EJP
European Journal of Pain

ORIGINAL ARTICLE

Characterization of a porcine model of post-operative pain

D. Castel[1], E. Willentz[2], O. Doron[2], O. Brenner[3], S. Meilin[4]

1 The Neufeld Cardiac Research Institute and Department of Physiology and Pharmacology, Sackler School of Medicine, Tel-Aviv University, Israel
2 Lahav Research Institute, Negev, Israel
3 Department of Veterinary Resources, The Weizmann Institute of Science, Rehovot, Israel
4 Neurology Division, MD Biosciences, Ness Ziona, Israel

Correspondence
Sigal Meilin
E-mail: sigal@mdbiosciences.com

Funding sources
This work has been funded by MD Biosciences and Lahav Research Institute.

Conflicts of interest
None declared.

Accepted for publication
10 August 2013

doi:10.1002/j.1532-2149.2013.00399.x

Abstract

Background: Management of acute pain related to surgical intervention, termed post-operative pain or POP, continues to be a major healthcare challenge. While the rat plantar incision model provides valuable data to researchers about the mechanisms mediating POP, the development of topical and localized treatments in small animal models is limited. To help address these issues, we describe here the characterization of a large animal model of incisional pain.

Methods: Pigs underwent full-skin incision or full-skin and muscle incision and retraction (SMIR). Withdrawal thresholds were determined using the Von Frey test at baseline, 0.5–12 h post-surgery and on days 1, 2, 3, 5 and 7 post-surgery. The analgesic effects of systemic morphine [0.1 or 1.0 mg/kg intramuscular (i.m.) dose] and local anaesthetic ropivacaine were studied. Spontaneous pain-like behaviours were scored and analysed. The effects on wound healing were evaluated by gross observation and by histopathological examination.

Results: Pigs incurring SMIR demonstrated significantly increased mechanical hypersensitivity compared with pigs that underwent full-skin incision only ($p < 0.05$). Maximal analgesia was achieved with morphine (1 mg/kg i.m. dose) at 0.5 h post-treatment. Local treatment with ropivacaine was effective at increasing the withdrawal threshold to Von Frey filaments compared with saline control ($p < 0.05$) for a period of at least 6 h. Wounds healed normally with no signs of infection, redness or swelling.

Conclusions: We propose that the pig model of incisional pain can provide an appropriate translational model for validating new topical and localized treatments for POP in humans.

1. Introduction

For all surgical procedures, acute post-operative pain (POP) is to be expected and should be adequately managed (Berry and Dahl, 2000). Unrelieved POP causes unnecessary patient suffering and discomfort, and leads to psychological and pathophysiological complications (Brennan et al., 2007). And yet, despite the availability of many therapeutic agents and advanced analgesic techniques (Wu and Raja, 2011), an alarming number of patients continue to report moderate to severe pain after surgery (Apfelbaum et al., 2003; Lorentzen et al., 2012). Therefore, continued research to understand the impact of analgesic agents on nociceptive mechanisms remains necessary (Wu and Raja, 2011).

Historically, the rodent has been the model of choice for developing new analgesic treatments in the study of POP (Curtin et al., 2009). Briefly, the rat model of incisional pain involves a 1-cm longitudinal incision of skin, fascia and muscle of the plantar aspect of the rat hindpaw, performed under anaesthesia (Brennan

What's already known about this topic?
- Pigskin is morphologically more homologous to human skin than is rodent skin.
- Neurotrophic factors play a role in injury-induced responses of peripheral sensory neurons in pigs.

What does this study add?
- This study provides an appropriate large animal model for the validation of new local and topical analgesics.
- This pig model enables three important parameters to be assessed in parallel: nociceptive sensitivity, spontaneous behaviour in the open pen and wound healing.

et al., 1996). Following this procedure, spontaneous and/or evoked nociceptive behaviours in the rat are examined for several days, corresponding to the time course of pain in post-operative patients (Dahl et al., 1992; Stubhaug et al., 1997). Classical measures of nociception include Von Frey (Whiteside et al., 2004) and paw pressure tests to assess mechanical hyperalgesia (Randall and Selitto, 1957), and hot-plate tests to assess thermal hyperalgesia (Bannon and Malmberg, 2007). However, one of the major disadvantages of the rodent model is its limited use in assessing topical and localized treatments. From a technical perspective, the location of the hindpaw is relatively small for topical application. Practically, rodents may lick or bite the injured paw, causing removal of any topical treatment that is applied.

The anatomy of rodent skin is significantly different from human skin, and healing occurs primarily by wound contraction rather than re-epithelialization (Sullivan et al., 2001). In contrast, porcine skin exhibits a higher degree of homology to human skin and heals by epidermal cell migration (Obreja et al., 2009; Swindle et al., 2011). Consequently, pigs have become a standard model of wound healing (Sullivan et al., 2001), and pig models of inflammatory pain have been most recently reported (Di Giminiani et al., 2013). There is also considerable correlation between contractile, metabolic and morphological features in skeletal muscle of humans and pigs, and, unlike the loose skin of rodents, pigskin is tightly attached to the muscle and subcutaneous tissue as in humans (McAnulty, 2012).

The aim of this study was to characterize a porcine model of POP through skin incision and skin and muscle incision and retraction (SMIR). We evaluate the nociceptive behaviour of pigs using the Von Frey test to determine the time course of mechanical hyperalgesia. Spontaneous pain-like behaviours are also observed. Wound healing was examined to eliminate any possible contribution from infection or inflammation. Finally, we report the responsiveness of the pigs to common analgesic treatments.

2. Materials and methods

2.1 Animals and housing

Danish Landrace × Large White cross-bred weaned male pigs ($n = 40$) from the domestic herd at Lahav Labs, Negev, Israel, were used. Before the beginning of the experiment, all of the animals were kept under conventional pig production conditions. All pigs were 7 weeks old and weighed 10 ± 1 kg at the start of the study. The animals were housed in open pens (1.4 × 2.4 m) 7 days prior to study initiation. The pigs were kept in groups of two or three during the acclimatization period and throughout the experiment. Feeding occurred three times daily using pigs special food (Dry Sows; Cat. No. 5420, Milobar, Oshrat, Israel), and pigs were provided opportunities to root and chew for enrichment. Fresh water was provided *ad libitum* by an automated system. The pigs were kept on a 12-h light/dark cycle. The study was approved by the Institutional Animal Care and Use Committee and adhered to guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain (Zimmermann, 1983). All tests, including observations of wound healing, response to a mechanical stimulus and behaviour observations were performed blind, i.e., the technician was unaware of the individual animal's treatment.

2.2 Habituation protocol

The pigs were habituated to the protocol for 5 days prior to surgery. The researchers, the veterinarian and the animal care technicians played with the pigs in their home pen for at least 15 min twice a day, for each day of the acclimatization period and throughout the experiment. As a result, the pigs became familiar with their observers, ensuring calm and consistent handling of the animals. The technicians remained the same throughout the entire study period; no other people were allowed into the housing facility. To familiarize the pigs with the protocol and technicians, the pigs were trained to walk to the preparation room daily during the habituation period. The pigs were always returned to their original pens with their original pen mates. The habituation process was intended to reduce the stress level of the pigs. The body weight of all the animals was measured at three time points: (1) 5 days prior to surgery, at the beginning of the acclimatization period (baseline); (2) on study day 0 (surgery day), prior to anaesthesia; and (3) 1 week post-surgery at study termination (study day 7). The experi-

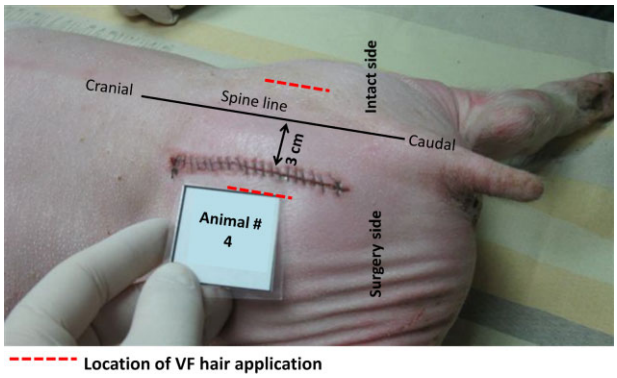

Figure 1 Incision location. A 7-cm incision was made on the left side of the lower back towards the caudal end of the pig and 3 cm lateral to the spine line. VF hair: Von Frey filament.

ment took place in June 2012, with the temperature in the surgery room kept at 19 °C (range 18–20 °C).

2.3 Anaesthesia and surgery

On the day of surgery, each pig walked freely to the preparation room before surgery. One of the technicians carried each animal in their hands and placed an anaesthetic face mask (Stephan Akzent Color, Gackenbach, Germany) on the pig's mouth and nose. Each animal was anaesthetized with a 3% isoflurane/100% oxygen mixture. The technician held the pig until it was relaxed and sleepy. Then the technician placed the pig in a sternal position, still connected to the anaesthesia mask. The pig was shaved and swabbed with 70% ethanol and then carried to the operation room. The pig was placed on the operating table, and a sterile environment was maintained. The area of the incision was swabbed with antiseptic liquid polidine solution (Polysept solution, Rekah Pharmaceutical Industry Ltd, Holon, Israel), and the non-operated areas were covered with sterile sheets. During anaesthesia, blood O_2 saturation was monitored (Spacelab Medical, Snoqualmie, WA, USA).

Two types of incisions were performed in this study: (1) full-skin incision: an incision of 6–7 cm was made through only the skin and fascia, keeping the muscle intact. The incision was made on the left side of the lower back towards the caudal end, approximately 3 cm lateral and parallel to the spine line of the pig (Fig. 1); and (2) full SMIR: an incision of 6–7 cm was made through the skin, fascia and muscle at the same location as described above. Incisions were closed using 3-0 silk sutures (Assut UK Ltd, West Yorkshire, UK) and a continuous suturing technique. Following the incision, all pigs received the antibiotic marbofloxacin (10% w/v) (Marbocyl®, Vétoquinol UK Ltd, Buckingham, UK) at a total dose of 0.5 mL per pig, which was administered via intramuscular (i.m.) injection into the neck muscle. Each animal was kept under anaesthesia for the duration of the surgery. The entire procedure, from the time when the animal was introduced to the face mask until the face mask was removed, was approximately 20 min. The animals were then returned to their home pen for recovery.

2.4 Study drug procedure

Forty domestic pigs were divided into seven treatment groups of five to six individual pigs (denoted G1–G7) (Table 1). G1 and G6 received saline i.m. injection in the neck region 30 min prior to Von Frey testing at each time point. G2, G3 and G7 received morphine at doses of either 0.1 or 1.0 mg/kg, administered by i.m. injection in the neck region 30 min prior to Von Frey testing at each time point. G4 and G5 received local dosing of 5-mL saline (Teva Labs, Netanya, Israel) or 0.5% ropivacaine HCl (Naropin®, APP Pharmaceuticals, LLC, Schaumburg, IL, USA), respectively. The saline or ropivacaine solution was carefully injected just below the incision area as follows: The incision was closed halfway, and then 2.5 mL of saline or 0.5% ropivacaine solution was injected under the skin into the pocket created following the closure. While injecting, the needle was pulled out slowly. Next, the incision was sutured closed almost completely and, immediately prior to the last stitches of the incision, another 2.5 mL of either saline or 0.5% ropivacaine solution (G4 and G5, respectively) was injected subcutaneously. Suturing of the incision and dosing was always performed in the same direction, i.e., cranial to caudal for each animal.

2.5 Assessment of mechanical sensitivity

Mechanical sensitivity was assessed using Von Frey filaments [Touch Test (Von Frey) Sensory Evaluator Kit, model 58011,

Table 1 Study groups, treatment and procedure.

Group #	*n*	Treatment	Procedure
G1	6	Saline (vehicle control) i.m.	Full-skin incision
G2	6	0.1 mg/kg morphine i.m.	Full-skin incision
G3	6	1 mg/kg morphine i.m.	Full-skin incision
G4	6	Local treatment with saline (control)	Full-skin incision
G5	6	Local treatment with 0.5% ropivacaine	Full-skin incision
G6	5	Saline (vehicle control) i.m.	Full SMIR
G7	5	1 mg/kg morphine i.m.	Full SMIR

i.m., intramuscular; SMIR, skin and muscle incision and retraction.

Stoelting Co., Wood Dale, IL, USA]. The tests were performed in the pig's home pen. Von Frey filaments ranging from a minimum of 1 g (diameter = 0.229 mm; force = 9.804 mN) to a maximum of 60 g (diameter = 0.711 mm; force = 588.253 mN) were used in this study. The intact side (contralateral to the side of incision) was introduced first to the Von Frey filaments as a control. The filaments were then applied approximately 0.5 cm proximal to the incision on the skin (Fig. 1). Each filament was applied three times with a 5–10-s interval between applications. If withdrawal was not achieved, a thicker filament was applied. If withdrawal was achieved, a thinner filament was applied. By alternating the filaments, the force required to achieve a withdrawal reaction was determined. This procedure was carried out at the following time points: 1 day pre-surgery (baseline), 0.5–12 h post-surgery (study day 0) and days 1, 2, 3, 5 and 7 post-surgery (study days 1, 2, 3, 5 and 7, respectively). On study day 7, animals from G2 to G7 were euthanized with an overdose of pentobarbitone. Animals from G1 (saline i.m. dosed pigs) were housed for a further 7 days (until study day 14) for wound closure and healing examination.

2.6 Wound healing examination

All animals were examined by trained technicians prior to surgery at baseline and then daily for a period of 7 days following Von Frey testing. The animals were scored according to two categories of inflammation: redness: 0 = none (normal); 1 = mild (slight redness at the area of the incision); 2 = moderate/severe (spreading of redness), and swelling: 0 = none (no swelling); 1 = mild (slight swelling); 2 = moderate/severe (pronounced swelling). The total score is the sum of points from each category. Therefore, the highest attainable wound score was 4 points.

2.7 Behaviour testing

Assessment of spontaneous behaviour required observation of the social and individual behaviour of the pig in the open pen. This was performed twice daily for a period of 5 days prior to the day of surgery. The scoring method used is based on a numerical rating scale (NRS) of multifactorial criteria and is modified from the method described by Reyes et al. (2002). Spontaneous behaviour of all animals in the open pen was observed at 1 and 3 h post-surgery, and then daily for an additional 4 days. The behaviour score was divided into two distinct categories: (1) solitary performance and (2) social behaviour (Table 2). The total score is the sum of all sub-score parameters; a higher score indicates a higher degree of pain. Solitary performance was assessed by an observer standing outside the pen recording the behaviour of the individual animal for a period of 10 min. Social behaviour was assessed by a technician stepping into the pen while an observer recorded the animal's response to the entrance of the technician.

Table 2 Spontaneous behaviour scoring of pigs in an open pen.

	Description	Score
Solitary performance		
Lameness	Normal walking	0
	Slight limping	1
	A lot of limping, kicking-like movements	2
Appearance	Normal lying and walking	0
	Guarding the injury	1
Lying	Normal lying	0
	Lying but alert	1
	No lying	2
Vocalization	Normal vocalization (low volume)	0
	Moderate-volume vocalization	1
	High-volume vocalization	2
Social behaviour		
Restlessness	Normal behaviour	0
	Pacing around the pen	1
	Jumps up and down and paces around the pen	2
Agitation	Normal behaviour	0
	Slightly moves away when approached	1
	Screaming and moves away when approached	2
Aggression	Friendly	0
	Moves away	1
	Biting and aggressive when approached by other pigs	2
	Attacking and biting pen mates	3
Isolation	Normal behaviour	0
	Moves away from pen mate	1
Total score	Range from 0–13 points	

The scoring criteria used are based on a numerical rating scale modified from Reyes et al. (2002). The behaviour score was divided into two distinct categories: (1) solitary performance and (2) social behaviour. The total score is the sum of all sub-scores.

2.8 Histopathological examination

On study day 14, the G1 animals were euthanized humanely according to animal welfare guidelines. The area of incision together with the surrounding skin was biopsied for wound analysis. The biopsy samples were cut in such a way that all incised skin layers were collected. Immediately, the individual samples were pinned to a flat piece of polystyrene to maintain their shape and placed into plastic histology cassettes with 10% neutral buffer formalin (4% formaldehyde) for 72 h at room temperature. The tissue samples were then processed routinely for light microscopy by dehydrating, embedding and cutting (Bancroft and Gamble, 2008). The samples were cut into 5-μm transverse increments with a microtome and stained with haematoxylin and eosin according to standard procedures (Bancroft and Gamble, 2008).

2.9 Data analysis

The results are presented as mean ± standard error of the mean (SEM). Comparisons between groups were performed

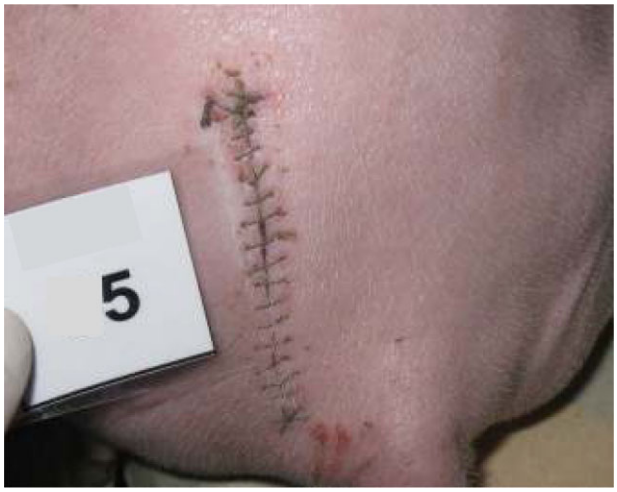

Figure 2 A representative wound picture taken on study day 7. The incision is closed with no sign of infection, swelling or redness. Healing discerned by gross observation is normal. The number shown on the left corresponds to the pig number.

by using unpaired Student's *t*-test (using GraphPad Prism® software, GraphPad Software Inc., San Diego, CA, USA) assuming a normal distribution of data. A *p*-value of <0.05 was considered significant.

3. Results

3.1 Weight gain

All animals gained weight during the study. Animals gained approximately 20% of their body weight (a total mean increase from 10 ± 1 to 12 ± 0.5 kg on study day 0 and 7, respectively). The increase in body weight recorded was in the normal range for pigs aged 7 weeks (de Grau et al., 2005), suggesting that the pigs were normal in health and behaviour. There was no statistical difference between the treated groups in weight gain ($p > 0.05$; data not shown), so the withdrawal response to the mechanical stimulus was deemed unrelated to sickness.

3.2 Wound healing observations

Seven days post-surgery, the wound had closed in all animals, with no signs of infection, swelling or redness (Fig. 2). The total mean inflammation score from study day 0 to 7 of three treated groups (G1, G3 and G6) are shown in Fig. 3, and denoted skin, morphine and muscle, respectively (Fig. 3). Results show that the inflammation pattern of the wounds analysed follows a normal acute inflammatory response of 2–7 days (Young et al., 2002). Consistent with previous data, acute administration of morphine (1 mg/kg, i.m.) reduced inflammation of the wound (Clark et al., 2007). Histological analysis of wound samples from G1 animals kept until study day 14, i.e., for 7 additional days, showed complete wound healing and normal scar formation (Fig. 4A–C).

3.3 Mechanical thresholds

Prior to incision, the animals did not respond to Von Frey filaments up to 60 g when applied to either the ipsilateral or contralateral side. However following the incision, the animals responded very strongly to the Von Frey filaments, and withdrew by twisting and turning 90–180° away from the investigator to avoid the stimulus. The withdrawal threshold of the incised skin to Von Frey filaments decreased 3 h post-surgery for the full-skin incision group (G1) (Fig. 5). The force required to achieve withdrawal was 3.37 ± 0.67 g ($n = 6$). In comparison, the withdrawal force required for the intact skin (contralateral control side of the same pig) was significantly greater 60.00 ± 00.00 g ($p < 0.05$), indicating increased sensitivity of the incised skin. Hypersensitivity to Von Frey filaments was still observed 7 days post-surgery, as demonstrated by a significantly lower withdrawal threshold of the incised skin compared with the intact skin of the same pig (10.00 ± 1.69 vs. 48.00 ± 7.20 g, respectively; $p < 0.05$) (Fig. 5). Animals in G6, incurring full SMIR, demonstrated significantly greater sensitivity to the mechanical force at 3 h post-surgery than animals that

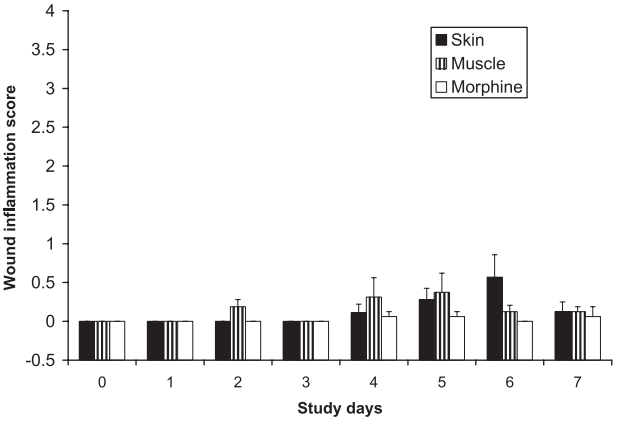

Figure 3 Wound inflammation scores. Values shown are the mean ± standard error of the mean total wound inflammation score of pigs that underwent full-skin incision (full black column denoted skin; G1), full-skin and muscle incision and retraction (vertical line column denoted muscle; G6), and full-skin incision following treatment with 1 mg/kg morphine intramuscular (white column denoted morphine; G3).

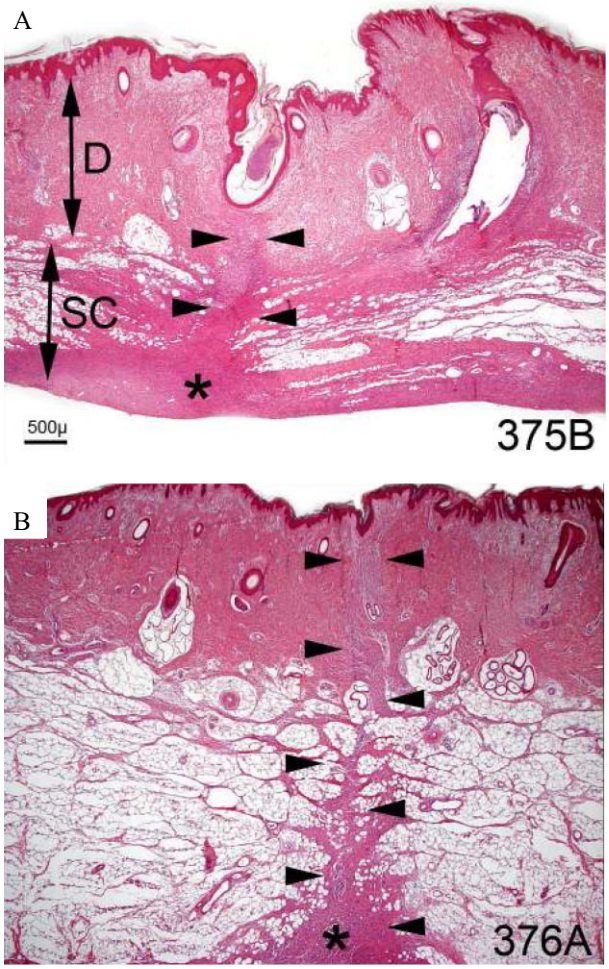

Figure 4 (A–C) Histological evaluation of wound healing on study day 14. Haematoxylin and eosin stained images from study day 14 representative of saline (vehicle control)-treated pigs incurring full-skin incision. Sample number and slice location are indicated on the bottom right: The letter A or B refers to the location of the slice in the trimming schematic shown in (C). (A) Red line layer indicates epidermis. The dermis (D) and the subcutis (SC) are indicated by a double-headed arrow next to the letters 'D' and 'SC', respectively. A thin and well-defined fibrous scar (flanked by arrowheads) extends through the dermis. The scar becomes wider at its base in the subcutis (indicated by an asterisk). (B) A thin and well-defined fibrous scar (flanked by arrowheads) extends through most of the dermis and subcutis. (C) Trimming schematic diagram.

underwent full-skin incision only (2.60 ± 1.20 vs. 5.33 ± 0.84 g, denoted muscle and skin, respectively; $p < 0.05$) (Fig. 6).

Treatment with 0.1 mg/kg morphine following full-skin incision (G2) was not effective in increasing the withdrawal threshold to Von Frey filaments (Fig. 7). Treatment with a higher dose, 1 mg/kg morphine, following full-skin incision (G3) was effective in reversing the low withdrawal threshold compared with saline (vehicle) treatment (G1) at 0.5 and 3 h post-treatment (48.7 ± 8.6 and 37.3 ± 7.2 g vs. 6.0 ± 1.0

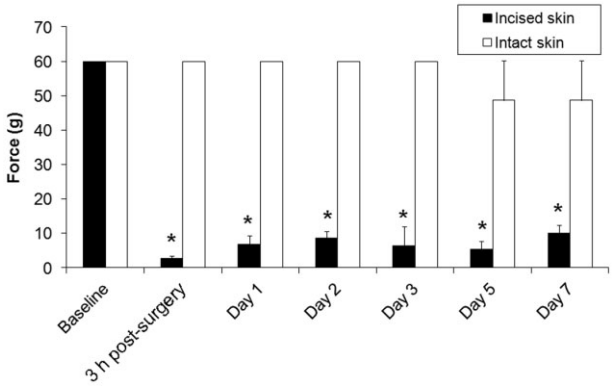

Figure 5 Time course of mechanical hyperalgesia measured by the Von Frey test following full-skin incision in pigs. The force (g) required to achieve a withdrawal response on the incised and intact sides was determined at the following time points: baseline (1 day pre-surgery), 3 h post-surgery and days 1, 2, 3, 5 and 7 post-surgery. Values are presented as mean ± standard error of the mean. *$p < 0.05$ incised versus intact side.

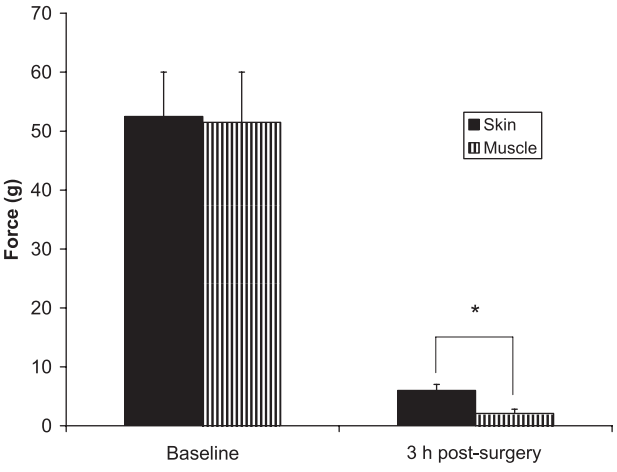

Figure 6 Withdrawal response threshold measure by the Von Frey test following full-skin incision or full-skin and muscle incision and retraction (SMIR) in pigs. The force (g) required to achieve a withdrawal response on the incised side for full-skin incision or SMIR pigs at baseline (1 day pre-surgery) and 3 h post-surgery. Values are presented as mean ± standard error of the mean. *$p < 0.05$ full-skin incision versus SMIR.

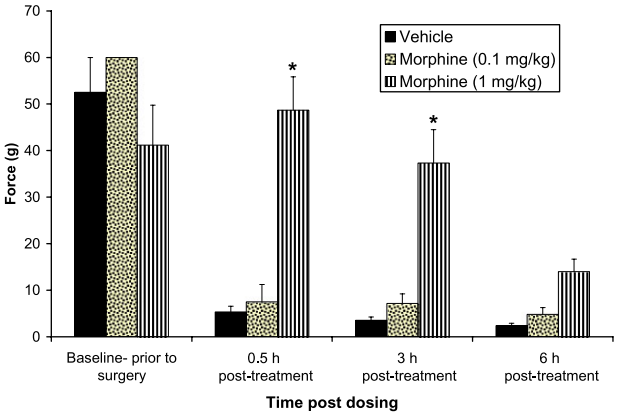

Figure 7 Analgesic effect of systemic treatment with morphine following full-skin incision in pigs. The Von Frey filament force (g) required to achieve a withdrawal response on the incised side for full-skin incision pigs treated with saline control (G1: full black column denoted vehicle), or treated with morphine at a dose of 0.1 or 1.0 mg/kg intramuscular (G2 and G3: pattern and vertical line columns, respectively) at baseline (1 day pre-surgery) and 0.5, 3 and 6 h post-treatment. Values are presented as mean ± standard error of the mean. *$p < 0.05$ morphine 1 mg/kg versus saline (vehicle control)-treated pigs.

and 2.4 ± 0.5 g, respectively; $p < 0.05$). At 6 h post-treatment, the analgesic effect of 1 mg/kg morphine was decreased, as the force required for withdrawal was not statistically different from the withdrawal force of the saline (vehicle)-treated animals (G1) (14 ± 2.7 vs. 2.4 ± 0.5 g, respectively; $p > 0.05$) (Fig. 7). In addition, a dose of 1 mg/kg morphine that was significantly effective for a period of 3 h following full-skin incision (G3) was not effective when the incision involved muscle retraction (G7). The mean withdrawal threshold with 1 mg/kg morphine recorded at 0.5 h post-treatment was significantly lower following SMIR than following full-skin incision (17.5 ± 5.1 vs. 48.7 ± 7.2 g, respectively; $p < 0.05$).

Local treatment with ropivacaine (G5) was effective in increasing the withdrawal threshold to Von Frey filaments following full-skin incision compared with local treatment with saline (control vehicle; G4); $p < 0.05$ (Fig. 8). Results of the effect of local treatment with ropivacaine at 1.5, 3 and 6 h post-treatment were 19.67 ± 1.47, 25.10 ± 2.20 and 18.75 ± 2.18 g, respectively. No significant difference in withdrawal response to Von Frey filaments was observed between the ropivacaine-treated (G5) and the saline-treated (G4) groups 8 h post-surgery (7.72 ± 1.42 vs. 2.00 ± 0.50 g, respectively; $p > 0.05$).

3.4 Behaviour observations

The pigs remained social and did not show any change in their walking ability post-surgery. However, transient changes in behaviour did occur following surgery. Transient changes, which included restlessness, isolation, moving away when approached (agitation) and vocalization, were observed for up to 3 h but were not observed at 6 h post-surgery (data not shown). The mean score of spontaneous pig behaviour 1 h post-surgery in G6 (full SMIR) was higher than the spontaneous behaviour score in G1 (full-skin incision only) animals (3.25 ± 0.63 vs. 1.67 ± 0.33, respectively; $p < 0.05$) (Fig. 9). Treatment of morphine at a dose of 1 mg/kg i.m. resulted in complete reduction of the spontaneous behaviour score in G3 animals that underwent full-skin incision. In animals with an incision that involved SMIR (G7), treatment with 1 mg/kg morphine i.m. resulted in a significant, yet incomplete reduction in the spontaneous behaviour score versus saline (vehicle)-treated animals (G6) (1.11 ± 0.31 vs. 3.25 ± 0.63; $p < 0.05$).

4. Discussion

This study was designed to evaluate the use of pig as a model of POP. Our results demonstrate that pigs experience enhanced nociceptive sensitivity to a mechanical stimulus, characterized by a significant decrease in withdrawal threshold to Von Frey filaments of the incised skin for 7 days post-surgery. Mechanical hyperalgesia after back shaving and full-skin incision in pigs was blocked in a dose-dependent manner for 3 h

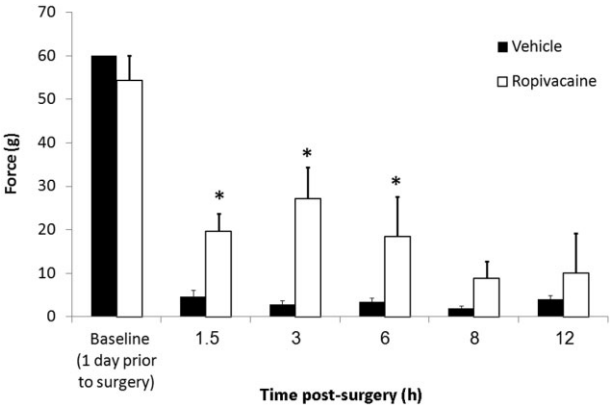

Figure 8 Analgesic effect of local treatment with ropivacaine following full-skin incision in pigs. The Von Frey filament force (g) required to achieve a withdrawal response on the incised side for full-skin incision pigs following local treatment with saline control (G4: full black column denoted vehicle) or 0.5% ropivacaine (G5: horizontal line column denoted ropivacaine) at baseline (1 day prior to surgery) and 1.5, 3, 6, 8 and 12 h post-surgery. Values are presented as mean ± standard error of the mean. At 8 h post dosing, no significant analgesic effect was measured. *$p < 0.05$ analgesic versus saline-treated pigs.

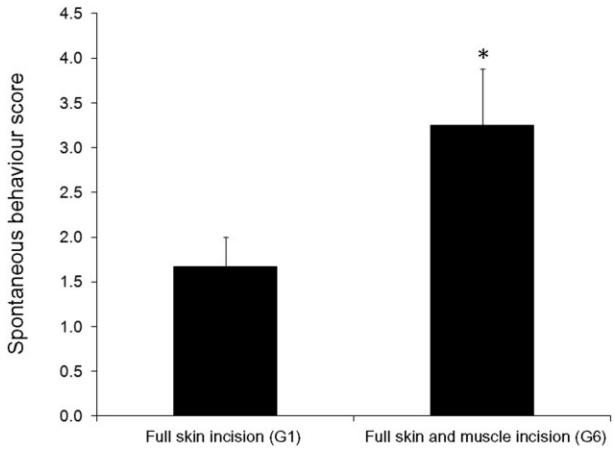

Figure 9 Mean spontaneous behaviour score. Total score of spontaneous pig behaviour 1 h post-surgery in G6 (full-skin and muscle incision and retraction) and in G1 (full-skin incision only) pigs. Values are presented as mean ± standard error of the mean. *$p < 0.05$ full-skin and muscle incision and retraction versus full-skin incision.

by systemic morphine (1 mg/kg), a common opioid analgesic administered to manage pain in post-operative patients (Li et al., 2001). However, systemic morphine administration after full SMIR was significantly less effective at the same dose. Local treatment with the anaesthetic drug ropivacaine partially reversed incision-induced mechanical hypersensitivity in pigs. Wound scoring of all animals and histology findings of the G1-treated group show that the incisions healed normally with no sign of infection or swelling. Although histopathological analysis was not performed on the other treated groups (G2–G7), wound examination by gross observation indicated that the mechanical hypersensitivity observed in pigs was not the result of interference from wound infection or inflammation. We also show that following incision, there was a transient increase in the pigs' spontaneous behaviour scores – behaviours that share a similar time course to pain measures in post-operative patients (Stubhaug et al., 1997; Brennan, 1999). Spontaneous behaviours were more pronounced when muscle retraction was involved.

Seven-week-old weaned pigs were used in our study for the following reasons: Young pigs are easier to handle, acclimatize and train better than adult pigs. Also, although pigskin is somewhat thicker overall than human skin, it becomes especially pronounced on the back in older pigs when they reach sexual maturity (Swindle, 2007). In addition, other similarities have been previously highlighted between pigs and humans, such as the similarity in the innervation patterns of the skin – characterized in the pig hindlimb (Lynn et al., 1995) – and the fact that the distribution and number of porcine hair follicles is closer to humans than is rodents. Such similarities make the pig an interesting model in the study of human outcomes of cutaneous events. In recent years, the translational potential of using large animal models within pain research has been emphasized (Henze and Urban, 2011). Indeed, a few studies have been published that support our findings using the pig as a translational animal model of nociceptive sensitivity (Swindle et al., 2011). For example, a study by Obreja et al. (2009) suggests that nociceptive and non-nociceptive fibre classes found in the skin of the pig correlate with human fibre classes, in both distribution and axonal excitability changes. Although this study refers to C-fibres and not to A-fibres that mediate tactile response, the investigation provides important evidence of the similarities of pigskin and its innervation to humans. Di Giminiani et al. (2012) showed that domestic pigs can be used to assess cutaneous nociception by behaviour responses to thermal and mechanical stimulations. More recently, a subsequent study by the same authors assessed the nociceptive responses in pigskin following cutaneous inflammation (Di Giminiani et al., 2013). Our present pig model of incisional pain may provide further information regarding nociceptive sensitivity and behaviour.

In a study by Reyes et al. (2002), blinded observation was used to compare the effects of analgesics in inguinal-incised domestic pigs. The authors suggest that the post-operative NRS scores used to measure the spontaneous behaviour of the pigs are related to the level of pain. Notably, the pigs used were similarly housed in open pens and were approximately the same weight and age on the day of surgery as the pigs used in our study. There are however disadvantages with the inguinal model of incision. For example, because pigs lie on their stomach, the possibility of successfully using local analgesics and keeping the groin area clean and uninfected is limited. In addition, our study, which uses a modified Reyes et al. (2002) NRS method for scoring spontaneous behaviour, shows that changes in pig behaviour are transient and limited to 3 h. Reyes et al. (2002) monitored pig's behaviour for 24 h and reported a 6-h time frame. The difference in the time frame of the NRS observation in our study may be due to the difference in wound location.

Human clinical studies using the Von Frey test to assess post-incisional mechanical hyperalgesia and pain have been reported (Bornemann-Cimenti et al., 2012). Bornemann-Cimenti et al. (2012) showed that the mechanical sensitivity range to Von Frey filaments

in patients at baseline (prior to surgical incision) and 48 h post-surgery was reduced from 93 ± 1.95 to 16 ± 3.63 g (mean ± standard deviation), respectively, in the placebo-treated group. Echevarria et al. (2011) also reported data suggesting that the Von Frey method is useful for comparing mechanical pain threshold changes between patient groups. Reported baseline pain thresholds to a mechanical stimulus were 69 (50.2, 95.1) g (i.e., pre-surgery) and an estimated 36 (27.0, 51.0) g post-surgery in the patient control group [values reported as mean (95% confidence interval)]. Hence, the data from previous human studies correlate to the mechanical sensitivity thresholds that were measured in pigs receiving saline (vehicle control) (G1: 60.00 ± 00.00 g pre-incision and 10.00 ± 1.69 g 7 days post-incision; mean ± SEM).

The hypersensitivity to the mechanical stimulus and the increase in spontaneous behaviour score were more pronounced in pigs that underwent SMIR. This result is consistent with findings in rats following incisions involving muscle as compared with those involving skin and fascia alone, and supported by studies that suggest a possible role for nerve growth factor (NGF) as an important mediator of pathologic pain (Wu et al., 2009). A study by Rukwied et al. (2010) proposed that direct injection of NGF into pigskin involves peripheral sensitization of skin nociceptors. Whether levels of NGF or other pain mediators are elevated following SMIR as compared with full-skin incision in the pig model has yet to be determined.

We observed that the analgesic activity of systemic morphine was dose dependent. A single dose of 0.1 mg/kg was not effective in relieving pain following full-skin incision; however, a single dose 10 times higher (1 mg/kg) was significantly effective in relieving pain ($p < 0.05$). The maximum analgesic effect of 1 mg/kg morphine administered using a single i.m. dose occurred at 0.5 h post-treatment and markedly decreased 6 h post-treatment. These observations are consistent with a previous study showing that the maximal analgesic effect of morphine at a dose of 1.5 mg/kg using an intravenous route occurred 1 h post-treatment in pigs; but at 8 h post-treatment, the effect of morphine was no longer detected (Risdahl et al., 1992).

Our study also assessed the effect of the local anaesthetic ropivacaine. We report that localized treatment with ropivacaine significantly reversed mechanical hypersensitivity for a period of at least 6 h. A previous pharmacokinetic study reported that ropivacaine clearance following systemic administration in pigs is also approximately 6 h (Betton et al., 2010). Additionally, a double-blind randomized controlled trial in humans showed that single-shot infiltration with ropivacaine transiently improves POP after breast cancer surgery (Vigneau et al., 2011). The study by Vigneau et al. (2011) reports that the analgesic effect of localized ropivacaine treatment in post-operative breast cancer patients was significant for 6 h ($p < 0.05$); at 8 h post-treatment, no analgesic effect was detected. Together, these findings suggest that the duration of analgesia from local treatments such as ropivacaine is similar in pigs and humans, and supports our proposal of the pig as an appropriate animal model for research and development of novel local analgesics.

In this present study, we demonstrate that incision-induced responses to Von Frey filaments before and after administration of analgesics were comparable with responses reported previously in humans. The observed changes of nociceptive sensitivity persisted for at least 7 days post-surgery, enabling possible assessment by repeated treatment of new analgesic agents. Moreover, the caudal location of the incision on the lower back is convenient for the application of patches, topical creams or gels, implants or other local treatments, as well as for injections. In conclusion, we propose that this pig model provides greater translational relevance for the evaluation of local treatments of POP compared with existing rodent models of incisional pain. We also propose that our model of incisional pain in the pig is applicable for the study of wound healing. Further research is needed to better understand the mechanisms underlying the difference in withdrawal response to mechanical stimulus following full-skin incision versus SIMR.

Author contributions

D.C. was the surgeon who conducted the *in vivo* phase. E.W. was responsible for the animals' general welfare. O.D. was responsible for the pig facility. O.B. was responsible for the histopathology analysis. S.M. directed the study group and takes final responsibility for the manuscript. All authors discussed the results and commented on the manuscript.

Acknowledgements

Klara Belzar, PhD, and XLR8 Health Ltd provided medical writing assistance for this manuscript.

References

Apfelbaum, J.L., Chen, C., Mehta, S.S., Gan, T.J. (2003). Postoperative pain experience: Results from a national survey suggest postoperative pain continues to be undermanaged. *Anesth Analg* 97, 534–540.

Bancroft, J.D., Gamble, M. (2008). *Theory and Practice of Histological Techniques* (Edinburgh: Churchill Livingstone) pp. 1–744.

Bannon, A.W., Malmberg, A.B. (2007). Models of nociception: Hot-plate, tail-flick, and formalin tests in rodents. *Curr Protoc Neurosci* 41, 8.9.1–8.9.16.

Berry, P.H., Dahl, J.L. (2000). The new JCAHO pain standards: Implications for pain management nurses. *Pain Manag Nurs* 1, 3–12.

Betton, D., Greib, N., Schlotterbeck, H., Joshi, G.P., Ubeaud-Sequier, G., Diemunsch, P. (2010). The pharmacokinetics of ropivacaine after intraperitoneal administration: Instillation versus nebulization. *Anesth Analg* 111, 1140–1145.

Bornemann-Cimenti, H., Lederer, A.J., Wejbora, M., Michaeli, K., Kern-Pirsch, C., Archan, S., Rumpold-Seitlinger, G., Zigeuner, R., Sandner-Kiesling, A. (2012). Preoperative pregabalin administration significantly reduces postoperative opioid consumption and mechanical hyperalgesia after transperitoneal nephrectomy. *Br J Anaesth* 108, 845–849.

Brennan, F., Carr, D.B., Cousins, M. (2007). Pain management: A fundamental human right. *Anesth Analg* 105, 205–221.

Brennan, T.J. (1999). Postoperative models of nociception. *ILAR J* 40, 129–136.

Brennan, T.J., Vandermeulen, E.P., Gebhart, G.F. (1996). Characterization of a rat model of incisional pain. *Pain* 64, 493–501.

Clark, J.D., Shi, X., Li, X., Qiao, Y., Liang, D., Angst, M.S., Yeomans, D.C. (2007). Morphine reduces local cytokine expression and neutrophil infiltration after incision. *Mol Pain* 3, 28.

Curtin, L.I., Grakowsky, J.A., Suarez, M., Thompson, A.C., DiPirro, J.M., Martin, L.B., Kristal, M.B. (2009). Evaluation of buprenorphine in a postoperative pain model in rats. *Comp Med* 59, 60–71.

Dahl, J.B., Erichsen, C.J., Fuglsang-Frederiksen, A., Kehlet, H. (1992). Pain sensation and nociceptive reflex excitability in surgical patients and human volunteers. *Br J Anaesth* 69, 117–121.

de Grau, A., Dewey, C., Friendship, R., de Lange, K. (2005). Observational study of factors associated with nursery pig performance. *Can J Vet Res* 69, 241–245.

Di Giminiani, P., Petersen, L.J., Herskin, M.S. (2012). Nociceptive responses to thermal and mechanical stimulations in awake pigs. *Eur J Pain* 17, 638–648.

Di Giminiani, P., Petersen, L.J., Herskin, M.S. (2013). Characterization of nociceptive behavioural responses in the awake pig following UV-B-induced inflammation. *Eur J Pain*. doi: 10.1002/j.1532-2149.2013.00340.x

Echevarria, G., Elgueta, F., Fierro, C., Bugedo, D., Faba, G., Iniguez-Cuadra, R., Munoz, H.R., Cortinez, L.I. (2011). Nitrous oxide (N(2)O) reduces postoperative opioid-induced hyperalgesia after remifentanil-propofol anaesthesia in humans. *Br J Anaesth* 107, 959–965.

Henze, D.A., Urban, M.O. (2011). Large animal models for pain therapeutic development. In *Translational Pain Research: From Mouse to Man*, L. Kruger, A.R. Light, eds. (Boca Raton, FL: CRC Press) pp. 371–385.

Li, X., Angst, M.S., Clark, J.D. (2001). Opioid-induced hyperalgesia and incisional pain. *Anesth Analg* 93, 204–209.

Lorentzen, V., Hermansen, I.L., Botti, M. (2012). A prospective analysis of pain experience, beliefs and attitudes, and pain management of a cohort of Danish surgical patients. *Eur J Pain* 16, 278–288.

Lynn, B., Faulstroh, K., Pierau, F.K. (1995). The classification and properties of nociceptive afferent units from the skin of the anaesthetized pig. *Eur J Neurosci* 7, 431–437.

McAnulty, P.A. (2012). *The Minipig in Biomedical Research* (Boca Raton, FL: CRC Press) p. 188.

Obreja, O., Ringkamp, M., Namer, B., Forsch, E., Klusch, A., Rukwied, R., Petersen, M., Schmelz, M. (2009). Patterns of activity-dependent conduction velocity changes differentiate classes of unmyelinated mechano-insensitive afferents including cold nociceptors, in pig and in human. *Pain* 148, 59–69.

Randall, L.O., Selitto, J.J. (1957). A method for measurement of analgesic activity on inflamed tissue. *Arch Int Pharmacodyn Ther* 111, 409–419.

Reyes, L., Tinworth, K.D., Li, K.M., Yau, D.F., Waters, K.A. (2002). Observer-blinded comparison of two nonopioid analgesics for postoperative pain in piglets. *Pharmacol Biochem Behav* 73, 521–528.

Risdahl, J.M., Chao, C., Murtaugh, M.P., Peterson, P.K., Molitor, T.W. (1992). Acute and chronic morphine administration in swine. *Pharmacol Biochem Behav* 43, 799–806.

Rukwied, R., Schley, M., Forsch, E., Obreja, O., Dusch, M., Schmelz, M. (2010). Nerve growth factor-evoked nociceptor sensitization in pig skin in vivo. *J Neurosci Res* 88, 2066–2072.

Stubhaug, A., Breivik, H., Eide, P.K., Kreunen, M., Foss, A. (1997). Mapping of punctuate hyperalgesia around a surgical incision demonstrates that ketamine is a powerful suppressor of central sensitization to pain following surgery. *Acta Anaesthesiol Scand* 41, 1124–1132.

Sullivan, T.P., Eaglstein, W.H., Davis, S.C., Mertz, P. (2001). The pig as a model for human wound healing. *Wound Repair Regen* 9, 66–76.

Swindle, M.M. (2007). *Swine in the Laboratory: Surgery, Anesthesia, Imaging, and Experimental Techniques* (Boca Raton, FL: CRC Press) p. 81.

Swindle, M.M., Makin, A., Herron, A.J., Clubb, F.J., Jr, Frazier, K.S. (2011). Swine as models in biomedical research and toxicology testing. *Vet Pathol* 49, 344–356.

Vigneau, A., Salengro, A., Berger, J., Rouzier, R., Barranger, E., Marret, E., Bonnet, F. (2011). A double blind randomized trial of wound infiltration with ropivacaine after breast cancer surgery with axillary nodes dissection. *BMC Anesthesiol* 11, 23.

Whiteside, G.T., Harrison, J., Boulet, J., Mark, L., Pearson, M., Gottshall, S., Walker, K. (2004). Pharmacological characterisation of a rat model of incisional pain. *Br J Pharmacol* 141, 85–91.

Wu, C., Erickson, M.A., Xu, J., Wild, K.D., Brennan, T.J. (2009). Expression profile of nerve growth factor after muscle incision in the rat. *Anesthesiology* 110, 140–149.

Wu, C.L., Raja, S.N. (2011). Treatment of acute postoperative pain. *Lancet* 377, 2215–2225.

Young, B., Stewart, W., O'Dowd, G., Wheater, P.R. (2002). *Wheater's Basic Pathology: A Text, Atlas and Review of Histopathology* (Edinburgh: Churchill Livingstone) p. 20.

Zimmermann, M. (1983). Ethical guidelines for investigations of experimental pain in conscious animals. *Pain* 16, 109–110.