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# 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 expressions and long-term effects on bone formation were evaluated in 18 male New Zealand White rabbits. A 10 × 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 days 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 days postoperatively. To assess pain, clinical examination, body weight recording and scoring of facial expressions from photos taken before, and 6–13 h after surgery were 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 days did not reduce facial expression scores after maxillary sinus augmentation in buprenorphine treated rabbits and did not affect long term bone formation.



## 1. 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, 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 expressions 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 expressions 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 results of NSAID on bone formation in humans (see Konstantinidis et al., 2013). Some authors recommend not using NSAIDs

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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., 2008; 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).

## 2. Materials and methods

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

### 2.1. Experimental design

The rabbits were randomised 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 days 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 randomised 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.

**Table 1**
Experimental 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 contralateral side.

| | 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) |

### 2.2. 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 were 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 (general 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.

### 2.3. 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 i.v. 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 prilocaine