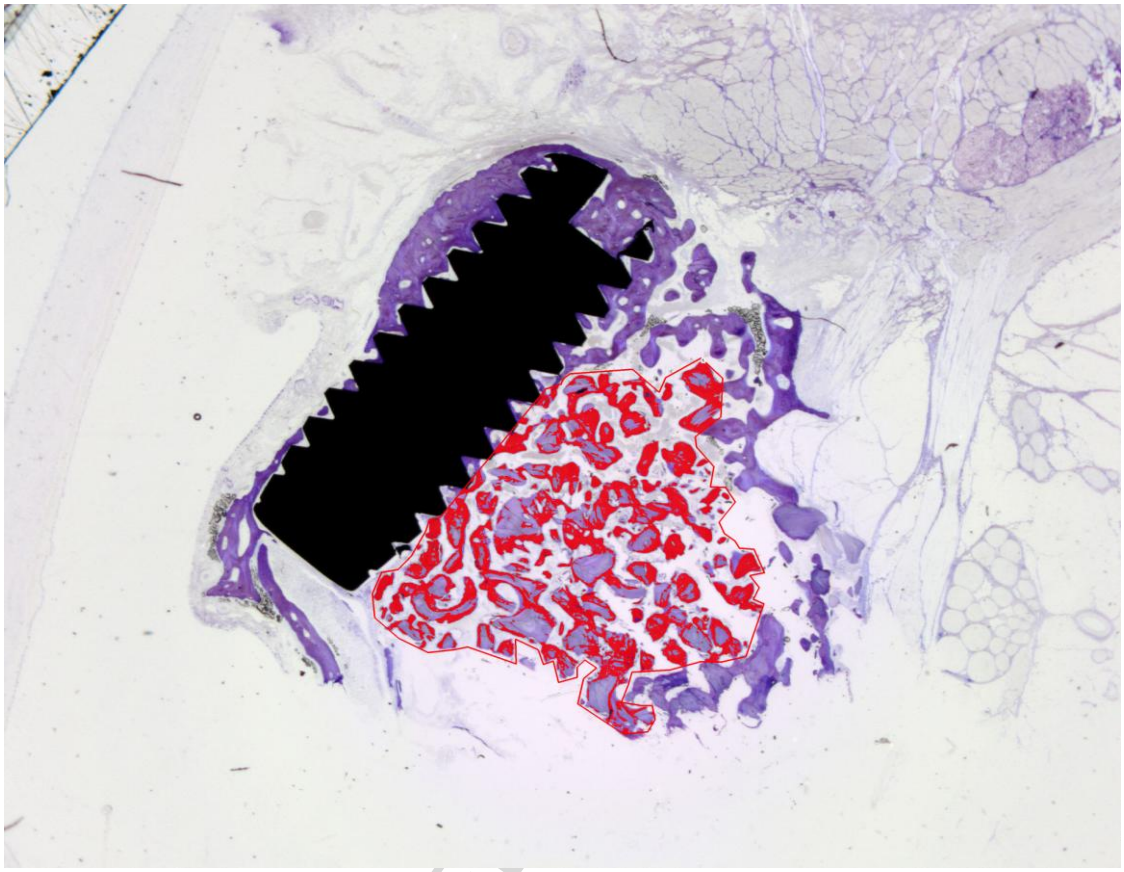


Fig. 3A

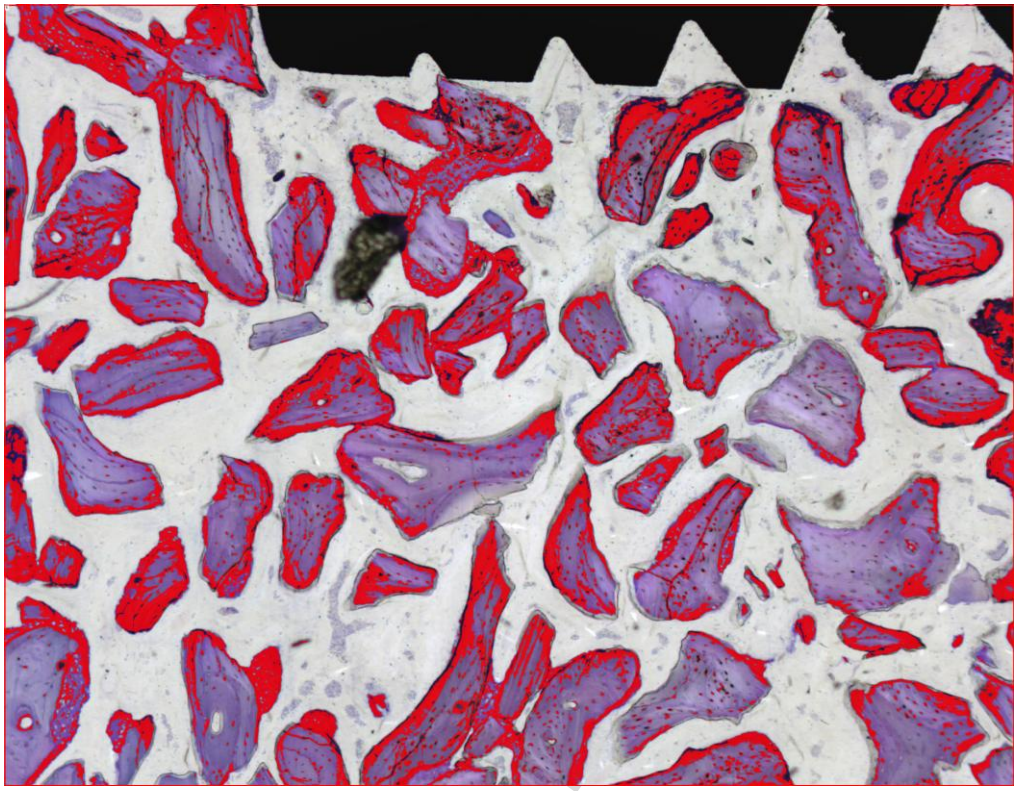


Fig. 3B

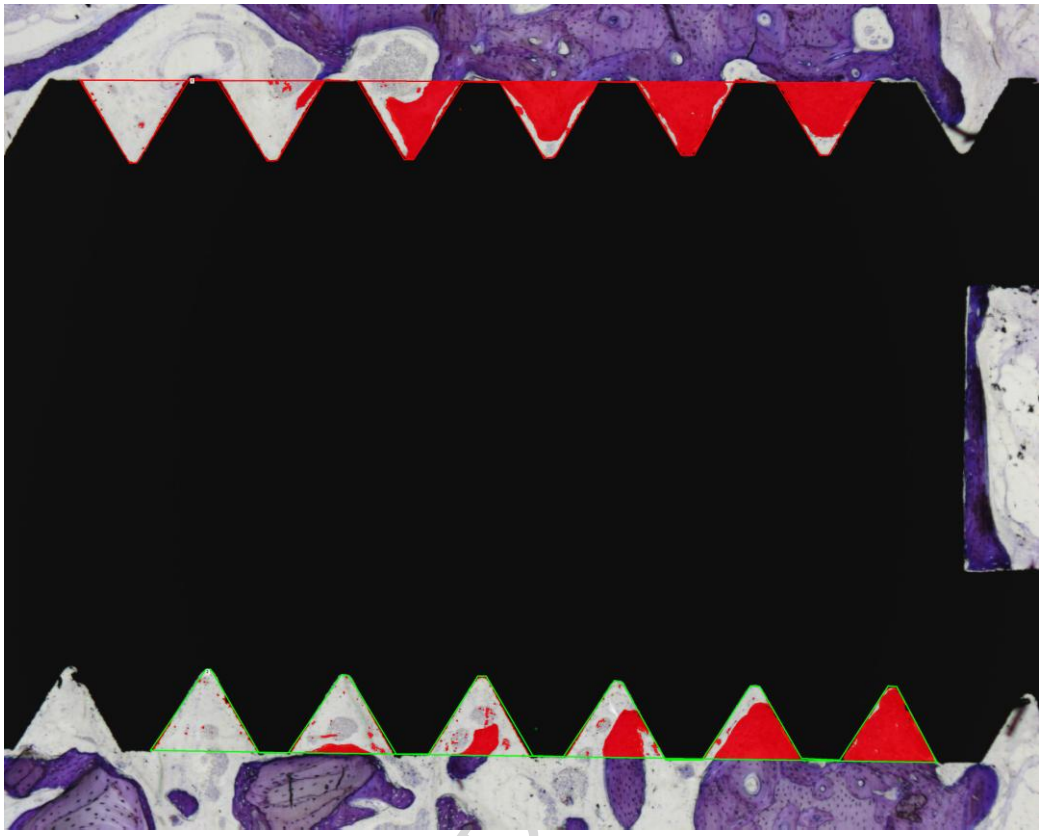


Fig. 3C

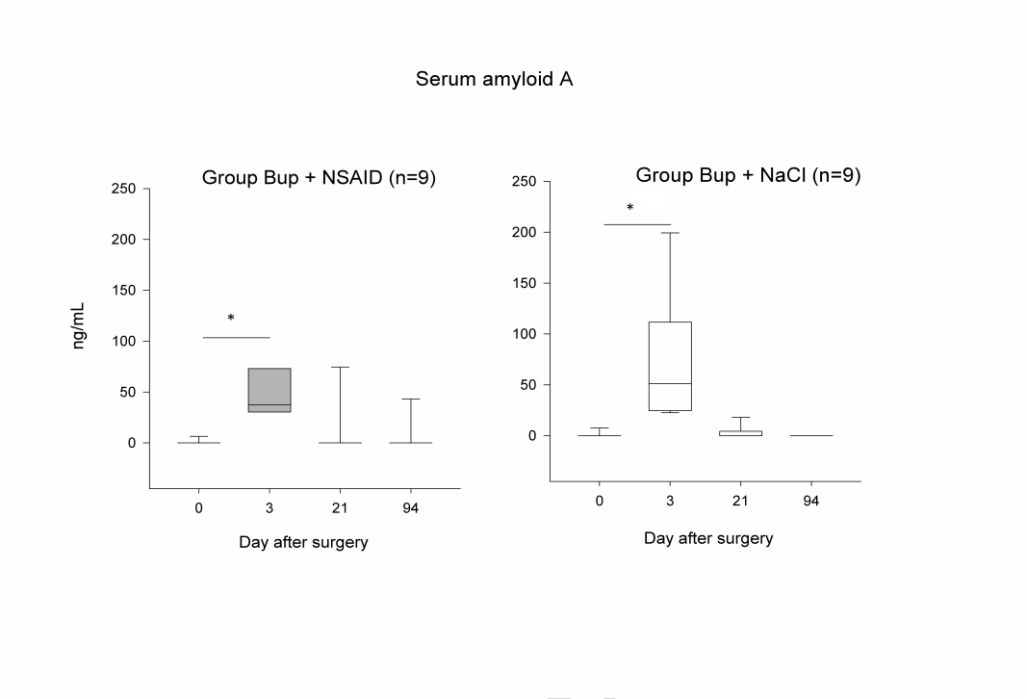


Fig. 4

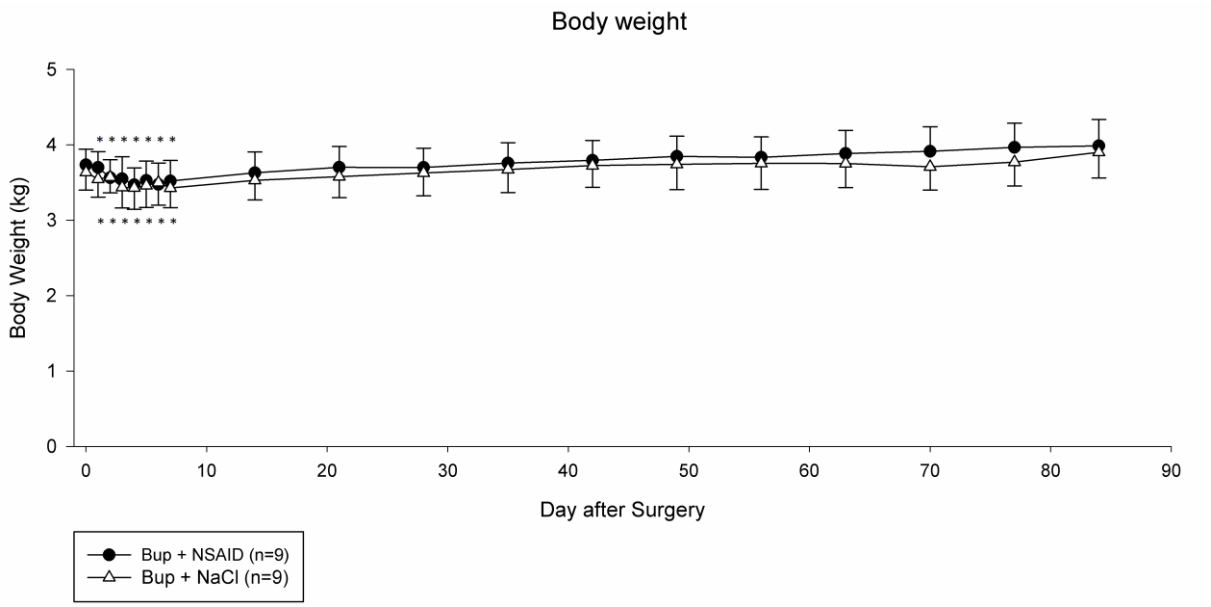


Fig. 5

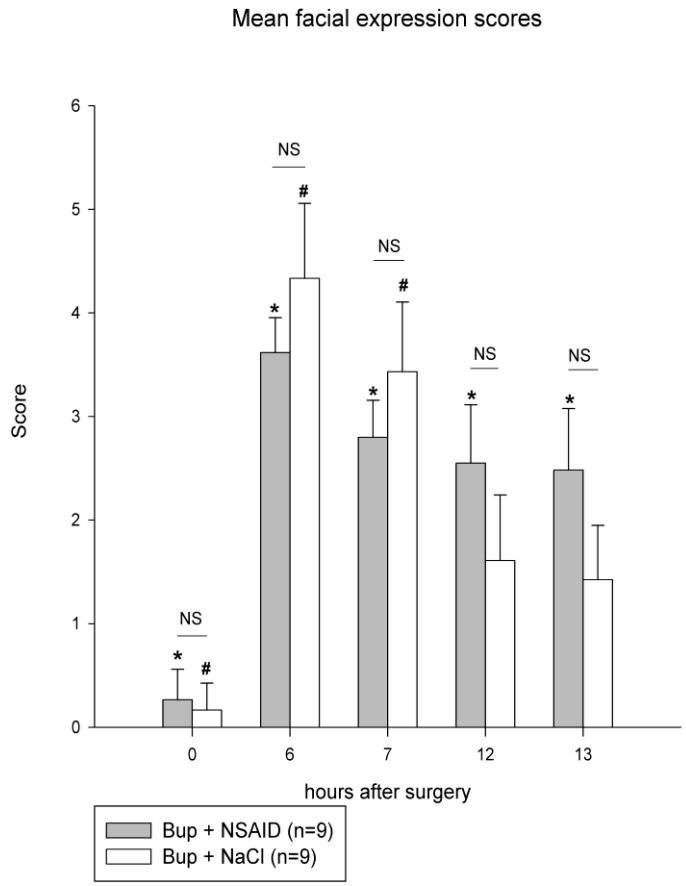


Fig. 6

Fig 7a.

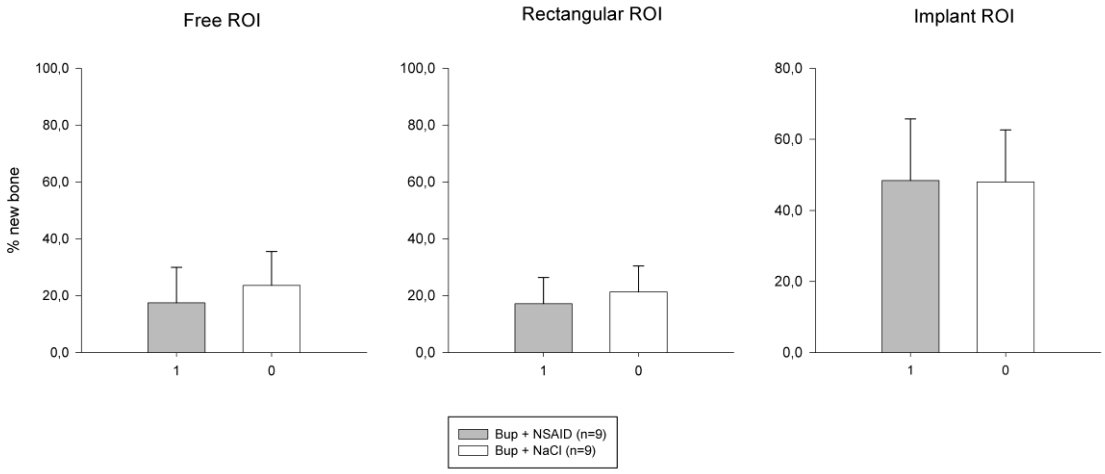
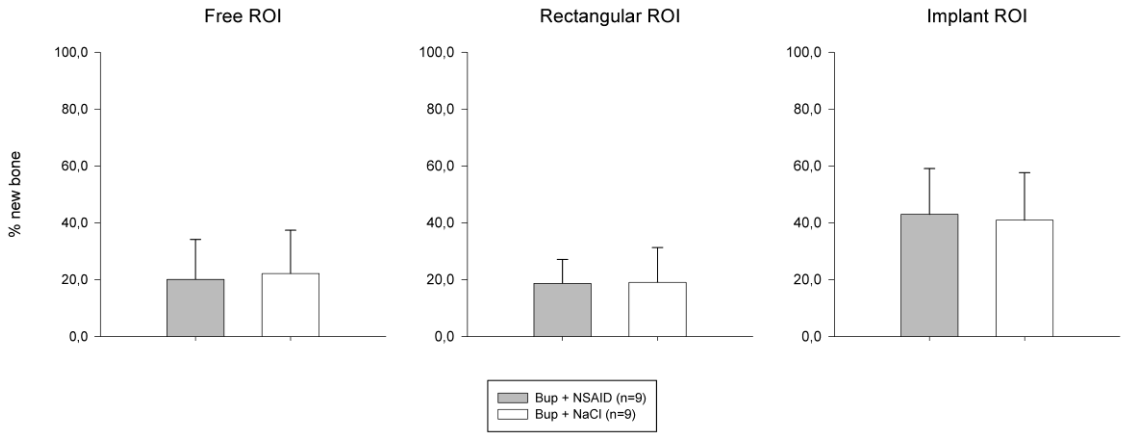


Fig. 7b.



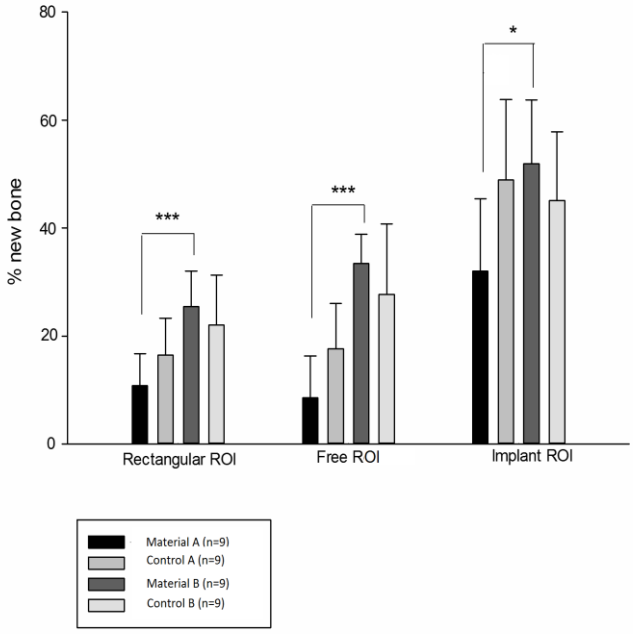


Fig. 7c

Table 1

	Analgesic treatment groups			
	Buprenorphine + carprofen (n=9)		Buprenorphine + NaCl (n=9)	
Sinus augmentation subgroups	Test material A (n=5)	Test material B (n=4)	Test material A (n=4)	Test material B (n=5)

Table 2

Quality parameter	Incorporation of implant in bone			Test material associated with bone	
Group	Good	Intermediate	Poor	Yes	No
Bup + NSAID (n=9)	6	3	0	9	0
Bup + NaCl (n=8)*	3	4	1	7	1
p-value (Fisher's exact test)		0.47		0.47	

* one section was excluded due to artefacts