Gentamicin-enriched, water-soluble polymer wax reduces the burden of infection after sternotomy in pigs

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Abstract

OBJECTIVES: One of the most frequent complications in cardiac surgery is postoperative bleeding from the sternum. To diminish the risk of bleeding, bone wax is frequently used for haemostasis. However, we have previously shown that bone wax impairs bone healing and induces inflammation in the sternum. A new, water-soluble polymer wax enriched with gentamicin has haemostatic properties similar to bone wax and may diminish the risk of infection. The purpose of this study was to determine whether the gentamicin-enriched, water-soluble polymer wax could reduce infection rates when compared with bone wax in a porcine model.

METHODS: Thirty-two Landrace/Yorkshire pigs were sternotomized and randomized to haemostasis by gentamicin-enriched, water-soluble polymer wax (Gen group) or bone wax (Wax group). After 4 weeks the pigs were euthanized. Blood samples were analysed for the fraction and concentration of neutrophil granulocytes and C-reactive protein and the surgical site was biopsied. Stereology was performed on histological samples, and the magnitude of infection was quantified as the areas of microabscesses, granulomas and tissue with acute inflammation compared with the total tissue area.

RESULTS: The temperature was 38.2°C in the Gen group vs 38.6°C in the Wax group, P < 0.05. No animals in the Gen group and three in the Wax group showed a temperature >39.3°C. Neutrophil granulocyte concentration was 5.00 × 10⁹/l in the Gen group and 6.92 × 10⁹/l in the Wax group, P = 0.277, with a leucocyte fraction of 20.9% vs 29.3%, P = 0.119. C-reactive protein (CRP) was 142 mg/l in the Gen group compared with 318 mg/l in the Wax group, P = 0.106. Histological samples showed acute inflammatory changes in 5.0% of the tissue in the Gen group vs 18.3% in the Wax group, P < 0.001. Microabscesses were present in 0.3% of the sample tissue in the Gen group vs 2.2% in the Wax group, P < 0.001. Concentrations of gentamicin were >100 mg/l in mediastinal fluid and <2 mg/l in venous blood.

CONCLUSIONS: When used for haemostasis after sternotomy in a porcine model, gentamicin-enriched, water-soluble polymer wax reduces sign of infection when compared with bone wax and therefore appears to be a more suitable choice for preventing postoperative, sternal osteomyelitis.

Keywords: Animal model • Infection • Mediastinal infection

INTRODUCTION

In cardiac surgery, one of the most serious complications is deep sternal wound infection (DSWI), which has a prevalence ranging up to 9% [1]. DSWI often necessitates reoperation and has a reported mortality rate eight times higher than in patients without DSWI [2]. Furthermore, DSWI leads to a prolonged and costly period of postoperative management along with increased pain and discomfort for the patient. One of the most frequent complications in cardiac surgery performed through a median sternotomy is intra- and postoperative bleeding which can cause haematomas predisposing to DSWI [3]. In patients with multiple risk factors, such as diabetes, obesity, male sex and chronic obstructive pulmonary disease, the risk of DSWI may be as high as 20–25% [4].

In order to diminish intra- and postoperative bleeding, it is common practice to use electrocauterization on the compact bone of the superficial and the profound surfaces of the sternum, and to apply bone wax to the spongy bone of the cut surfaces to achieve mechanical haemostasis. Although this technique efficiently controls perioperative bleeding, the use of bone wax is associated with postoperative side effects. Bone wax, a blend of beeswax with a softening agent such as paraffin, is not readily resorbed from the surgical site [5]. It has been shown in experimental models that bone wax may act as an infectious nidus reducing the number of bacteria needed to initiate an infection [6, 7]. The use of aggressive, preoperative systemic antibiotic regimens has reduced the incidence of DSWI, and the high mortality rate has improved with modern therapy such as vacuum-assisted closure therapy [8, 9]. More recently the local application of anti-
biotics has shown promise, combining the advantage of reduced systemic exposure to antibiotics with a highly potent local effect [1, 10]. However, even with the best modern care, high-risk patients still have a high mortality rate from complications related to bleeding and infection [11].

A water-soluble polymer wax has recently been introduced to reduce bleeding [12]. This polymer wax is used the same way as bone wax to immediately induce mechanical haemostasis by sticking to the bone surface and thereby creating a physical barrier. Polymer wax has been shown to be eliminated in an unchanged state through renal clearance within 48 h [13, 14]. Because it is water soluble, polymer wax has the potential to be a good vehicle for local delivery of an antibiotic to the sternum. In previous studies, gentamicin-enriched, collagen sponges (GCS) have been used to apply local antibiotics. These sponges were placed between the two sternal halves before closure. A meta-analysis of four randomized control trials using GCS showed a decreased prevalence of DSWI compared with untreated controls [15]. GCS is an efficient way of delivering a local antibiotic, but without the haemostatic properties of bone wax or polymer wax.

Polymer wax is approved for use as a medical device for providing haemostasis on cut bone surfaces. We have previously shown that treatment with bone wax inhibits bone healing after sternotomy when compared with polymer wax in a porcine model [16]. We also found that bone wax induces inflammation in the sternum, and that polymer wax does not [16]. The objective of the present study is therefore to determine whether an antibiotic-haemostatic polymer wax combination can reduce the risk of infection and inflammation when compared with bone wax in a human-compatible, experimental animal model. We hypothesize that pigs treated with gentamicin-enriched, water-soluble polymer wax will have reduced infection rates compared with pigs treated with bone wax.

MATERIALS AND METHODS

A total of 32 Danish Landrace female pigs with a weight of 50 kg comprised the study material. The project was conducted according to the guidelines given by the Danish Inspectorate for Animal Experimentation and approved by this institution.

Surgical procedure and postoperative care

At induction of anaesthesia, the animals were given 1000 mg procaine benzylpenicillin and 1250 mg dihydrostreptomycin (NOVO, Copenhagen, Denmark) intramuscularly to ensure comparable baseline colonization and to prevent infections of the airways.

The animals were sedated using 125 mg zolazepam, 125 mg tiletamine, 125 mg xylazine, 125 mg ketamine and 25 mg butorphanol (Virbac, Carros, France).

Anaesthesia was maintained with Propofol 20 mg/ml at 50 ml/h (Fresenius kabi, Bad Homburg, Germany) and Fentanyl 50 µg/ml at 40 ml/h (B. Braun Melsungen AG, Melsungen, Germany).

Using an aseptic technique, the animals were subjected to a midline sternotomy using an oscillating saw without severing the two cranial costae. Each animal was randomized to haemostasis using either the gentamicin-enriched, water-soluble polymer wax (Gentamicin-enriched Ostene®, Ceremed, Inc., Los Angeles, CA, USA) (Gen group) or bone wax (B. Braun Melsungen AG, Melsungen, Germany) (Wax group). The gentamicin concentration in the water-soluble polymer wax was 40 mg/g, giving a maximum total dose of ~140 mg gentamicin per 3.5 g packet. In both groups, wax was applied until haemostasis was achieved. The pleura and pericardium were not opened. Before closing the sternum, a drain (20F Portex, Smith Medical International Ltd, Hythe, UK) was placed in the thoracic cavity in the midline close to the sternum. The sternum was closed using rigid osteosynthesis by 12 steel wires (Monofilament 316L Stainless steel non-absorbable sutures, Syneture, Mansfield, MA, USA). Fascia, muscle, and skin tissues were closed in three layers using 0 Polysorb for the fasciae and muscle layers, 3-0 Biosyn for intradermal sutures and 0 Surgipro for the skin (all sutures Syneture, Mansfield, MA, USA). Fluid was collected from the drain at specific time-points; at closing, and at 1 h, and 2 h after closing. The drains were then removed. At the same time venous blood samples were collected. The fluids obtained from the drain and the blood samples were analysed for gentamicin concentration (VITROS Chemistry products GENT-reactant with VITROS Chemistry products calibration-kit 13 on VIROS 5,1 FS Chemistry System and VITROS 5600 integrated System), with a potential positive bias of up to 15%. A swab was taken from the wound and the catheter tip and sent for bacterial cultivation.

Anaesthesia was stopped 2.5 h after closing the sternum and the animals returned to the housing facilities for 4 weeks of post-surgical care by trained veterinary staff. Analgesics were given until the third postoperative day. Flunixin 100 mg (Sigma-Aldrich, St. Louis, MO, USA) and buprenorphine 1.5 mg (Arepharma GmbH, Radebeul, Germany) were given at the end of the operation. For the first 3 postoperative days, flunixin 1.5 mg/kg and buprenorphine 0.012 mg/kg were given three times daily or as needed. During surgery, two animals were excluded from the study. One was excluded due to intubation problems and the second, due to ventricular fibrillation at extubation. All animals surviving surgery and the transport to the housing facilities were included in the study. Fifteen pigs were included in each of the two groups. During the next 4 weeks, three animals in the Wax group and one in the Gen group developed infection resulting in poor thriving and were euthanized prior to the end of the observation period. Two of the animals in the Gen group could not have their serum-gentamicin measured, leaving 13 animals in the Gen group for final analysis.

The primary end-point was spontaneous sternal wound infection within 4 weeks postoperatively. Clinical infection was defined as fever (temperature >39.3°C in young pigs), failure to thrive (no eating or drinking despite sufficient analgesics), presentation of redness of the sternal area and purulent secretion. The presence of clinical infection was judged by a single person (T.K.M.) at the time of euthanization and verified by culture and/or microscopy of the Gram-stained aspirate. No specific test for drug sensitivity was performed.

After 4 weeks, the pigs were euthanized by captive bolt and exsanguination. Body weight and temperature were measured in the morning before the pigs were transported from the housing facilities to the experimental facilities for termination. At the same time, blood samples were taken for leucocyte count and CRP. Several biopsies ad modum Kamme were taken from both anterior and posterior surfaces of the sternum. After bacterial culture, results were obtained from these biopsies, and the segment with the highest bacterial load and a matching clinical impression was chosen from each sternum for further histological evaluation. Thus, one segment per sternum was sent for histology.
Specimen preparation

Chosen sternal segments were fixated undecalcified in formalin and embedded in methylmethacrylate (all chemicals; Sigma-Aldrich, St. Louis, MO, USA). Using a microtome, 7 µm thick slices were cut parallel to the frontal plane of the segment and stained with Goldner’s trichrome, which stains calcified bone matrix green, uncalcified bone matrix (or osteoid) red and calcified cartilage pale green.

Stereology

Microscopic evaluations were performed using a motorized Proscan 11™ stage (Prior, Rockland, MA, USA) and a MT1201 microcator (Heidenhain, Traunreut, Germany) coupled to a Nikon ECLIPSE 80i microscope (Nikon, Tokyo, Japan). The images were captured using a DP72 camcorder (Olympus, Ballerup, Denmark) coupled to a computer with the newCAST™ software (version 3.4.0.1; Visiopharm, Hørsholm, Denmark) which was used to generate a set of counting frames and a point grid. The region of interest was defined as the diastasis, considered as the area between the two closest edges of the cortical bone plus 1500 µm to each side from the most medial bone edge. Counting frames included 100% of the region of interest. The setup used was a point grid with a ×92 magnification and grid constant of 1 927 529 µm² per point. Any type of tissue cutting the upper right quadrant of the cross was counted. Types of tissue counted were: inflammatory cells, granulomas (spherical conglomerates of inflammatory cells, including giant cells, surrounded by fibrous tissue) and abscesses (gatherings of inflammatory cells with a central necrosis). The different types of tissue were expressed as a volume fraction of the total amount of tissue in the sampling area, including healthy tissue.

Statistics

Continuous values were reported as means and standard deviations (SDs). Statistical analyses were performed using Student’s t-test for all parametric variables. Fisher’s exact test was used for categorical data. A P-value of <0.05 was considered statistically significant. With an anticipated decrease in infection rate of at least 15%, with a statistical power of 80% (α = 0.05), sample size was calculated to 14 animals in each group.

RESULTS

Clinical infection was present in three pigs treated with the gentamicin-enriched, water-soluble polymer wax and in seven pigs treated with bone wax, P-value = 0.123. Body temperature was significantly higher in pigs treated with bone wax compared with pigs treated with gentamicin-enriched, water-soluble polymer wax (Table 1). Although not statistically significant, all of the infection parameters were lower and weight gain was higher in the Gen group compared with the Wax group (Table 1). The same animals that had increased temperature and increased biomarkers in their serum showed signs of clinical infection. Details of histological results are given in Table 2. The fractional area of inflammation was significantly lower in the Gen group than in the Wax group (P < 0.001). The fractional area of Microabscesses was also lower in the Gen group (P < 0.001). Chronic inflammation as defined by granulomas also tended to be lower in the Gen group (P = 0.0601).

Multiple species of bacteria were found in all pigs. The concentration of gentamicin in venous blood samples peaked 1 h after closing the sternum at a concentration of <2 mg/l for pigs treated with gentamicin-enriched, water-soluble polymer wax (Fig. 1). The concentrations of gentamicin were >100 mg/l in samples of draining fluid from pigs treated with gentamicin-enriched, water-soluble polymer wax.

DISCUSSION

We found that pigs treated with gentamicin-enriched, water-soluble polymer wax had histologically significantly less inflammation and fewer microabscesses compared with pigs treated with bone wax. The temperature was significantly lower in pigs treated with gentamicin-enriched, water-soluble polymer wax, and other systemic indicators of infection, CRP and leucocytes, were also lower. Our findings did not confirm our hypothesis of reduced infection rates, but showed a reduced burden of infection in pigs treated with gentamicin-enriched, water-soluble polymer wax compared with pigs treated with bone wax. We have previously shown that bone wax increases inflammation when compared...
with polymer wax due to impaired fracture healing [16]. In this study, inflammation due to infection was investigated, showing similar results.

Due to its human-like physiology and its compatibility with existing techniques and equipment used for heart surgery in humans, the pig model has shown to be a suitable choice for research on sternal healing [17]. One of the major differences when comparing the porcine with the human model is the environment where the pigs are kept postoperatively. The operative wound is much more exposed to bacteria and contamination than in humans. In addition to this, the pig, being a four-legged animal, also exposes the sternal wound to contamination when lying down. The effect of these differences was obvious since DSWI was prevalent among our study animals whereas this complication is less frequently seen in humans, making this a suitable animal model in which to investigate infection.

The shown temperature difference between the two groups is a vague indication of infection. However, we found a trend of increased rates of clinical infection, increased infectious parameters and decreased weight gain in the bone Wax group. These trends, combined with the difference in temperature, give a strong indication of a clinical correlation to the difference in infection rates found by histology.

Studies on smaller animals have shown that the utilization of water-soluble polymer wax results in reduced infection rates [18]. Using a larger animal model, our study supports these earlier findings. Gentamicin-enriched, water-soluble polymer wax has previously been shown to have a protective effect against single-bacterial inoculated infections [19]. Our study supports these results using a model with spontaneous infection with multiple bacteria. Although the bacterial exposure in this study differs from the clinical setting, local antibiotics may need to be considered to prevent DSWI in high-risk patients associated with the use of haemostatic bone wax.

Osteomyelitis is an infection that is difficult to treat, particularly when associated with the implantation of foreign material (e.g. prosthesis, electrodes or as in our case; steel wires and bone wax) [20]. In orthopaedic surgery, osteomyelitis is a relatively common problem, for which treatment with localized gentamicin has been used with good results [21]. However, a problem when working with localized antibiotics is the development of resistance to the chosen drug. This was found by Neut et al. [22] when working with chains of gentamicin-loaded polymethylmethacrylate beads. They found that in patients treated for prosthesis-related infection, 19 of 28 developed resistance to the gentamicin-loaded polymethylmethacrylate beads [22].

High doses of systemically administered gentamicin are ototoxic and nephrotoxic, but little is known about the prevalence of adverse effects of locally applied gentamicin [23, 24]. We found a localized concentration of gentamicin in the sternum 10 times higher than therapeutic concentrations (5–10 mg/l). Corresponding concentrations in blood samples peaked after 2 h and were found to be several times lower than therapeutic concentrations. Low, systemic concentrations of gentamicin are thought to cause fewer adverse effects, while high concentrations in the mediastinal fluid give a more localized effect in the area where bacteria is most likely found. This is suitable since the risk of infection is greater here due to haematomas and necrosis following the sternotomy, electrocauterization, and insertion of foreign material.

The areas of inflammation and abscesses were larger in the pigs treated with bone wax than in those treated with gentamicin-enriched, water-soluble polymer wax, indicating a more severe on-going infection and inflammation in these pigs. When presented with foreign material, the immune system responds by attempting to wall off this material, thereby creating a granuloma. If this foreign material is relatively non-toxic this will create a ‘low turnover’ granuloma [25]. One of the weaknesses of this study was the short follow-up time and this might have been too short to show the true amount of granuloma formation in the two groups. Another limitation is the lack of a study-arm with polymer wax without gentamicin. This would have been interesting since we have previously shown that bone wax induces inflammation in the sternum, and polymer wax does not [16]. It was estimated that 14 animals in each group would be enough to detect a significant difference between the two groups, but in accordance with our present results, the level of significance would have profited from a greater study population. The results of this study imply that the clinical use of gentamicin-enriched, water-soluble polymer wax may present an opportunity to deliver a localized antibiotic in combination with a haemostatic treatment and thus may lead to fewer postoperative infections for patients undergoing heart surgery.

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REFERENCES


