AnestaGel – P™ Provides Greater Analgesia in a Rat Model of Post-Operative Incisional Pain with Mechanical Allodynia than Exparel® at 24 and 48 Hours

Authors: William Taylor, Daniel P. Sipple, D.O., F.A.B.P.M.R., D.A.B.P.M., Stefano M. Sinicropi, M.D., F.A.A.O.S.

ABSTRACT

The feasibility and duration of AnestaGel-P™, a novel hydrogel-based drug delivery system to provide sustained analgesia was evaluated in a post-operative incisional pain rat model when compared to a positive control, Exparel®. Twenty-eight male Sprague-Dawley rats were prepared for surgery and an approximately 1 cm long incision was made in the left hind paw. The incision was closed and the animals were treated with either AnestaGel–P or Exparel in a randomized manner. The animals recovered from the procedure and monitored until euthanasia at 96 hours post-surgery. Tissues were taken at the injection site and local lymph nodes, and analyzed to determine tissue impact as a result of sample injection. No abnormal tissue response was detected from or in either test sample and was generally well tolerated. AnestaGel–P delivered a statistically significant greater analgesic effect at 24 and 48 hours post incision and AnestaGel–P provided an equivalent analgesic effect at < 24 hours post-surgical incision, and equivalent analgesic effect at >72 hours.

INTRODUCTION

Pain is one of the few medical conditions that affects nearly every human being in the world. The development of opiates, cocaine and derivatives of these compounds has done much to reduce human suffering. The coca leaf's importation to Europe in the 1880s, combined with the emergence of modern organic chemistry, led to an explosion in regional anesthesia, the foundations of modern pre-operative, peri-operative and post-operative pain management. Cocaine, while effective, was highly addictive, short-acting, and not without central nervous system and cardiovascular toxicity. Early local anesthetic derivatives of cocaine were also of limited duration, leaving opium derivatives, such as cocaine, highly addictive, and as the mainstay of post-operative pain management.

Over 100 million Americans suffer from chronic pain each year. The annual economic impact of pain in the U.S. is estimated to exceed $635 billion (Stith Butler, Adrienne ; Xi, Jing ;Cox, Thelma L; Pope, Andrew M.; Randall, Donna; Bowman, Victoria), affecting more lives than heart disease, diabetes and cancer combined. The NIH has declared development of new, safer medications to alleviate pain with lower risk a top priority. Pain is a major reason for physician visits, prescription medications, disability claims and a significant decrement to economic productivity and quality of life. Appropriate management of pain is high-risk and time intensive for trained specialists, who are in short supply.

Opiates, the mainstay of treatment for severe pain, have been utilized for centuries. Persons over 65, representing the largest demographic of consumers of health care in the United States, tolerate opiates poorly (Manchikanti M. D., Pampati MSc and Boswell). Opiate related constipation, bowel obstruction, respiratory suppression, and delirium increase length of stay, cost, mortality and reduce patient satisfaction. Declared an epidemic by the Centers for Disease Control, over 47,000 deaths were reported in 2014, a 6.5 % increase from the previous year and a 200% increase since 2000. (Rudd MSPH, Aleshire JD and Zibbel PhD.) In addition, opiates are mostly ineffective in treating neuropathic pain and headache, which constitutes a disproportionate sample of the most painful conditions known: cluster headache, complex regional pain syndrome, migraine headache, phantom limb pain and trigeminal. Patients with these conditions often remain collateral damage from the opiate epidemic, as fear of opiates coinciding with a limited pharmacological armamentarium limits effective treatment options.

By 1957, the effective duration of local anesthetics was significantly increased with the introduction of bupivacaine. While infusion pumps with bupivacaine are utilized to deliver continuous nerve block, until the arrival of liposome-based Exparel, there was not a significant improvement in the duration of action of local anesthetics for over fifty years. Exparel is a leading analgesic used to treat post-operative pain. It has been shown to be an effective analgesic up to 72 hours. Exparel is a liposomal formulation that is injected directly into the target tissue and placed in the interstitial spaces. Its action depends on the breakdown of the carrier liposomes and subsequent elution of Bupivacaine into the surrounding

tissues. It has been reported that in some cases the product does not last the full 72 hours (Schnacky PharmD and Kelley PharmD) (VHA Pharmacy Benefits Management Services, Medical Advisory Panel and VISN Pharmacist Executives). Migration from the injection site, tissue type, and mechanical action may all play a role in causing variability in liposome breakdown and duration.

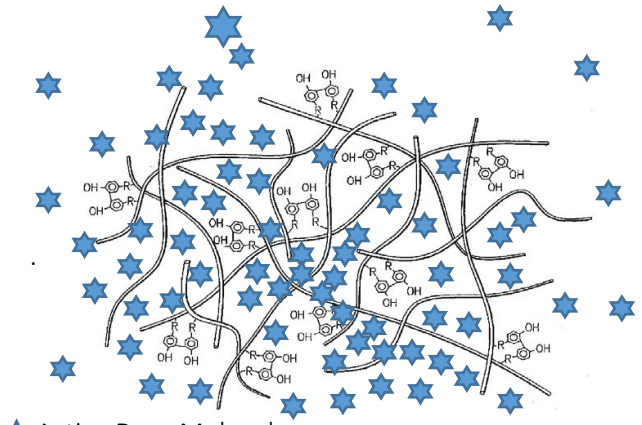
Matrix™ BioHydrogel is a tunable, biocompatible and physiologically neutral platform technology. This flexible implantable carrier platform can be used in stem cell and drug delivery, soft tissue, nerve, bone and other regenerative applications. Via customized tunable unique crosslinking, Matrix BioHydrogel engineered AnestaGel-P exceeds Exparel in efficacy, in a pre-clinical study. It performed as well as Exparel < 24hrs and exceeded Exparel analgesic effectiveness at 24 and 48 hours, and again matched performance > 48 hrs. AnestaGel-P also remained present in the injection location while Exparel was no longer present at the end of the study. Due to its consistency, AnestaGel-P will not migrate from the injection site.

AnestaGel-P utilizes a new approach to elution of analgesics into target tissues. AnestaGel-P utilizes the tried and true method of using a hydrogel as a drug reservoir, but is unique in that it is able to crosslink in the presence of the drug molecule without interacting or reacting with it. This allows for a much higher drug load capacity for the drug reservoir and allows for tuning the material to target desired properties in delivery rate, persistence, duration and stability. AnestaGel-P also ensures that the material stays in place where it is delivered into the target tissues. This is due to its physical form that resists extrusion from the injection site and migration through interstitial tissue spaces.

MATERIALS AND METHODS

The test article for this study was AnestaGel-P, a bupivacaine-loaded injectable hydrogel mixture indicated for administration into a surgical site to produce post-surgical analgesia. AnestaGel–P was administered to animals assigned to one test group and compared to a positive control arm that was administered Exparel. AnestaGel-P is comprised of 35 mg/mL bupivacaine-dense hydrogel sustained release particle contained in a loose continuous phase binding hydrogel matrix. The positive control article for this study was Exparel (1.33%, NDC 65250-266-20), a bupivacaine liposome injectable suspension indicated for administration into a surgical site to produce post-surgical analgesia. The test and control articles were in a sterile, ready-to-use form prior to study procedures. Exparel 13.3 mg/mL was used for the positive control and supplied as a vial. 1mL BD syringes with 0.1mL demarcations were used to deliver the test articles to the sciatic nerve between the greater trochanter and ischial tuberosity. AnestaGel-P was supplied in gel form in 1mL BD syringes with 0.1mL demarcations. Animals were transferred to the procedure room, anesthetized, and the left hind paw prepared for aseptic surgery. Surgical creation of a 1 cm longitudinal incision along the plantar aspect of the foot was performed and the incision closed in standard fashion. At the completion of the 96-hour testing session, animals were humanely euthanized and submitted for gross necropsy by a testing facility veterinarian. The injection sites of each animal and local lymph nodes were collected, processed for histology, and submitted to a board certified veterinary pathologist for analysis.

The key to Matrix BioHydrogel technology is its tunable, three-dimensional amino acid crosslinking. Through crosslinking density, AnestaGel's viscosity is intentionally tailored to a consistency approaching peanut butter, less prone to migration. Unlike Exparel, whose lipid structure is compartmentalized, Matrix BioHydrogel-based AnestaGel possesses contiguous three-dimensional molecular uniformity in a three-dimension lattice. Such molecular precision produces precise, reliable elution mechanics. In contrast, Exparel's lobulated, fat globule reservoir is amorphous, its surface to volume ratio, being in liquid form, is variable, affecting degradation, elution and migration. This raises concerns for Marcaine toxicity through bolus release, migration away from the surgical site.



AnestaGel-P particles are created by crosslinking a high molecular weight polysaccharide in the presence of a drug molecule. The relatively mild crosslinking reaction allows the drug to be captured within the crosslinked molecular mass at a desired concentration. The newly

formed drug reservoirs are then processed to form a uniform particle size and then bound in a loose second formulation of hydrogel that binds the particles together forming a thick gelatinous mass ready for delivery to target tissues.

Rat Model and Methods

The Sprague-Dawley rat is a widely used and established animal model for behavioral and nociceptive research. Rats are suitable for testing and widely accepted by the appropriate regulatory agencies.

This study was designed to evaluate the effects of AnestaGel-P on mechanical allodynia in a post-operative incisional pain model when compared to a positive and negative control. The model was based on an allodynia assessment technique in which an incision is created in the hind paw of a rat and pain response was assessed using pressure applied to the wound site using von Frey hairs. (Brennan) (Chaplan, Bach and Yaksh). Two arms of this study utilized sixteen (16) male rats (Sprague-Dawley), weighing approximately 150-250 grams on the day of the implant procedures. Age was dependent on weight for all animals. They were verified to be in good health through a physical exam performed by testing veterinary care staff at the time of arrival and within two days prior to the study procedure. Any animals showing signs of disease, which may affect the outcome of the study, were excluded from the study.

Animals must have met the following criteria in order to be enrolled in the study:

- Pre-injury baseline 50% response threshold of ≥ 7 grams of force on both the ipsilateral and contralateral paws
- Full recovery from the surgical procedure

Animals were given free access to food and water. Prior to dosing and surgery, each animal underwent a baseline behavioral test assessing the 50% response threshold to mechanical stimulation using von Frey filaments.

Sixteen (16) animals meeting inclusion criteria were assigned to treatment groups (test group I (n=8) or positive control (n=8), ensuring even distribution based on the pre-injury baseline 50% response thresholds.

Animals were transferred to the procedure room, anesthetized, and the left hind paw prepared for aseptic surgery. A 1 cm longitudinal incision along the plantar aspect of the foot was performed and the incision closed in standard fashion. Following the incisional procedure, each animal received an injection of the corresponding treatment (with the exception of the negative control group who did not receive an injection) targeting the sciatic nerve between the greater trochanter and the ischial tuberosity. All animals recovered from anesthesia and returned to general housing.

The 50% response threshold was measured for all groups at 3, 6, 9, 24, 48, 72, and 96 hours post-dosing according to the up-down method using von Frey filaments. Animal observations occurred at least once daily for the duration of the study. Analgesic and antibiotic therapy was not administered during the conduct of the study. At the completion of the 96-hour testing session, animals were humanely euthanized and submitted for gross necropsy by a testing facility veterinarian. The injection sites of each animal and local lymph nodes were collected, processed for histology and analysis.

HISTOLOGY/PATHOLOGY

Sixteen (16) male Sprague-Dawley rats were prepared for surgery and an approximately 1 cm long incision was made in the left hind paw. The incision was closed and the animals were treated as appropriate for their assigned group (AnestaGel–P or Exparel). The animals recovered from the surgical procedure, then were tested and monitored per the protocol until euthanasia at 96 hours post-surgery. The animals were then humanely euthanized and tissues were taken from the injection site and nearby local lymph nodes for histology. Tissue samples were prepared and hematoxylin and eosin (HE) slides were made. Samples of the injection site and nearby lymph nodes were collected for analysis.

AnestaGel-P

Eight injection sites were evaluated. Three sections of muscle were normal. Five sections had minimal to moderate histiocytic inflammation in the muscle fascia and occasionally in the muscle. Within the inflammation, there were a few to multiple, small irregularly shaped deposits of pale blue, acellular, non-birefringent material consistent with the test article. Three sections had minimal myofiber regeneration.

Eight sections of lymph node were evaluated. All of the sections of lymph node were normal.

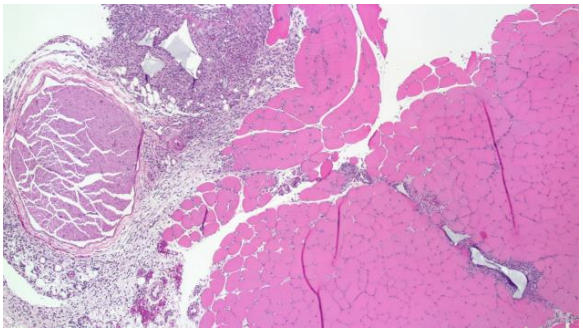


Figure 1. AnestaGel-P, Animal #16V144, Slide 1, Overview of the muscle and facia with inflammation and test article material. HE stain, 40x total magnification.

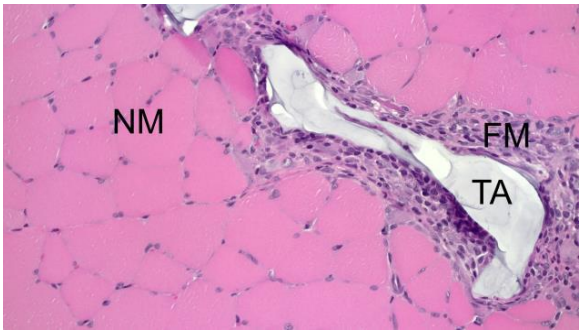


Figure 2. AnestaGel-P, Animal #16V144, Slide 1, Higher magnification of Figure 5 showing normal muscle (NM) infiltrated by foamy macrophages (FM) surrounding test article material (TA). HE stain, 200x total magnification.

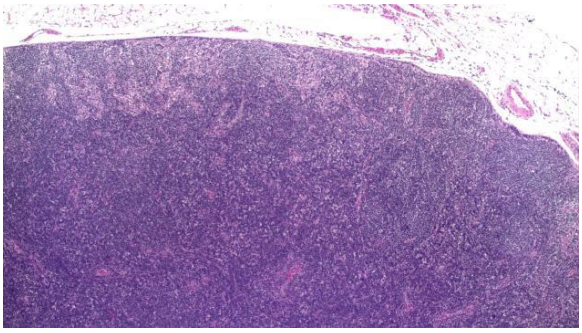


Figure 3. AnestaGel-P, Animal #16V157, Slide 2, Overview of the lymph node. HE stain, 40x total magnification.

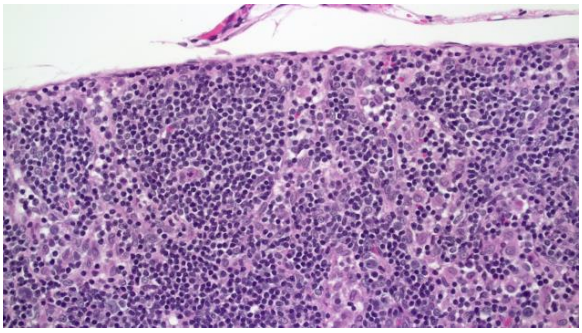


Figure 4. AnestaGel-P, Animal #16V157, Slide 2, Higher magnification of Figure 7 showing normal lymph node. HE stain, 200x total magnification.

Exparel

Eight injection sites were evaluated. Five sections of muscle were normal. Three sections had minimal to mild histiocytic inflammation in the muscle fascia.

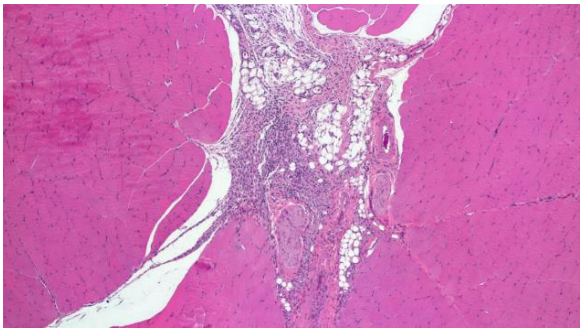


Figure 5. Exparel, Animal #16V150, Slide 1, Overview of the muscle and facia with inflammation. HE stain, 40x total magnification.

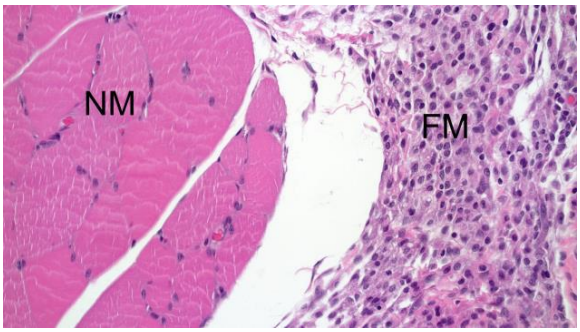


Figure 6. Exparel, Animal #16V150, Slide 1, Higher magnification of Figure 9 showing normal muscle (NM) adjacent to fascia infiltrated by foamy macrophages (FM). HE stain, 200x total magnification.

Histiocytic inflammation was present in the fascia of the muscle at the injection site in the test group and the positive control group. This is not an unexpected finding at a site in which a vehicle suspended drug has been injected. The AnestaGel-P had more histiocytic inflammation and also had deposits of an extracellular material consistent with the test material in the area of inflammation. No extracellular material was seen in the Exparel group sites.

All of the lymph nodes were normal in both test groups.

Eight sections of lymph node were evaluated. All of the sections of lymph node were normal.

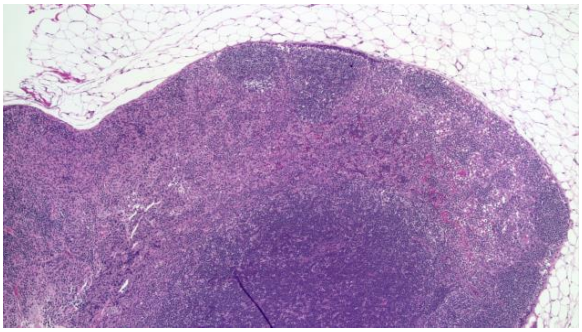


Figure 7. Exparel, Animal #16V161, Slide 2, Overview of the lymph node. HE stain, 40x total magnification.

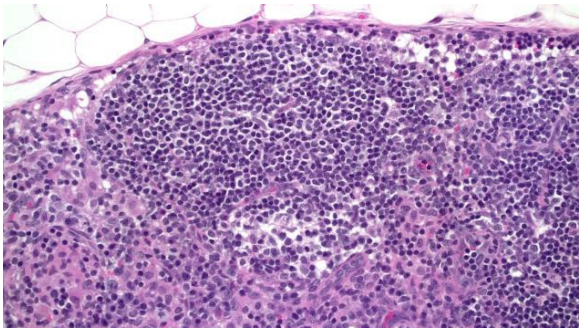
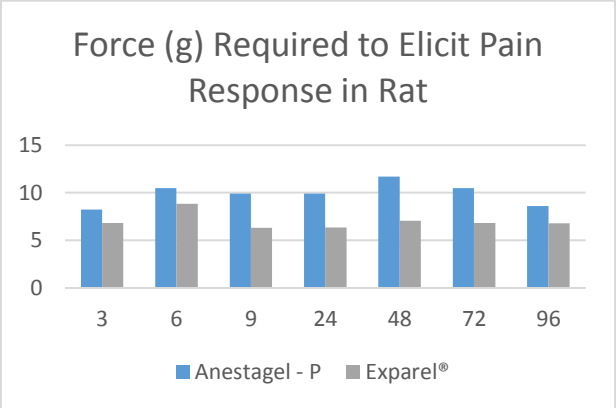


Figure 8. Exparel, Animal #16V161, Slide 2, Higher magnification of Figure 11 showing normal lymph node. HE stain, 200x total magnification.

RESULTS



Force applied within 1mm of incision wound of a Male Sprague-Dawley Rat. $p<0.05$ at 24 and 48 hr. time points. AnestaGel-P consistently exceeded Exparel in efficacy at every time interval from 3 to 96 hours and maintained the 10 g force threshold from 6 to 72 hours.

DISCUSSION

AnestaGel-P provided superior analgesic effectiveness 24 and 48 hours post administration of the hydrogel to Exparel Liposome suspended solution. Histology results demonstrate the AnestaGel-P test article was still present at conclusion of the study, but the Exparel was not present in tissue samples. Delivery vehicle migration away from the injection site will reduce analgesic drug delivery to the surgical site as it will carry the drug material within it as it migrates away.

AnestaGel-P was loaded with more bupivacaine and demonstrated it was able to supply pain relief superior to Exparel up to 72 hours. AnestaGel-P is designed to quickly release drug from the binding matrix short term and then continuously supply the drug to the injection site by elution from the dense hydrogel particle reservoirs. Exparel is dependent upon the continuous degradation of the liposomes and is dosed at a lower concentration.

CONCLUSION

AnestaGel-P was determined to be a superior drug delivery vehicle for pain treatment when compared to Exparel. The physical properties of a homogenius, densely crosslinked molecular structure facilitate superior elution mechanics. Demonstrating non-Newtonian fluid properties, the viscosity of AnestaGel-P increases with resistance, retarding migration from the targeted site of placement. Exparel, in contrast, possesses classical low viscosity fluid mechanics, freely migrating through the interstitial space away from the target tissue, diminishing efficacy.

AnestaGel-P is a tunable bio-hydrogel, offering customizable concentrations of local anesthetics with consistent elution mechanics of superior duration. Liposomal delivery systems are not crosslinked. Low viscosity exposes greater surface area relative to volume, accelerating degradation, with resultant less consistent elution mechanics.

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