Research in Veterinary Science 100 (2015) 252–256

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

# Transdermal fentanyl and its use in ovine surgery

Chris Christou *, Rema A. Oliver, John Rawlinson, William R. Walsh

*Surgical and Orthopaedic Research Laboratory, Prince of Wales Clinical School, University of New South Wales, Level 1 Clinical Sciences Building Avoca St, Randwick 2031 Australia*

ARTICLE INFO

*Article history:*
Received 5 January 2015
Accepted 6 April 2015

*Keywords:*
Analgesia
Fentanyl
Pre-emptive
Sheep
Ovine

ABSTRACT

Fentanyl delivered via a transdermal patch has the potential to decrease the need for post-operative handling of sheep undergoing surgical procedures. Two studies were performed to test: (1) the ideal timing for the application of pre-emptive analgesic patches and (2) the efficacy of a 2 μg/kg/h dose, as extrapolated from other species.

The first study had sheep divided into two groups. Group 1 had a fentanyl patch applied for 24 h prior to a patch change and group 2 had a fentanyl patch applied 72 h prior to a change.

The second study applied the results obtained in the first and tested the efficacy of 2 μg/kg/h as an effective dose in an orthopaedic surgical environment.

Results indicated that the ideal time for pre-emptive fentanyl patch administration is 24–36 h prior to surgery and that 2 μg/kg/h is an effective minimum therapeutic dose rate for the use of fentanyl as an analgesic in an orthopaedic surgical environment.



## 1. Introduction

With the use of sheep as pre-clinical orthopaedic research models gaining momentum, the ethical considerations regarding their husbandry and the control of pain becomes paramount. The Australian code for the care and use of animals for scientific purposes states “The use of local and general anaesthetics, analgesics and sedatives must be considered as part of a plan to manage pain and distress, and such use should at least parallel their use in current veterinary or medical practice.”(Australian Government, 2013)

The definition of pain has been debated philosophically over the ages and has changed as knowledge has increased. Pain has been described as an unpleasant sensory or emotional experience associated with actual or perceived tissue damage (Rollin, 1999).

Pain can arise from two physiological processes, tissue damage and the associated release of prostaglandins and other inflammatory cytokines, and from direct nerve damage or stimulation. Sensitisation to pain occurs as a result of a decreased threshold of nociceptor peripheral nerve terminals and an increased excitability of central neurones (Woolf and Chong, 1993). There are two major classes of systemic analgesics, non-steroidal anti-inflammatory drugs (NSAIDs) and opioids. NSAIDs assist with minimising the effects of the inflammatory cytokines in the peripheral tissues. Opioids work by increasing the threshold of excitability of the peripheral and central neurones (Lotsch et al., 2013). The daily need for injection of NSAIDs and the short 3–6 h duration of action for the commonly used opioids increases the demand of handling the sheep after any operative procedure which is expected to induce ongoing pain. This increased post-operative handling and injecting of the sheep does not only increase stress, it can also lead to further injury should the animal panic during the handling process. The effects NSAIDs can have on bone healing, via their anti-prostaglandin action, can also lead to complications in result reporting and is a significant consideration in fracture healing models (Barry, 2010; Pountos et al., 2012). The opioid mechanism of action is via receptors in the central and peripheral nervous system and therefore opioid analgesics have no direct impact on bone healing (Sehgal et al., 2011). Three major opiate receptor types are recognised within the central nervous system (CNS) and were named based on the compound that originally resulted in specific receptor binding. These are the mu (μ), delta (δ), and kappa (κ) opiate receptors that bind to morphine, dynorphin, and ketocyclazocine, respectively (Kukanich and Clark, 2012). mu-Receptor agonists are noted for their ability to produce profound analgesia with mild sedation (Hofmeister and Egger, 2004).

Fentanyl is an opioid analgesic developed as an alternative to morphine in 1960 in an effort to provide increased potency and fewer side effects (Stanley, 1992). Like morphine, fentanyl binds to mu receptors, but it has a potency 80–100 times greater than morphine (Ahern et al., 2009). The major drawback of opioid analgesics, however, is their short half-life after IV or IM injection which again raises the issue of increased post-operative handling.

Since 1991 fentanyl has become available as a slow release transdermal patch sold under the trade name Durogesic by Janssen

* Corresponding author. Surgical and Orthopaedic Research Laboratory, Prince of Wales Clinical School, University of New South Wales, Level 1 Clinical Sciences Building Avoca St, Randwick 2031, Australia. Tel.: +61 2 93824040; fax: +61 3 93822660.

*E-mail address:* c.christou@unsw.edu.au (C. Christou).

http://dx.doi.org/10.1016/j.rvsc.2015.04.006
0034-5288/

Pharmaceuticals (Hofmeister and Egger, 2004). While developed for use in human medicine, fentanyl patches have also found favour as an off label analgesic in the veterinary world. Fentanyl has been studied in many species including dogs, cats, horses and sheep at a dose rate of 2 µg/kg/h (Ahern et al., 2010; Hofmeister and Egger, 2004; Orsini et al., 2006). A drawback of the fentanyl patch is its slow uptake to therapeutic levels following application (Ahern et al., 2010). In an effort to provide effective analgesia when required, that is intra and post operatively, the timing for the pre-emptive application of the fentanyl patches was investigated.

The use of pre-emptive analgesia to assist in the control of post-surgical pain is a concept that has been introduced and debated for many years; while no consensus has been reached, many studies show a beneficial effect (Mishra et al., 2013; Ong et al., 2005; Woolf and Chong, 1993). With the potential benefits of anticipating a noxious stimulus outweighing any contraindications, our studies aim to find an effective and practical regimen for the use of fentanyl patches in an ovine orthopaedic research situation. The optimal analgesic concentration of fentanyl in sheep has not been determined and all reported effective blood concentrations are based on extrapolation from other species (Ahern et al., 2010). In humans, Lane reported that the minimum blood concentration required to achieve effective analgesia is 1 ng/ml of blood (Lane, 2013), however Gourlay et al. have demonstrated it to be as low as 0.23 ng/ml in some human patients (Gourlay et al., 1988).

The aims of the studies were to evaluate the optimal pre-emptive regimen for the application of fentanyl patches in an effort to maximise post-surgical analgesia and to test the dose rate required to achieve effective analgesic effects in a surgical environment.

## 2. Methods

### 2.1. Study 1 – To determine optimal pre-operative timing of patch application

Ethical approval was gained from the Animal Care & Ethics Committee of the University of New South Wales (12/115A). Eight, 2 year old cross-bred Merino wethers were divided into pairs and acclimatised in their respective stalls (6 $m^2$) for 1 week prior to the study; the duration of the study was 6 days. The sheep were maintained on a diet of lucerne hay, chaff and water *ad libitum*.

The sheep were randomly allocated into 2 groups of $n = 4$, each group received two patches throughout the course of the study.

For group 1 the second fentanyl patch was applied 24 h, following removal of the first patch and for group 2 the second patch was applied 72 h after removal of the first (Fig. 1). No surgical procedures were performed as only the drug's absorption and elimination curve was investigated in this study.

One 100 µg/kg/h fentanyl patch was assigned to each sheep per application; given their weights were 85.9 kg ± 7.5 kg this produced a dose rate range of 1–1.6 µg/kg/h.

All patches were applied to the left antebrachium on the cranial aspect just below the elbow (Fig. 2). To apply the patches the sheep were manually restrained by one person placing one arm behind the rump and the other under the jaw, while using lateral pressure to constrain the sheep against the wall of the pen. Each leg was clipped with no. 40 clipper blades with care taken so as not to graze or cut the skin. The skin was cleaned with a 70% v/v chlorhexidine/alcohol solution and was allowed to fully dry before the patch was applied. Following patch application, the leg was then wrapped with a single layer of elastic conforming bandage (Vetrap, 3M) and this was held in place with sticking plaster (Tensoplast Vet, Smith & Nephew). This ensured good patch/skin contact with no slippage.

For blood sampling each sheep was held by the same means used for patch application; sampling was performed via jugular venipuncture using a 19G needle according to the time points described in Fig. 1; 4 ml of blood was collected into a plain vacuum blood tube (Vacutiner, BD Medical). The whole blood was allowed to stand for 10 min for the clotting process to begin, before being centrifuged at two times the force of gravity (2*g*) for a further 10 min. The serum was then extracted using a micropipette and placed into cryotubes for immediate freezing at −18 °C.

Fentanyl levels were measured in duplicate using a commercially available, human enzyme-linked immunosorbent assay (ELISA) Fentanyl kits (BQ Kits), according to the manufacturer's instructions. A negative and positive standard was provided with each kit. The negative standard was tested against normal sheep serum. The absorbance was read at 570 nm/650 nm using a microplate absorbance reader (Tecan Sunrise). Each sample was tested in triplicate, the ELISA results were collated and mean drug levels along with their SD were calculated based on conversion factors obtained from the negative and positive standards results.

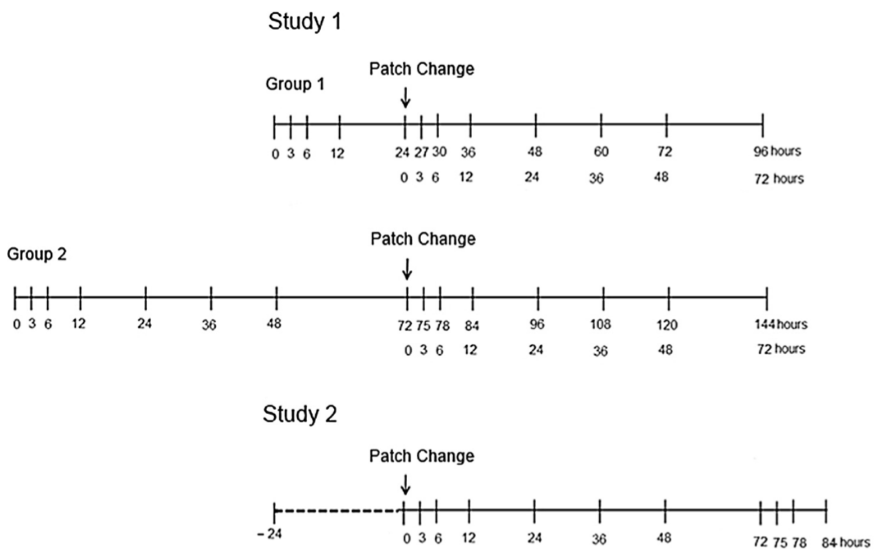


**Fig. 1.** Study designs – Timelines of the groups and their allocated blood collection times.

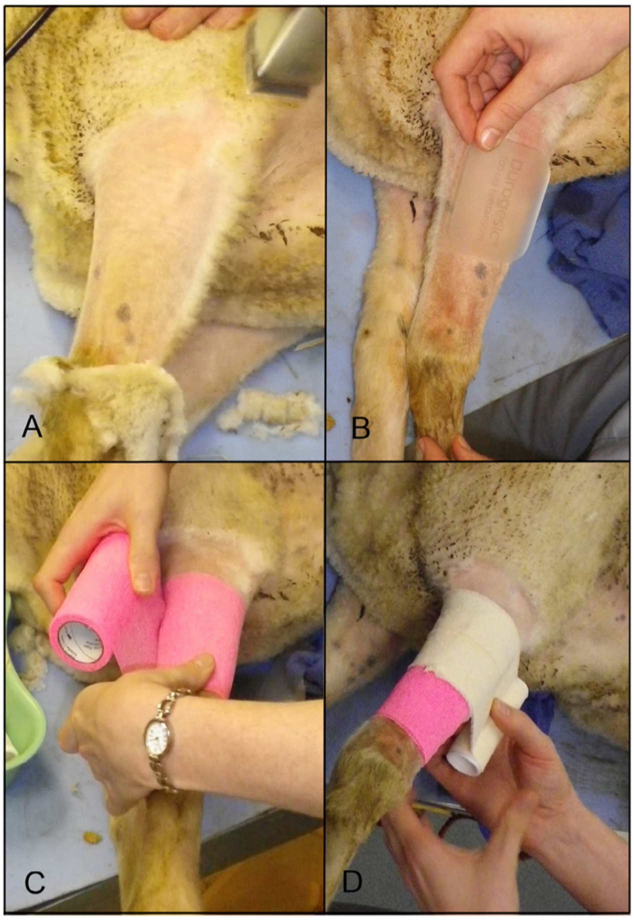


**Fig. 2.** Stages of patch application. A: Clipping the wool with fine clippers; B: application of the patch after cleaning of the skin; C: securing the patch with non adhesive Vetrap; D: final layer of Tensoplast to ensure good skin adhesion and durability.

The data were analysed using SPSS (IBM SPSS Statistics 20), significance levels were set at $P = 0.05$. After testing for normality of distribution using the Shapiro–Wilk test, the means were compared across corresponding time points from each group following application of the second patch using a generalised linear model with a post-hoc Bonferroni correction.

Clinical observations, such as the demeanour of the sheep, their responsiveness to environmental stimuli and their alertness, were recorded during the course of the study to ascertain any behavioural changes or sedative effects as a result of the fentanyl. These were performed by the authors CC and JR.

### 2.2. Study 2 – Effectiveness of 2 µg/kg/h as a recommended dose rate

Following Ethical approval from the Animal Care & Ethics Committee of the University of New South Wales (14/51B), the four sheep enrolled in this study were due to undergo a multilevel lumbar intervertebral spinal fusion. The sheep were weighed and patches applied to achieve a minimum dose rate of 2 µg/kg/h. The average weight of the sheep was 68.5 kg ± 8 kg. Fentanyl patches come in 100 µg/kg/h, 50 µg/kg/h and 25 µg/kg/h sizes. Given the range of weights, each sheep received a 100 µg/kg/h and a 50 µg/kg/h patch per application. This provided an effective dose range of 2–2.5 µg/kg/h.

Pre-emptive patches were applied 24 h before surgery based on the results obtained in the first study. Following pre-anaesthetic sedation with tiletamine/zolazepam (Zoletil Virbac) at 5 mg/kg intramuscularly, general anaesthesia was induced using isoflurane (Forthane Abbott) via mask induction prior to intubation. The first patches were removed and the second patches applied at this time achieve a minimum 72 h of post-operative analgesia. Blood sampling and fentanyl levels were measured as described earlier, following time points described in Fig. 1. At the end of the prescribed 72 h effective life of the fentanyl patch a further 3 blood samples were added at 75, 78 and 84 h, to investigate any residual capacity of the patches.

The behavioural responses of the sheep were monitored and recorded from the time of the first patch application pre-surgery. Post-surgical monitoring concentrated on the relative comfort of the animals based on posture, demeanour, ambulation and food and water intake. All observed parameters were recorded as part of the animal's surgical record. The observations were tabulated and used as comparative measures throughout the study.

## 3. Results

### 3.1. Study 1

After acclimatisation, all of the sheep were accustomed to human presence, they did not panic when the holding room was entered, but remained reluctant to be cornered and caught in their pens. Once caught the sheep were co-operative, however they maintained high muscle tone while they were manually restrained to be clipped and have their patches applied. Following the application of the first fentanyl patch, at the blood sampling time of 3 h, cornering the sheep became easier, they stood calmly while blood was being drawn however muscle tone was high. By the 6 h time point the sheep were relaxed with normal muscle tone and were easily approachable. They stood calmly and were even curiously approaching while blood was being drawn from their partner in the pen. This behaviour remained up to the 36 h time point at which time cornering the sheep in the pen became marginally harder, they still stood calmly for the blood sampling and curiosity still persisted. At 48 h post patch application, cornering the sheep was similar to the 3 h time point; they stood well, but were no longer curious as to the blood being drawn from their partner. By 72 h, all sheep co-operation had dissipated. This behavioural cycle was repeated following the application of the second patch. The group 1 sheep did not appear to be more heavily sedated than the group 2 sheep following the application of their second patch. (See Table 1 for summary.)

The ELISA analysis indicated a rapid rise in serum levels to the 12 h point post first patch application in both groups (Figs. 3 and 4). This was followed by a gradual increase to peak levels at approximately 36 h for group 2. In both groups the application of the second patch resulted in a further elevation of drug levels with a second peak in the blood profile curve, indicative of the drug pre-loading provided by the first patch, this second peak coincided with the 6–12 h time points.

No significant differences were seen between the corresponding mean blood levels for each of the time points between the two groups post second patch, for the peak serum levels or duration of fentanyl presence post application.

### 3.2. Study 2

Following application of the first patch, behavioural changes and sedation of the sheep followed the same time-line pattern as in the first study, where by 24 h, 3 of the 4 sheep were no longer concerned by our presence and head carriage was lower than normal. At the time of patch change it was discovered that the patch of the fourth sheep had slipped under its bandaging material, explaining

**Table 1**
Relative degrees of sedation as recorded by observers CC and JR in the first study. (Note the 24 hour point and time zero for the second patch in group 1 are the same observation. The 72 hour point and time zero for the second patch in group 2 are the same observation.)

| | Study 1 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Sheep | Time after first patch | | | | | | | |
| | | 0 | 3 | 6 | 12 | 24 | 36 | 48 | 72 |
| Group 1 | 2485 | 0 | + | ++ | ++ | ++ | | | |
| | 2486 | 0 | + | ++ | ++ | ++ | | | |
| | 2487 | 0 | + | ++ | ++ | ++ | | | |
| | 2488 | 0 | + | ++ | ++ | ++ | | | |
| Group 2 | 2481 | 0 | + | ++ | ++ | ++ | ++ | + | + |
| | 2482 | 0 | + | ++ | ++ | ++ | ++ | + | + |
| | 2483 | 0 | + | ++ | ++ | ++ | ++ | + | + |
| | 2484 | 0 | + | ++ | ++ | ++ | ++ | + | + |
| | Sheep | Time after second patch | | | | | | | |
| | | 0 | 3 | 6 | 12 | 24 | 36 | 48 | 72 |
| Group 1 | 2485 | ++ | ++ | ++ | ++ | ++ | ++ | + | + |
| | 2486 | ++ | ++ | ++ | ++ | ++ | ++ | + | + |
| | 2487 | ++ | ++ | ++ | ++ | ++ | ++ | + | + |
| | 2488 | ++ | ++ | ++ | ++ | ++ | ++ | + | + |
| Group 2 | 2481 | + | ++ | ++ | ++ | ++ | ++ | + | + |
| | 2482 | + | ++ | ++ | ++ | ++ | ++ | + | + |
| | 2483 | + | ++ | ++ | ++ | ++ | ++ | + | + |
| | 2484 | + | ++ | ++ | ++ | ++ | ++ | + | + |

Key: 0 = no sedation.
+ = Mild sedation – standing, pliable, head position normal.
++ = Moderate sedation – standing, pliable, head position lowered.
+++ = Heavy sedation – standing, not concerned with surroundings.
++++ = Recumbent.

its relative lack of sedation. The study was continued as prescribed with a second patch application for this animal and its surgery being performed per the protocol. Extra care was taken to ensure no discomfort was evident post-operatively for this animal due to the possibility of lower blood levels due to patch slippage. The effective single patch application protocol for this animal showed an overall reduced drug level profile over the course of the study (Fig. 5, 2588). Post-operatively all the sheep remained calm and did not show any outward signs of discomfort such as reduced appetite, reduced ambulation or teeth grinding. Sheep 2588 remained more alert than the others, but still did not appear anymore distressed or in pain and required no ancillary analgesia for breakthrough pain.

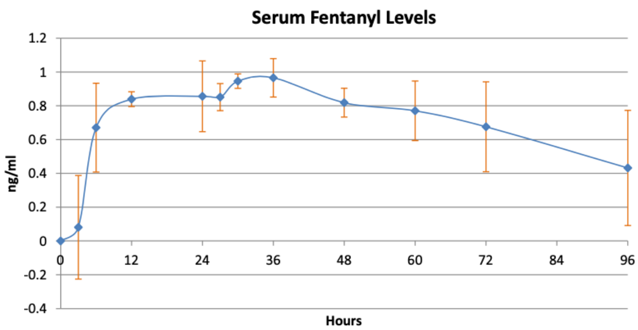


**Fig. 3.** The mean (±SD) serum concentration of fentanyl from group 1 animals where the second patch was applied after 24 h.

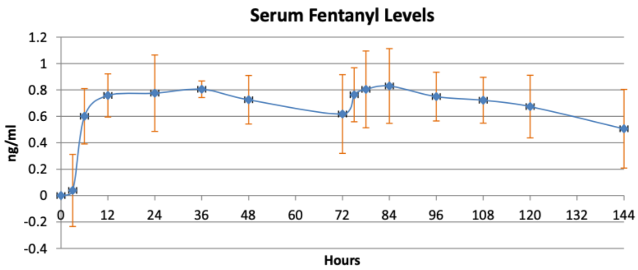


**Fig. 4.** The mean (±SD) serum concentration of fentanyl from group 2 animals where the second patch was applied after 72 h.

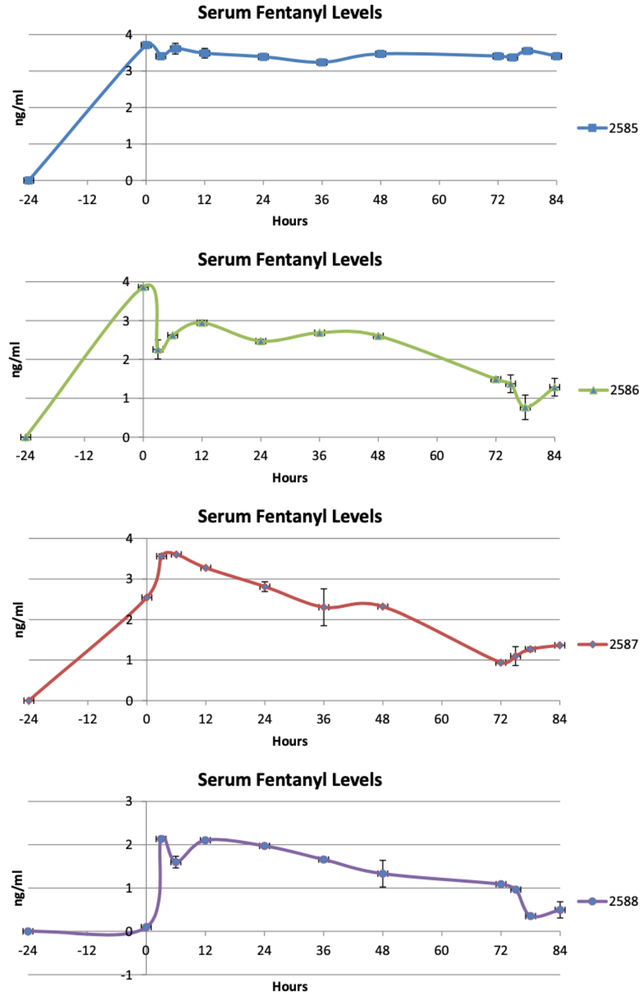


**Fig. 5.** The mean (±SD) serum concentration of fentanyl of each sheep in the second study. Note different upper limits of scale for animal 2588.

The fentanyl blood levels measured in the four sheep enrolled in this study are presented in Fig. 5. As can be seen, all the animals achieved measured blood concentrations of fentanyl above 1 ng/ml (the minimum therapeutic level suggested by Lane, 2013). Sheep 2588 had a lower peak blood concentration and a shorter duration of action as a result of the first patch not adhering effectively, but its levels still remained in the reported therapeutic range. Continued drug release for a minimum of 12 h post the prescribed 72 h period was observed in all four sheep, however maintenance of therapeutic levels cannot be guaranteed (Fig. 5).

## 4. Discussion

The aim of the first study in this series was to quantify the drug absorption and elimination curves of fentanyl using a strict application method to see if there was a difference between the two nominated application protocols. No statistical differences were detected between the application protocols when peak drug levels and duration of action were compared. The concept of changing patches at the time of surgical intervention helps to provide a minimum of 72 h post-surgical analgesia without the need for stressful post-operative re-handling, especially when orthopaedic procedures are performed and the risk of post-operative injury is high. The effective dose rate for this study, though below values extrapolated from other animals, was enough to produce mild sedation and malleability in handling commensurate with the drug levels attained in the blood. When the findings of the first study were applied to a clinical scenario in the second study, greater sedation and effective analgesia were achieved; this was reflected by the increased drug levels of fentanyl recorded. The results of the second study confirm a minimum dose rate of 2 µg/kg is required to achieve effective therapeutic levels as extrapolated from other species (Hammond et al., 2008). The animal where the first patch did not adhere to the skin provided valuable insight into the benefits and effectiveness of pre-emptive application of fentanyl to achieve therapeutic blood levels. Without pre-loading the peak blood levels attained and their duration were both reduced.

When observations regarding an animal's welfare and behaviour are made, much is dependent on the skill level and experience of the observer. The conclusion on whether an animal is in any pain or discomfort is a relative conclusion based on previous surgical cases. In our studies CC and JR have over 60 years of clinical experience with ovine husbandry and surgery between them.

The ethical treatment of animals leads to a limitation of the second study in that it precluded the use of a control surgical group with no post-operative analgesia. The aim of this study was to evaluate the pharmacokinetics and clinical efficacy of fentanyl patches for analgesia thereby reducing the need for post-operative handling and the injecting of drugs. Another potential limitation is that, while the comfort and wellbeing of each animal were observed and recorded based on clinical observations, no relative comparisons were performed within the study. The use of a human based ELISA test kit may also be considered a limitation to this study. However negative controls were tested using sheep serum and no cross reactivity was found. The kits were used consistently across all of the animals and studies.

Ahern et al. (2009, 2010) have provided valuable data regarding the use of fentanyl in sheep however none of the studies have included the use of a pre-emptive loading dose and as such the resultant fentanyl blood levels attained were lower than those realised in our studies.

The manufacture of a fentanyl patch is such that the rate limiting step in the release of the drug is controlled at the patch in an effort to reduce patient based absorption variables (Lane, 2013). The variables can include skin temperature, skin thickness and preparation (Hofmeister and Egger, 2004; Knutson et al., 1987; Roy and Flynn, 1990). These variables were kept constant as possible in this protocol by applying the patches to the same area on the sheep in a temperature controlled environment.

## 5. Conclusion

The first study showed that no statistical differences were detected between the two protocols when fentanyl blood levels were compared. We can therefore conclude that for pre-emptive analgesia the timing for changing of a fentanyl patch is not critical when replacement occurs between 24 and 72 h after the first.

The second study showed that a dose rate of 2 µg/kg in combination with a pre-emptive patch blood levels above 1 ng/ml of fentanyl was maintained for a minimum of 72 h post second patch application. At this dose rate and blood levels, clinical observations indicated that the animals appeared comfortable and did not show signs of distress.

## Acknowledgements

The authors would like to thank Greg Mitchell for his assistance in all the husbandry and animal handling procedures.

## References

Ahern, B.J., Soma, L.R., Boston, R.C., Schaer, T.P., 2009. Comparison of the analgesic properties of transdermally administered fentanyl and intramuscularly administered buprenorphine during and following experimental orthopedic surgery in sheep. American Journal of Veterinary Research 70, 418–422.
Ahern, B.J., Soma, L.R., Rudy, J.A., Uboh, C.E., Schaer, T.E., 2010. Pharmacokinetics of fentanyl administered transdermally and intravenously in sheep. American Journal of Veterinary Research 71, 1127–1132.
Australian Government., 2013. Australian code for the care and use of animals for scientific purposes. In: National Health and Medical Research Council (Ed.), eighth ed.
Barry, S., 2010. Non-steroidal anti-inflammatory drugs inhibit bone healing: a review. Veterinary Comparative Orthopaedics and Traumatology 23, 385–392.
Gourlay, G.K.P., Kowalski, S.R.B., Plummer, J.L.P., Cousins, M.J.M.D., Armstrong, P.J.M., 1988. Fentanyl blood concentration-analgesic response relationship in the treatment of postoperative pain. Anesthesia & Analgesia 67, 329–337.
Hammond, R.C., Christie, M., Nicholson, A., 2008. Opioid analgesics. In: Maddison, J.P., Page, S.W., Church, D. (Eds.), Small Animal Clinical Pharmacology, second ed. Elsevier, pp. 309–329.
Hofmeister, E.H., Egger, C.M., 2004. Transdermal fentanyl patches in small animals. Journal of the American Animal Hospital Association 40, 468–478.
Knutson, K., Krill, S.L., Lambert, W.J., Higuchi, W.I., 1987. Physicochemical aspects of transdermal permeation. Journal of Controlled Release 6, 59–74.
Kukanich, B., Clark, T.P., 2012. The history and pharmacology of fentanyl: relevance to a novel, long-acting transdermal fentanyl solution newly approved for use in dogs. Journal of Veterinary Pharmacology and Therapeutics 35, 3–19.
Lane, M.E., 2013. The transdermal delivery of fentanyl. European Journal of Pharmaceutics and Biopharmaceutics 84, 449–455.
Lotsch, J., Walter, C., Parnham, M.J., Oertel, B.G., Geisslinger, G., 2013. Pharmacokinetics of non-intravenous formulations of fentanyl. Clinical Pharmacokinetics 52, 23–36.
Mishra, A., Afzal, M., Mookerjee, S., Bandyopadhyay, K., Paul, A., 2013. Pre-emptive analgesia: recent trends and evidences. Indian Journal of Pain 27, 114–120.
Ong, C.K.-S., Lirk, P., Seymour, R.A., Jenkins, B.J., 2005. The efficacy of preemptive analgesia for acute postoperative pain management: a meta-analysis. Anesthesia & Analgesia 100, 757–773.
Orsini, J.A., Moate, P.J., Kuersten, K., Soma, L.R., Boston, R.C., 2006. Pharmacokinetics of fentanyl delivered transdermally in healthy adult horses – variability among horses and its clinical implications. Journal of Veterinary Pharmacology and Therapeutics 29, 539–546.
Pountos, I., Georgouli, T., Calori, G.M., Giannoudis, P.V., 2012. Do nonsteroidal anti-inflammatory drugs affect bone healing? A critical analysis. Thescientificworldjournal 2012, 606404.
Rollin, B.E., 1999. Some conceptual and ethical concerns about current views of pain. Pain Forum 8, 78–83.
Roy, S.D., Flynn, G.L., 1990. Transdermal delivery of narcotic analgesics: pH, anatomical, and subject influences on cutaneous permeability of fentanyl and sufentanil. Pharmaceutical Research 7, 842–847.
Sehgal, N., Smith, H.S., Manchikanti, L., 2011. Peripherally acting opioids and clinical implications for pain control. Pain Physician 14, 249–258.
Stanley, T.H., 1992. The history and development of the fentanyl series. Journal of Pain and Symptom Management 7, S3–S7.
Woolf, C.J., Chong, M.S., 1993. Preemptive analgesia-treating postoperative pain by preventing the establishment of central sensitization. Anesthesia & Analgesia 77, 362–379.