IN VITRO ELUTION OF ANALGESIC MEDICATIONS FROM AN ABSORBABLE GELATIN SPONGE

by

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Abstract

Objective: To compare the *in vitro* elution characteristics of six common analgesic medications from a commercially available absorbable gelatin sponge.

Study Design: Experimental study.

Methods: Gelatin sponges were loaded with various analgesic medications, including two opioids, preservative-free morphine and fentanyl, two local anesthestics, bupivacaine and lidocaine, and two α_2-adrenergic agonists, dexmedetomidine and xylazine. The loaded sponges were placed in dishes containing phosphate-buffered saline (PBS) and maintained at 37° C with constant agitation. Concentrations of each drug were determined at various time points up to 24 hours post-inoculation using high-pressure liquid chromatography. Two phases were conducted, utilizing undried loaded sponges (phase one) and dried loaded sponges (phase two).

Results: In both phases, all analgesic medications eluted from the gelatin sponge and equilibrated rapidly with the PBS, achieving maximal concentration within 30 minutes. In phase two, the rapid nature of the release was captured by increasing sampling within the initial 30 minutes. Results were consistent for each medication with minimal variation. Steady state concentrations were significantly higher in phase two with four out of six medications.

Conclusions: Analgesic medication elution from an absorbable gelatin sponge was rapid and consistent. Drying the loaded sponge prior to use will likely substantially increase the amount of medication eluted but not prolong release.

Clinical Relevance: The rapid release of analgesic medications from the gelatin sponge makes a prolonged analgesic effect unlikely without further modification. Toxicity may be a concern. Further study is required to investigate efficacy *in vivo*, safe dosing regimens and prolongation of duration of action.

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CHAPTER 1 -

Systemic versus regional drug administration

The ability to deliver measured amounts of a substance continuously over an extended period of time to a local environment has advantages over systemic administration. Such local delivery allows administration of lower volumes of medications, reduces or eliminates potential adverse effects, and achieves higher drug concentrations at the target tissue[1-5]. Appropriately selected topical, local and regional techniques have been investigated for providing analgesia to veterinary patients while minimizing physiologic insult. Drugs which are unable to be used systemically due to severe toxicity may be administered regionally due to lower systemic concentrations. Theoretically nearly any drug could be administered via regional delivery instead of systemic, and many have been evaluated. In most situations, regional drug administration promotes a longer duration of therapeutic action than intravascular or intramuscular administration, as these more traditional methods typically are rapidly distributed and metabolized[6]. When administered orally, many drugs commonly undergo first-pass metabolism and biotransformation at the liver which inactivates the molecule, which is summarily excreted via renal, biliary or fecal routes[46]. Preferably regionally-delivered drugs would exert their local effects the first time the drug reaches the target tissue, as once the drug leaves the target tissue and enters systemic circulation, behavior and efficacy are similar to systemic administration. Many factors which will be further discussed in this manuscript, both relating to the drug and the *in vivo* environment, determine the pharmacokinetic and pharmacodynamic properties of each drug in an individual patient.

Disadvantages of common regional drug delivery systems include the expense, invasiveness, expertise and equipment often required to prepare and implement these techniques. Controlled-release systems are often intricately and meticulously created in a laboratory setting, making them impractical and cost-prohibitive for use in a typical veterinary clinical setting. In nearly all situations, these devices require at a minimum heavy sedation and often general anesthesia for implantation, frequently in conjunction with major surgical procedures. Depending on the type of delivery, an additional hospital visit and anesthetic event may be necessary to remove the device and eliminate persistent adverse effects. Standardizing the rate and duration of drug release as well as minimizing the patient's immune response *in vivo* are vitally important to efficacy. Implants can predispose the local surgical site to inflammation, tissue reaction and infection, and these risks must be addressed and minimized. Another limiting factor in utilizing certain delivery systems is establishing the necessary technical expertise and obtaining the equipment required to perform these techniques[7].

Based on the discussed advantages and disadvantages of regional drug delivery, the ideal delivery vehicle would be biocompatible, absorbable, reproducible, inexpensive, practical for in-hospital use, and structurally consistent with a functional porosity from which to elute drugs. Scientists and engineers have strived to attain these vehicle characteristics for many years, but few if any have achieved the production of an ideal delivery vehicle.

Delivery Vehicles

The most common drugs investigated for regional elution in both the human and veterinary medical realms are antimicrobials[8-14,16,28,29,33,34,37,38,123]. This phenomenon has been most extensively explored in both prophylactic and therapeutic models, using a variety of vehicles with varying levels of success. The first description of regional antibiotic delivery was by Buchholz and Englebrecht in 1970, who described the elution of gentamicin and other antibiotics from polymethylmethacrylate (PMMA) beads[8]. Literally thousands of scientific studies have been performed since then, scrutinizing various modifications of PMMA and other biomaterials to maximize the benefits of these drug-release vehicles. Interestingly, even with decades of research and significant financial investment, the PMMA beads originally described by Buchholz are very similar to those routinely used today, mainly in orthopedic applications. In several *in vitro* veterinary studies, PMMA-antibiotic beads have been shown to elute various antimicrobials with concentrations at least attaining minimum inhibitory concentration (MIC) over several weeks[9-11]. The choice of which antimicrobial to combine with the PMMA is dependent on clinical experience, drug stability and culture and sensitivity results. Because these antibiotics are eluted regionally and typically do not achieve high systemic concentrations, antibiotics which may not have been safe to use systemically become viable options with this route. One example is aminoglycosides which have known systemic limitations due to renal tubular toxicity, but often can be beneficial for multidrug-resistant infections[9,10]. Regional administration via PMMA beads often dramatically decreases the quantity of antibiotic required as compared with systemic administration which facilitates utilization of more expensive antibiotics such as meropenem, which otherwise might not be financially feasible in veterinary medicine[11]. One major disadvantage of PMMA implants is the requirement for surgical removal

as they are not absorbable. In addition, if the indication for PMMA-antibiotics beads is treatment of osteomyelitis and the beads are placed within an osseous defect, bone healing cannot occur until the PMMA is extracted.

Another biomaterial evaluated for use as an antibiotic delivery vehicle is calcium sulfate, or plaster of Paris. Advantages include osteoconductivity and absorbability, obviating later surgical removal. Calcium sulfate has been shown to neither stimulate nor inhibit bone formation[12]. In an *in vitro* experimental study evaluating elution from calcium sulfate beads impregnated with either vancomycin, amikacin or both, only the vancomycin-impregnated beads were effective at inhibiting growth of a *Staphyloccocus* species for an extended period of time, up to 56 days[12]. In this study, amikacin elution occurred over the 84 day study period but only achieved MIC for less than 24 hours. Another study investigated a more rapidly absorbing calcium sulfate pellet impregnated with gentamicin demonstrated complete dissolution of pellets within 12-16 hrs which corresponded with 50-70% of antibiotic eluted within 4 hrs[2].

With the recent advent of interventional radiology and stenting procedures in both human and veterinary fields, drug-eluting implants have been developed. Especially in the cardiovascular realm, the risk of infection becomes paramount as catastrophic infectious complications occur in more than 25% of aortic prosthesis cases[13,123]. Commercial knitted polyester grafts have been modified by immersing the graft in a gelatin sealant loaded with rifampin and tobramycin. These modified aortic grafts have been shown to resist infection by *Staphylococcus aureus* in an *in vivo* canine model[14]. Besides infection, another complication encountered with coronary arterial stenting is chronic inflammation and hypersensitivity reactions, potentially leading to thrombosis and restenosis[4,15]. In an attempt to minimize the inflammatory response, coronary stents coated with a bioabsorbable polymer coating of salicylic

acid and sirolimus have been shown to elute these medications rapidly over the initial 48 hrs, followed by a gradual release over the following 6 days[15]. However, localized overdose has been seen in cases of stent fracture[4]. Self-assembled monolayers (SAMs) are polymers approximately 1-10 nm thick which are developed on a molecular level and added onto 316L surgical steel; various medications have been attached and, in one *in vitro* study with ibuprofen, demonstrated steady elution over a 35 day duration[4].

The ophthalmic route of drug administration has also been developed, both for ophthalmic and systemic drug use. One study, aimed at developing a novel treatment for endophthalmitis, evaluated a custom-made biodegradable polymer scleral plug which was impregnated with vancomycin, amikacin and dexamethasone[16]. When assessed *in vitro*, the device eluted efficacious concentrations of the antibiotics for up to 14 days, and results suggested that potential methods to prolong the elution period included decreasing the antibiotic/steroid to polymer ratio, increasing the sintering temperature and/or increasing the compression pressures during device manufacture[16]. Another study evaluating a custom polymer ophthalmic device found that elution was diffusion controlled and was altered by the pH of the eluent and the molecular weight and charge of the eluted drug[17]. Using an equine recurrent uveitis model, Bilger et al. demonstrated prolonged release of cyclosporine and control of disease utilizing a custom-made biodegradable polymer pellet implanted surgically in the deep scleral tissue[124].

In human and veterinary neurosurgery, application of a biomaterial to laminectomy sites has become standard of care. This procedure was initially thought to decrease the likelihood of formation of fibrous adhesions and potential compression and/or pain between incised musculature healing adjacent to dura mater, the so-called laminectomy membrane[18,19]. Materials

utilized include subcutaneous adipose tissue autograft, cellulose membranes, absorbable gelatin sponge and various gels. Since these materials were being placed in the peridural region, researchers became interested in the addition of analgesic drugs to be subsequently eluted for therapeutic purposes. The most extensively investigated human product is Oxiplex® gel (FzioMed Inc, San Luis Obispo, CA), which is an absorbable combination of carboxymethylcellulose and polyethylene oxide utilized to prevent epidural fibrosis, although not approved by the United States FDA. In one small prospective human study conducted in Italy, morphine was mixed with Oxiplex® and applied epidurally in conjunction with microdiscectomy, leading to dramatically decreased pain scores for approximately 24-36 hrs, but worsening of pain after that time frame[20]. Due to the apparent lack of prolonged analgesia, the authors could not recommend clinical use. Another material utilized to deliver prolonged spinal analgesia is a viscous lidocaine-hyaluronate formulation which was demonstrated in a canine model to significantly prolong absorption kinetics, with an apparent half-life of 56 min versus 4 min for lidocaine solution[21]. Unfortunately, this change in lidocaine absorption did not correlate with an increased duration of action as measured by loss of hindlimb motor function.

Gelatin has been extensively investigated as a medium for drug delivery, and is produced by partial hydrolysis of collagen. A multitude of studies have investigated various derivatives of this substance for drug delivery. One commonly available absorbable gelatin sponge (Vetspon, Novartis Animal Health US, Inc., Greensboro, NC) is prepared from purified pork skin gelatin and is routinely used as a hemostatic agent in surgical procedures due to its ability to absorb and hold within its interstices up to 45 times its weight of fluid[22]. Its use in the surgical site is well tolerated due to relatively fast absorption (four to six weeks in soft tissues) and lack of inflammatory or toxic side effects[22]. Complete implant absorption avoids the surgical trauma

and anesthesia associated with physical extraction. Studies have shown that these sponges have minimal cytotoxic and genotoxic characteristics and effectively induce platelet adhesion and release of α-granules in the formation of platelet aggregates[23].

The release of drugs from commercially available gelatin sponges as well as custom-made sponges has been explored. Utilizing a canine tracheomalacia model, scientists from Japan used commercial absorbable gelatin sponges impregnated with both basic fibroblast growth factor (bFGF) and bone morphogenetic protein (BMP-2) to slowly elute these substances into cartilaginous defects over several weeks and demonstrated significant improvements in regeneration compared with controls[24-26]. Gelatin sponges used in a rabbit ulnar osteotomy model to elute BMP-2 led to 33% faster bone healing times than controls with either no sponge or saline-impregnated sponges[27]. A similar study using a canine tibial osteotomy model illustrated improved bone healing when gelatin sponges impregnated with rhBMP-2 were placed at the osteotomy[117]. The elution kinetics of commercial sponges are frequently altered by performing various sponge modifications.

Gelatin sponge modifications

Gelatin sponges have been utilized for many regional drug delivery systems, and most are either custom-made laboratory preparations or modifications of commercially available sponges. The most common challenges encountered when using commercial sponges to elute drugs are excessively rapid release of the drug and quick deterioration of the sponge material[28,29]. Strategies to retard drug elution include slowing the absorption of release medium, delaying the

release of drug molecules from the vehicle, and slowing the outward diffusion away from the vehicle into the local environment.

The most effective approach to prolonging drug elution appears to be the addition of various diffusion restrictors to the gelatin sponge construct. By crosslinking gelatin with alginate (a polysaccharide found in brown algae cell walls), prolonged drug release over four days was achieved in one study, which corresponded with decreased sponge porosity and decreased water uptake ability[30]. Another study illustrated a slower penetration of eluent medium into the deeper portions of a gelatin sponge with the addition of polyethylene glycol monostearate and cetyl ester wax[31]. These diffusion restrictors not only slowed water absorption, but simultaneously prevented rapid outward elution of drug as well. Takemoto et al. created a collagen and gelatin sponge scaffold to facilitate sustained elution of bFGF, finding that varying the percentage composition of gelatin had a direct effect on duration of drug release[32]. By coating these sponges with a thin silicone membrane, diffusion was further prolonged[32]. Other intensely technical modifications which have demonstrated positive effects include blending chitosan (byproduct of chitin) or fibrin with gelatin, plasticizing sponge constructs, adding polyelectrolyte complexation between molecules, crosslinking matrices to decrease permeability, implanting drug-loaded gelatin microspheres, increasing collagen content, adding hydroxyapatite or other minerals, and altering drug molecule solubility, among others[3,33-40].

Ophthalmic preparations have been shown to elute insulin and pilocarpine via scleral absorption, delaying release of these drugs compared with traditional liquid eyedrop administration[41,42]. In both of these studies, loaded gelatin sponges were dried by evaporation under vacuum for at least 72 hrs prior to implantation to remove the solvents and possibly prolong drug elution[41,42].

Epidural analgesia via gelatin sponge

Many different epidural analgesics have been considered for use in human spinal surgery and delivered via several methods which are described in the epidural administration section of this manuscript. The most common indication for spinal surgery in dogs is intervertebral disc disease and subsequent compressive radiculopathy, and numerous therapeutic variations of discectomies and laminectomies have been described. One study in humans evaluated the feasibility of using a morphine- and methylprednisolone-impregnated gelatin sponge for postoperative analgesia following lumbar discectomy[1]. Patients with this treatment option required significantly less postoperative parental narcotics, and were discharged from the hospital sooner than historical control patients not receiving the sponge[1]. Few complications were noted, including intermittent bladder catheterization and presumptive discitis[1]. Another study in humans evaluating the efficacy of buprenorphine-soaked gelatin sponges following thoracolumbar laminectomy illustrated a significant difference in pain relief scores postoperatively when compared with saline-soaked controls[43]. In addition, the duration of pain relief until supplemental analgesia was requested was significantly longer in study patients, approximately 15 hours, when compared with controls at less than one hour[43]. Side effects were minimal, although the study group experienced a higher incidence of nausea[43].

A similar study was performed by Domnick et al. investigating the efficacy of a medetomidine and preservative free morphine-impregnated gelatin sponge on postoperative analgesia following hemilaminectomy for thoracolumbar intervebral disc disease in dogs[44]. This double-blinded prospective analysis demonstrated a significant analgesic effect of the impregnated sponge, as measured by pain scoring and rescue analgesic requirements[45].

It should be noted that leaving an absorbable gelatin sponge in a peridural location is against the manufacturer's recommendations, presumably for the concern of spinal compression as the sponge absorbs fluid and expands[22].

CHAPTER 2 -

Analgesic drug pharmacokinetics

Several important factors determine the changing concentrations of a drug within an animal. One of the critical factors which affects the concentration of active drug at the receptor site is plasma protein binding affinity. Only drug which is free within the plasma can exit the extracellular fluid space and diffuse across membranes. Clinically, albumin is the most common protein to which drugs bind, α_1-acid glycoprotein (AAG) is the second biggest contributor, and others include globulins, mucopolysaccharides, hemoglobin, nucleoproteins and phospholipids. This binding process is usually reversible, and as unbound or free medication is excreted or diffuses out from the plasma, the weak chemical forces of the remaining albumin-drug complex are overcome to release additional free drug and restore the concentration of free drug circulating in the plasma[46,47].

A normally functioning and anatomically-intact glomerulus should not allow albumin to pass, thus exposing only unbound drug to the possibility of renal clearance by filtration. A similar effect is appreciated in the hepatic parenchyma, as only unbound drug is able to diffuse to biotransformation sites[46]. Thus, increased protein-binding may decrease hepatic metabolism and maintain plasma concentrations as well as prolong duration of action of the drug. Different drugs have variable affinities for plasma albumin, and those with higher affinities can displace those with lower affinities, but clinical consequences are rare.

Hypoalbuminemia and hypoproteinemia are common sequelae to a plethora of disease processes, and significant decreases in albumin and other plasma proteins can have a profound

effect on circulating drug concentrations. Especially with highly protein bound drugs, the more severe the hypoalbuminemia, the greater the free drug concentration. This could lead to prolonged or increased drug effect, and could predispose to toxicity issues that would not be of concern in euproteinemic states. However, it may also lead to greater elimination.

Diffusion of drugs across biological membranes is an important process which facilitates drug absorption, distribution, metabolism and excretion. The rate and degree of absorption is dependent on the physiochemical properties of the drug (size, conformation, pH, pK_a, solubility), the vehicle in which it is released, and on the biochemical nature of the membranes to be crossed[46]. The influence of pH on diffusion is not completely understood, but the ionized form of a drug is usually more water soluble (hydrophilic) and less lipid soluble, making diffusion across the cell membrane less likely[46]. On the contrary, non-ionized substances more readily cross the phospholipid bilayer. The non-ionized to ionized drug ratio is dependent on the drug pK_a, or dissociation constant, and the pH of the medium. With drugs that undergo renal elimination, urinary pH can affect clearance. In the face of alkaline urine, basic drugs tend to exist in the non-ionized form, thus are more lipid soluble and more likely diffuse back into the vasculature[48]. Alkaline urine leads to ionized forms of acidic drugs, and consequently increased excretion. The opposite phenomenon exists if acidic urine is present. In addition, acidic drugs preferentially bind to albumin, while basic drugs tend to bind lipoproteins or α_1-glycoproteins or both[48].

Volume of distribution is another variable which often has a dramatic effect on the pharmacokinetics of a drug. Apparent volume of distribution is defined as the theoretical volume of fluid in which the total amount of drug administered would need to be uniformly distributed to achieve the desired plasma drug concentration[49]. Larger volumes of distribution

are commonly found with lipophilic drugs, as these drugs easily diffuse and accumulate in body tissues, especially adipose tissue. Prolonged release of drug from these stores may be appreciated. Drugs which are hydrophilic and/or highly protein-bound are less likely to diffuse across cell membranes to access adipose stores, thus tend to have lower volumes of distribution[49].

Another pharmacokinetic variable to consider is clearance, which is defined as the volume of plasma cleared of drug by metabolism and excretion per unit time. Clearance correlates precisely with the quantity of milliliters of the volume of distribution cleared per unit time. Several variables can affect clearance, including protein binding, glomerular filtration rate, hepatic disease, afferent blood flow to the kidney, renal tubular reabsorption rate and others. Utilizing the two parameters of clearance and volume of distribution enables the half-life of a drug to be calculated, which is essential for clinical decision making. Decreased renal function, increased drug protein binding and increased drug volume of distribution are three examples of situations which may lead to a drug half-life being significantly prolonged[46,48].

Morphine sulfate is a pure opioid agonist which acts at the μ and κ receptors. It is a hydrophilic molecule with a pH of 2.5 to 6.0 for a solution of morphine sulfate. It exhibits relatively low protein binding (36%), with higher skeletal muscle tissue binding observed at approximately 54%[50]. The drug also concentrates in the renal, hepatic and pulmonary parenchyma, with lower levels found in the central nervous system when administered systemically. Metabolism consists mainly of hepatic glucuronidation, and these metabolites are subsequently eliminated via renal excretion[51]. Morphine is absorbed when given by intramuscular, intravenous, epidural and subcutaneous routes. When administered orally, morphine is poorly and unreliably absorbed and likely undergoes significant first-pass

metabolism, substantially reducing bioavailability to approximately 5-18%[52,53]. Similarly, when morphine is administered per rectum, only 19.6% becomes systemically bioavailable, making this route unlikely to be clinically efficacious[54]. When administered intravenously in dogs, apparent volume of distribution is 4.5-7.5 L/kg, clearance is 62-83 mL/min/kg, and elimination half-life is approximately 1.1 hr[50,53]. The plasma profile appears to correlate with a two compartment model, with a rapid initial distribution and subsequent slower elimination phase[53]. When administered via the epidural route, morphine is rapidly absorbed into the systemic circulation and detectable in the serum within five minutes, reaching a maximal concentration within 30-40 minutes[55]. The elimination half-life of morphine from the lumbar CSF following epidural administration is approximately 3.5 hrs[56]. Interestingly, cerebrospinal fluid (CSF) morphine levels following lumbosacral epidural injection achieve maximal concentrations sooner at the lumbar subarachnoid space than the cerebellomedullary cistern, at 60 minutes and 180 minutes, respectively[55,57]. Morphine appears in much higher concentrations in CSF than in plasma when administered epidurally, with a CSF: plasma concentration ratio of about 100-200[58]. A custom-made multivesicular liposome-based preparation of morphine administered via epidural catheter dramatically extended duration of efficacy, leading to a 17-fold increase in CSF mean residence time and three-fold increase in elimination half-life[56]. Potential mechanisms responsible for the delayed CSF absorption are discussed later in this manuscript in the epidural administration section.

Fentanyl citrate is a highly lipophilic phenylpiperidine derivative with a pH of 4.0 to 7.5 in solution. It is a full opioid agonist with activity at the μ receptor and is at least 100 times more potent than morphine. It is highly protein bound. Routes of administration include intravenous, intramuscular, epidural and transdermal. Plasma fentanyl concentrations decrease rapidly for

approximately 20 minutes following intravenous injection due to redistribution throughout adipose and muscle tissue and elimination from the central compartment, exhibiting a short distribution half-life of 4.5 min[59]. This rapid initial concentration decrease is followed by a more gradual decline as the drug is redistributed and eliminated from the body, thus best fitting a two compartment model[59]. Fentanyl is metabolized via hydroxylation and N-dealkylation in the liver, and these metabolites are predominantly eliminated by the kidney, with less than 10% excreted in the feces[60]. When administered intravenously, apparent volume of distribution is about 3-5 L/kg, clearance is 10-20 mL/min/kg, and elimination half-life is approximately 45 min to 4 hrs[59]. Studies evaluating epidurally-administered sufentanil demonstrated vastly higher concentrations in CSF than those in plasma (44-fold), yet a shorter terminal elimination half-life in CSF of 2.8 hrs, compared with 4.1 hrs in plasma[61,62].

Lidocaine is an amide-type local anesthetic which acts at fast sodium channels inhibiting recovery after repolarization, and lidocaine solution has a pH of 6.5. Routes of administration include intravenous, intramuscular, subcutaneous, local, transdermal and epidural. Intramuscular administration is highly effective, with 91.9% of the dose absorbed, but in a clinical setting, regulating and monitoring dosing is more difficult than intravenous administration[63]. Oral dosing is ineffective due to high first-pass effect. Lidocaine undergoes plasma protein binding based on drug concentration, with the fraction of unbound drug increasing as overall drug concentration increases[63]. Approximately 60-80% of lidocaine is protein bound, particularly to AAG, and this relationship can be dramatically affected by inflammatory disease states, with AAG increasing during the acute-phase response, and by cytokines, prostaglandin E_2 and cyclic adenosine monophosphate[64]. A significant negative correlation exists between the fraction of unbound lidocaine and AAG concentrations, such that increasing AAG concentrations as seen

with inflammation lead to decreased percentage free lidocaine[65]. Metabolism occurs rapidly in the liver as biotransformation via oxidative N-dealkylation, ring hydroxylation, conjugation and cleavage of the amide linkage, producing metabolites which are eliminated in the urine. Approximately 10% of lidocaine is excreted unchanged in the urine[63]. Following an intravenous bolus, the terminal elimination half-life is about 48 min, the mean specific clearance is approximately 1.2-2.4 L/kg/hr and the apparent volume of distribution is 4.5 L/kg[21,63]. Severe hepatic dysfunction can lead to a prolonged lidocaine half-life. Epidural administration of lidocaine appears to instigate a biphasic absorption process, with an initial fast phase exhibiting an apparent half-life of 4 min, followed by a slower absorption phase with a half-life of 131 min[21].

Bupivacaine is a local anesthetic with a similar mechanism of action as lidocaine, although with a slower onset and longer duration of action. It has a pH of 4.0 to 6.5 in solution and is more lipophilic than lidocaine, contributing to its longer duration of action and increased potency (four fold). An intermediate hepatic extraction ratio of 0.38 may contribute to prolonged effect, compared with lidocaine's hepatic extraction ratio of 0.65[66]. Routes of administration include subcutaneous, epidural, intra-articular and intradermal[69]. It is approximately 95% protein bound, and achieves high concentrations in highly perfused organs such as liver, brain, heart and lungs. Metabolism occurs in the liver via dealkylation and hydrolysis, as well as partial detoxification by conjugation with glucuronic acid[67]. This could lead to clinically significant toxicity in cats or patients with hepatic dysfunction due to limited capability to produce glucuronide conjugates[67]. Excretion occurs through the kidney, with only 6% of bupivacaine excreted unchanged. When administered with a short (15 min) intravenous infusion, the terminal half-life was about 53 min, clearance was about 9.5 mL/min/kg, and apparent

volume of distribution was 0.7 L/kg[68]. A three-compartment open model appears to be in effect, with the first compartment represented by rapid distribution to highly perfused tissues such as brain, liver, kidneys, and myocardium, followed by equilibration of drug to the deep compartment of muscle, fat, skin and gastrointestinal tract[68]. The third compartment represents elimination and redistribution from deep compartments. Epidural administration has been shown to be a highly effective adjunct modality of analgesia with few side effects and a prolonged duration of action[70-74], becoming efficacious within 2 minutes and having an elimination half-life of nearly three hours[75].

Xylazine is an α_2-adrenergic agonist with sedative, analgesic and muscle relaxant properties. It is a lipophilic compound with a pH in solution of 5.5, and it is generally administered via intravenous, intramuscular, epidural or subcutaneous routes. After intramuscular administration, xylazine is rapidly absorbed and has a half-time of absorption of 3.4 min, although absorption can be quite variable and incomplete, with a bioavailability between 52-90% in the dog[76]. Following a single intravenous dose, the apparent volume of distribution is 2.5 L/kg, clearance is 81 mL/min/kg and the half-life of elimination is 24-30 min[76,77]. The pharmacokinetic data from these studies best fit a two-compartment open model. Xylazine undergoes rapid metabolism at the liver via conjugation and hydrolysis yielding about 20 metabolites which are mostly excreted in the urine, and less than 8% is excreted in the urine unchanged[78,79]. Epidurally administered xylazine has been shown to have a potent analgesic effect likely of local spinal origin in dogs, as analgesia was not impaired by administration of the α_2-adrenergic antagonist atipamezole[80,81]. Prospective studies have demonstrated that epidural xylazine administration decreases the minimum alveolar concentration of isoflurane in a dose-

dependent manner in anesthetized dogs and is associated with minimal cardiovascular side effects[82,83].

Dexmedetomidine is the most potent α_2-adrenoceptor selective agonist available in veterinary medicine. It is not a pure α_2 agonist as it also binds noradrenergic imidazoline receptors found in the brain, kidney and pancreas, potentially leading to hypotension and anti-arrhythmogenic action[84,85]. It can be administered via the intravenous, intramuscular, epidural and transmucosal routes, while subcutaneous administration is not recommended due to erratic absorption and unreliable response[86]. It is lipophilic and in solution has a pH of 4.5 to 7.0[87]. For many years, medetomidine was the major commercially available detomidine-related sedative used in small animal practice, with the majority of the α_2-adrenoceptor activity from its d-enantiomer, dexmedetomidine. Only within the past two years was dexmedetomidine available commercially in veterinary medicine. As demonstrated in receptor binding studies, medetomidine has an α_2:α_1 selectivity factor of 1620, which is significantly more selective than detomidine at 260, clonidine at 220 and xylazine at 160[88]. Dexmedetomidine undergoes hepatic biotransformation via glucuronidation, hydroxylation, N-methylation and hydrolysis with subsequent excretion in the urine. A small amount of metabolites is excreted in the feces and less than 5% excreted unchanged in the urine[89]. Pharmacokinetic parameters of intravenous medetomidine include an apparent volume of distribution of approximately 3.0 L/kg, clearance of about 27-33 mL/min/kg, and terminal half-life ranging from 0.97 to 1.6 hrs[89]. When given intramuscularly in dogs, peak plasma levels are achieved in about 35 min with 60% bioavailability, apparent volume of distribution is 0.9 L/kg, and elimination half-life is approximately 40-50 min[86]. Kuusela et al. compared the pharmacokinetic parameters of medetomidine, dexmedetomidine and levomedetomidine in dogs and found very little difference

between medetomidine and dexmedetomidine, while the L-enantiomer had a significantly larger volume of distribution at steady-state and a more rapid clearance[90]. Interestingly, the clinical effect of dexmedetomidine was similar but slightly more intense and longer acting than equipotent doses of medetomidine, while levomedetomidine appeared to be pharmacologically inactive. The authors suggested this phenomenon could exist if the L-enantiomer interacted with or even antagonized the dextro-form, competed at the same receptor sites, or exhibited an effect on α_1-receptors[90]. Several studies have illustrated the efficacy and duration of action of epidurally administered medetomidine and dexmedetomidine for prolonged analgesia, often in combination with an opioid[91-94].

Epidural administration

Analgesic drugs administered via the epidural route have been successfully utilized in both human and veterinary patients[119-122]. Traditional methods of administration include spinal needle placement into the epidural space at the lumbosacral intervertebral space or caudal lumbar vertebrae, preferably avoiding intrathecal deposition. Alternatively an epidural catheter can be placed at the lumbosacral space using palpation, or radiographic or fluoroscopic guidance. Analgesic drugs can also be administered directly to the dura mater as a local splash block or within a delivery vehicle in association with surgical procedures.

Goals of epidural administration include decreasing or eliminating the need for systemic analgesia, decreasing the amount of general anesthetic medications necessary during surgical procedures, and providing potent pain relief directly at the level of the spinal nociceptive fibers. Additionally, minimizing or avoiding systemic adverse effects and reducing voluntary motor

function deficits are preferred. The specific fibers involved in impulse transmission have been extensively investigated, and consist of nociceptive (C and A-δ), sympathetic (B), motor (A-α) and tactile sensory (A-β) fibers[5,118]. Several factors influence the rapidity, duration and degree of blockade on these fibers, including the size and myelination of each fiber type, which affects medication diffusion[5].

Epidurally administered drugs are absorbed and distributed via at least four distinct processes: transdural absorption into the cerebrospinal fluid and pia mater, sequestration by epidural adipose tissue, lymphatic uptake, and systemic absorption through the epidural vasculature and spinal radicular arteries[57,95]. The degree of absorption with each route is highly dependent on the relative lipophilicity or hydrophilicity of the drug. Highly lipophilic drugs will be absorbed rapidly in the vasculature and have systemic (supraspinal) effects, as well as a slightly prolonged effect likely from sequestration within the epidural fat[95]. Hydrophilic drugs undergo minimal vascular uptake and thus have a longer mean residence time in the epidural space and prolonged elimination half-life, leading to increased spinal effects and less pronounced systemic effects[96,97]. Spinal absorption of epidurally-administered drug requires movement through the meninges, which is mediated by the measured permeability coefficient. Bernards and Hill scrutinized which factors influenced a certain drug's coefficient, including molecular weight, molecular surface area, molecular volume, length of the major molecular axis, and octanol:water distribution coefficient, and found only the latter to have any correlation[98]. A potential explanation for the biphasic relationship between hydrophilicity and the permeability coefficient involves the two layers of the meningeal barrier. The arachnoid mater has both a hydrophilic aqueous region with extra- and intracellular fluid and a hydrophobic lipid domain with cell membrane lipids which need to be traversed to access the pia mater[98]. Hydrophilic

drugs dissolve rapidly to penetrate the aqueous region, but have difficulty diffusing at the lipid interface. Conversely, lipophilic drugs readily traverse the phospholipid bilayer but encounter the aqueous phase on the opposite side, thus encountering the rate-limiting step in diffusion[96,97]. For example, only 0.3% of epidural morphine is estimated to be able to cross the meninges due to the drug hydrophilicity[57]. The octanol:buffer$_{7.4}$ distribution coefficients for the drugs we analyzed are fentanyl 955, morphine 1.0, lidocaine 110, bupivacaine 560, dexmedetomidine 2.8, and xylazine 0.15, with a higher coefficient correlating with increased lipophilicity[5].

The specific gravity of CSF and the administered drug affect the distribution of drug throughout the subarachnoid space. Normal canine CSF ranges between 1.005 and 1.017 and feline CSF between 1.005 and 1.021[5]. In humans, hyperbaric drugs tend to spread caudally toward the lower spine, while hypobaric drugs spread rostrally against gravity[5]. Most drugs administered epidurally are hypobaric, such as morphine (1.002) and bupivacaine (1.003), or isobaric, such as lidocaine (1.010)[5]. When epidural opioids are administered, diffusion rates through the spinal meninges and rostral spread to the thoracic spine are inversely related to the drug molecular weight[96-98].

The μ, κ and δ opioid receptors are present in elevated concentrations in laminae I and II of the dorsal horn of the spinal cord[99]. When bound, these G-protein-linked receptors inhibit intracellular cAMP and voltage-gated calcium channels resulting in prevention of substance P release from C and A-δ nociceptive fibers, which subsequently inhibits ascending transmission via excitatory pathways to the brain. Opioids induce minimal blockade of B-sympathetic, A-α and A-β fibers, thus having little to no effect on vascular tone, motor, and tactile sensory modalities, respectively[5].

Local anesthetic medications act by blocking voltage-gated sodium channels in the nerve membrane, thus inhibiting the generation and propagation of action potentials. Because these channels are present in all nerve fibers, preferential blockade is not possible. Nerve fibers differ substantially in their susceptibility to blockade, as well as the onset and duration of effects, due to nerve fiber size and myelination. In general, smaller shorter fibers (B and C) are blocked before larger fibers (A) fibers, and unmyelinated fibers are blocked before similarly sized myelinated fibers[5,67]. The order of blockade seen clinically is B, C, A-δ, A-γ, A-β and A-α, corresponding to pain, warmth, touch, deep pressure and motor function, respectively[5,100]. In addition to lipid solubility, pK_a has also been shown to affect the rate of penetration of a drug through lipid membranes[100]. Three important effects that local anesthetics have on axonal excitability include: tonic impulse depression leading to conduction failure, as sub-blocking concentrations lead to progressive decreases in excitability until conduction is halted; use-dependent conduction failure, where the axon's capability to propogate action potentials is inhibited by the presence of local anesthetic-bound sodium channels; and activity-dependent suppression of impulses[101].

Spinal α_2-receptors initiate several mechanisms in a similar fashion to opioids, including blockage of substance P release from C nociceptive fibers, presynaptic binding on primary afferent terminals, and postsynaptic action on dorsal horn neurons[91]. Sensory blockade via C and A-δ fibers occurs, as well as moderate tactile sensory and mild motor blockade. Systemic α-effects can be quite dramatic depending on drug dose and distribution, but B sympathetic fibers are not directly affected. Xylazine has to been shown to have an isoflurane sparing effect when administered epidurally[83], and epidural medetomidine and dexmedetomidine have significant antinociceptive effects in cats[102] and dogs[92]. Several studies have suggested that epidural α_2-

agonist administration may potentiate epidural opioid analgesic effects[91,103]. Branson et al. demonstrated in a canine tail clamp model that a single dose of medetomidine administered epidurally had minimal analgesic effect alone, but when combined with morphine, more than doubled the duration of analgesia compared with morphine alone[91]. Similar results were seen in a tail withdrawal reaction test in rats, where epidural medetomidine was found to have minimal intrinsic antinociceptive effects, but produced significant potentiation of analgesia with epidural fentanyl[104]. A similar synergistic interaction with opioids was demonstrated with epidural morphine and medetomidine in dogs anesthetized for cranial cruciate ligament stabilization[105].

CHAPTER 3 - Experimental Study

PURPOSE

The purpose of this study was to compare the drug release characteristics of six common analgesic medications from a commercially available absorbable gelatin sponge *in vitro*, and extrapolate this data to determine the feasibility of a future *in vivo* prospective clinical study utilizing a mammalian surgical model. We hypothesized that the absorbable gelatin sponge would elute analgesic medications at a consistent, rapid rate which could be quantified *in vitro*.

MATERIALS AND METHODS

PHASE 1

Absorbable gelatin sponges were cut into approximately 10x20x7 mm pieces from commercially available slabs. The mass of each sponge was determined prior to drug addition. The mass of a sponge of the aforementioned dimensions was 0.0197 g, and to minimize variability, each sponge was trimmed until it weighed within 2.5% of this mass (range 0.0192 to 0.0202 g; mean 0.0197 g; SD 0.0003). Five milliliters of each of six medications was placed into a six milliliter glass test tube. Medications evaluated included two opioid medications, preservative-free morphine sulfate (Duramorph, Hospira, Inc., Lake Forest, IL) (25 mg/ml) and fentanyl citrate (Fentanyl citrate, Hospira, Inc., Lake Forest, IL) (50 μg/ml), two local anesthestics, bupivacaine hydrochloride (Bupivacaine HCl, Hospira, Inc., Lake Forest, IL) (5 mg/ml) and lidocaine hydrochloride (Lidocaine HCl, Hospira, Inc., Lake Forest, IL) (20 mg/ml), and two α_2-adrenergic agonists, dexmedetomidine hydrochloride (Dexdomitor, Pfizer Animal

Health, New York, NY) (0.5 mg/ml) and xylazine hydrochloride (AnaSed, Lloyd Laboratories, Shenandoah, IA) (100 mg/ml). Each of the different drugs was assessed in triplicate. Each sponge was completely immersed in the specific medication for five seconds, squeezed entirely to expel air bubbles, and immediately replaced in the test tube. After soaking for one hour, the sponges was carefully removed with thumb forceps and placed directly in an individual petri dish filled with twenty milliliters of sterile phosphate-buffered saline (PBS) at pH 7.4. The medium was maintained at pH 7.4, 37° C and constantly agitated throughout the study. The PBS was sampled at 0, 0.5, 1, 2, 4, 8, 12 and 24 hours by removal of 200 microliters of the eluent fluid. After each sample was collected, 200 microliters fresh PBS was added, and each test tube was returned to the agitator. The collagen sponges were not rinsed with PBS between transfers to more closely approximate physiologic conditions. Eluent samples were immediately frozen and stored at -70˚ C. Concentrations of each drug were determined in triplicate via high-pressure liquid chromatography (HPLC) with UV detection or HPLC with mass spectrometry as detailed below.

PHASE 2

Phase two was similar to phase one with the exception of altering sponge loading and timing of sample collection. Due to the high concentrations of xylazine observed in phase 1, the concentration of xylazine in phase two was 20 mg/mL. Instead of immersing the sponges in each analgesic medication for one hour, the sponges were loaded by placing the test tubes with the sponge and five milliliters of each medication in an evaporator unit (TurboVap LV evaporator, Zymark Inc., Portland, OR). This unit subjected the test tubes to continuous air flow (15 psi) and a warm water bath (37° C) until the medications had evaporated and the sponges

were visibly dry. This loading phase took between 24 and 36 hours. The loaded sponges were then placed in PBS as previously described in phase one. Eluent samples were collected at the following time points: 0, 10, 20, 30, 45, 60 and 90 minutes and 2, 4, 8, 12 and 24 hours.

Determination of drug concentrations in PBS

The concentrations of fentanyl (*m/z* 337.14 →105.3), dexmedetomidine (*m/z* 201.231 →95.1), and morphine (*m/z* 286.08 → 152.1) were determined using liquid chromatography with mass spectrometry. The internal standard solution contained fentanyl d5 (*m/z* 342.158 → 105.0) 100 ng/mL for fentanyl, ondansetron (*m/z* 294.206 → 170.3) 1000 ng/mL for dexmedetomidine, and morphine d6 (*m/z* 292.13 → 151.90), 1000 ng/mL for morphine in 50% methanol. Standard solutions were made in PBS. The analyte ranges were 0.1-10.0 µg/mL for fentanyl, 1.0 - 50.0 µg/mL for dexmedetomidine, and 1.5625 – 50.0 µg/mL for morphine. Standard curves were constructed daily and run at the beginning and the end of the sample batches and accepted if the predicted value was within 15% of the actual concentration and the correlation coefficient (R) was at least 0.99. The sample preparation for fentanyl consisted of adding 25 µL of the sample or standard and 50 µL of the internal standard solution to 425 µL 0.1% formic acid and vortexed. The sample preparation for dexmedetomidine consisted of adding 50 µL of the sample or standard and 50 µL of the internal standard solution to 400 µL 0.1% formic acid and vortexed. Due to the high concentrations of morphine in all samples, they were initially diluted by adding 20 µL of the sample to 980 µL PBS. The diluted samples or standards, 20 µL, were added to 50 µL of IS and 930 µL of 0.1% formic acid. The mobile phase consisted of A: acetonitrile and B: 0.1% formic acid at a flow rate of 0.4 mL/min. A gradient starting at 100% B from 0-1 minute, then a linear gradient to 80% B at 3 minutes which was held until 6 minutes, then a linear

gradient to 100% B 6.5 minutes with a total run time of 7.5 minutes. The column used was a C18 column (Supelco Discovery 2.1x50 mm, 5.5 μM, Sigma, St. Louis, MO, USA).

Lidocaine, bupivacaine and xylazine were measured by HPLC with UV detection. Lidocaine and bupivacaine standards and samples were prepared by adding 10 μL to 990 of 0.05% trifluroacetic acid. The standard curve for lidocaine was linear from 0.125 – 2 mg/mL, for bupivacaine from 0.03125 – 1 mg/mL, and for xylazine from 0.3125 – 10 mg/mL. Standard curves were accepted if the predicted concentration was within 15% of the actual concentration and $R>0.99$. The UV absorption was monitored at 203 nM for lidocaine and 215 nM for bupivacaine and xylazine. The mobile phase consisted of A: acetonitrile and B: 0.02% TFA at a flow rate of 1 mL/min. The mobile phase for lidocaine, bupivacaine and xylazine were 82% B, 70% B, and 78% B, respectively.

The resultant data were evaluated with a regression analysis. Statistical analysis was performed using repeated measures ANOVA. $P<.05$ was considered significant for all statistical calculations.

Based on the structural variation observed when cutting the gelatin sponges to size, we used sponge mass to minimize variability. We then evaluated the relationship of sponge mass to sponge volume with unused, unloaded gelatin sponges. Twenty sponges were cut into rectangular prisms weighing precisely 0.0200 g. The linear dimensions of these sponges were then measured in millimeters using a surgical ruler (Kendall Devon skin marker & ruler, Tyco Healthcare Group LP, Mansfield, MA) to simulate a type of measurement device routinely available in a surgical environment. The volume of each sponge was then calculated in cubic millimeters using the formula V= length x width x height.

RESULTS

In phase one, all analgesic medications eluted from the gelatin sponge and equilibrated rapidly with the PBS (Figure 1). Results were consistent for all medications, with minimal variation within each trial. Maximal concentration of each medication was achieved within 30 minutes for all medications.

In phase two, maximum elution also occurred by 30 minutes (Figure 2). By increasing the sampling frequency within this initial 30 minute period, we were able to capture the rapid nature of this release. Results were again consistent for all medications, with a similarly minimal amount of variation within each trial.

Steady state concentrations were achieved within two hours in both phases, and were significantly higher in phase two than in phase one with four out of six medications. The mean steady state concentration of fentanyl in phase two was 8.37 μg/mL, which was significantly higher than the mean steady state concentration of 1.45 μg/mL in phase one ($p<.001$) (Figure 6). The mean steady state concentration of lidocaine in phase two was 2718 μg/mL, which was significantly higher than the mean steady state concentration of 554.9 μg/mL in phase one ($p<.001$) (Figure 3). The mean steady state concentration of bupivacaine in phase two was 729.9 μg/mL, which was significantly higher than the mean steady state concentration of 208.7 μg/mL in phase one ($p<.001$) (Figure 4). The mean steady state concentration of dexmedetomidine in phase two was 60.4 μg/mL, which was significantly higher than the mean steady state concentration of 22.5 μg/mL in phase one ($p<.001$) (Figure 8). In contrast to the other medications, the mean steady state concentration of xylazine in phase one was 4966.4 μg/mL, which was significantly higher than the mean steady state concentration of 964.5 μg/mL in phase two ($p<.001$) (Figure 7). The difference in xylazine concentration, five-fold, is similar to the

difference in concentration used in phase one (100 mg/mL) and phase two (20 mg/mL). The mean steady state concentration of morphine was 664.7 μg/mL in phase one and 720.3 μg/mL in phase two, which were not significantly different (p=.18) (Figure 5). The sponges remained intact throughout the sampling in phase one, but in phase two, some of the dried sponges broke apart when transferred.

Twenty gelatin sponges each weighing precisely 0.0200 g had volumes ranging from 1260 to 1540 mm^3. The corresponding linear width dimension of each sponge was quite variable, ranging from 9 to 11 mm, while the height and length were consistently 7 mm and 20 mm, respectively.

DISCUSSION

A method to deliver controlled amounts of analgesic medications in a local environment is desirable for a number of reasons. Decreased systemic side effects, decreased medication quantities, and potential increased efficacy can be seen with such a delivery technique[1-5]. Having an inexpensive, practical local analgesic option rapidly available during the immediate perioperative period would be beneficial to veterinary surgeons. Various delivery devices have been investigated for a plethora of medications with mixed results. The most commonly used compounds to elute medications in veterinary surgery are polymethylmethacrylate and calcium sulfate beads, often placed in infected surgical environments to elute antibiotics over weeks to months[9-12]. Several morphine-eluting gels and pastes have been investigated for use in human spinal surgery, with mixed results regarding analgesic efficacy and prevention of post-surgical scarring[20,106-110]. Recently, some veterinary surgeons have augmented their standard post-operative hemilaminectomy pain protocol with the addition of an analgesic-impregnated gelatin

sponge placed peridurally within the hemilaminectomy window[44]. This modification anecdotally improved patient comfort, and prospective clinical analysis of this technique appears promising[45]. To the authors' knowledge, elution of veterinary analgesic medications from an absorbable gelatin sponge has not been quantified. We chose two medications from each of the following classes: μ-opioid receptor agonists (fentanyl, morphine), α_2-adrenoceptor agonists (dexmedetomidine, xylazine) and sodium-channel blocking local anesthetics (lidocaine, bupivacaine). The results of this pilot study confirm that analgesic medications were eluted from an absorbable gelatin sponge at a consistent, albeit rapid, rate.

The concentration of each medication appeared to be directly related to the eluted steady state concentration achieved. Fentanyl, the medication with the lowest concentration used to load the sponges (0.05 mg/mL), achieved the lowest steady state concentration (1.45 μg/mL). As the concentration of the medication increased, the corresponding steady state concentration achieved was higher. This applied for all medications sampled in phase one, with the most concentrated medication, xylazine at 100 mg/mL, achieving the highest steady state concentration at 4966.4 μg/mL. In phase two, this effect was similar with the three least concentrated medications: fentanyl, dexmedetomidine and bupivacaine, respectively. However, the most concentrated medication, morphine at 25 mg/mL, did not achieve the highest steady state concentration. This was achieved by lidocaine (20 mg/mL) with a mean steady state concentration of 2718 μg/mL. The reason for this result is unknown, although variable drug stability while exposed to light and increased temperatures during evaporation, or differing affinities of each medication for the gelatin sponge may be factors.

By drying the loaded sponges before insertion into the elution medium, significantly higher steady state concentrations were consistently achieved compared with undried sponges.

This effect was seen with fentanyl, lidocaine, bupivacaine and dexmedetomidine. In contrast to these medications, significantly greater steady state concentrations were achieved with undried sponges than with dried sponges when utilizing xylazine. This is very likely the result of the altering of the concentration of xylazine used to load the sponges between phases one and two, with 100 mg/ml solution utilized for phase one and 20 mg/ml solution for phase two. This change in xylazine concentration between phases was made because of the massive absolute quantity of xylazine eluted in the first phase. Toxicity concerns would be a major factor, as discussed further in the next paragraph. If the same concentration of xylazine was used for both phases, a similar effect may have been seen. Assuming a linear relationship existed between the drug concentration and the steady state concentration achieved, we would have expected a five-fold increase in steady state xylazine concentrations in phase two if the 100 mg/ml solution had been used instead of the 20 mg/ml solution. The steady state concentration of morphine was not statistically significant between phases.

When quantifying the eluent concentrations of all medications, toxicity concerns become readily apparent. For example, the steady state concentration of xylazine achieved in phase one was approximately 5 mg/ml, which equates to approximately 100 mg of drug eluted from each sponge. This total quantity of xylazine would likely have a substantial potential for toxicity, especially in small animals. Xylazine administration using this concentration and vehicle could have profound and potentially lethal consequences, as recommended doses range from 0.5 to 2.2 mg/kg in dogs[86]. Even larger dogs weighing 30 to 40 kg would be overdosed with this protocol. Depending on the level of systemic absorption and distribution, described side effects of xylazine include emesis, muscle tremors, bradycardia, bradypnea, and even fatal cardiac arrest[111,112]. The toxic effects of lidocaine have also been extensively investigated in animals,

with concerns arising at serum levels above 8 μg/mL in dogs[113-116]. Described adverse effects include central nervous system stimulation, muscle tremors, ataxia, depression, nystagmus, hypotension, bradycardia (PR and QRS interval prolongation), seizures and circulatory collapse[86]. Similar toxicity concerns could exist with all medications, especially if utilizing loaded sponges prepared as in phase two, as significantly greater mean steady state concentrations of eluted medication are achieved. Critical evaluation of these variables with *in vivo* pharmacokinetic and pharmacodynamic analyses as well as establishment of efficacious dosing regimens would be prudent prior to clinical implementation.

Unfortunately, achieving prolonged elution was not accomplished. By drying the analgesic-loaded sponge prior to placement in the medium, we were able to achieve higher steady state concentrations but the speed of elution was not altered. Further modifications of the gelatin sponge may be considered to prolong medication elution. In one study investigating ophthalmic pilocarpine release from Gelfoam, researchers were able to extend release for five hours by impregnating the sponge with two diffusion restrictors, cetyl ester wax and polyethylene glycol 400 monostearate, which are relatively inexpensive and nonirritating substances[31]. By embedding the sponge with these retardants using an extensive production protocol, prolonged drug elution may be attained by slowing release medium penetration and simultaneously preventing rapid outward diffusion of the drug. Other techniques known to change the local elution kinetics from collagen-based matrices include cross-linking the matrix to decrease permeability, increasing collagen content, and making the drug molecule less water soluble[37]. According to the manufacturer, a gelatin sponge will absorb completely in four to six weeks when placed in an appropriate environment[22]. Thus, it appears that achieving prolonged release beyond this time frame is unlikely without significant vehicle modifications.

The gelatin sponge device we utilized is composed of a network of interstitial pores which allow release medium to rapidly penetrate the matrix interior. We hypothesize that the eluent quickly penetrated the pores and dissolved the medication, with the medication subsequently diffusing throughout the external PBS. The rate at which eluent penetrates the sponge is dependent on the tortuosity of the pores, the sponge density, solubility and diffusion coefficient in the solvent[31]. In phase one, this process appears to have occurred instantaneously. Our first sampling point was 30 minutes, and additional sampling within the initial 30 minutes may have revealed differences between each medication elution rate. However, these potential differences within the initial 30 minutes would be highly unlikely to be clinically relevant. In phase two, we adjusted our sampling times to further scrutinize the drug release kinetics with focus on the first hour. The release was again rapid for all medications, although sampling points within the first hour demonstrated an exponential release curve with maximal steady state concentrations achieved after approximately one to two hours.

Determination of the time to onset and duration of action for medications delivered into a wound environment is likely multifactorial, involving surface area, volume and porosity of the delivery device, volume and concentration of the eluted medication, relative lipo- or hydrophilicity of the medication, eluent fluid turnover rate in the local environment, and pH and temperature of the environment. Relative lipophilicity and hydrophilicity did not seem to affect the elution rate in our study. Fentanyl is a highly lipophilic drug, while morphine is relatively hydrophilic, and the elution pattern was identical. This finding suggests that the medication lipophilicity did not affect binding to the gelatin sponge nor dissolution in the release media. It is unknown whether this variable would influence elution in a closed *in vivo* situation. However, a local environment with a low fluid turnover rate may lead to prolonged elution.

The relationship of sponge mass to sponge surface area and volume is variable as demonstrated by the aforementioned results. It appeared that the structural integrity of each sponge was variable, and the effects of these inconsistencies are unknown. The seemingly rapid penetrability and relative porosity of the gelatin sponge suggest that sponge mass and volume may not have an appreciable impact of elution rates, while these measurements may be a more determinant factor in mean steady state concentrations or the total amount of medication eluted. If used clinically, maintaining a precise sponge size may be important to minimize variability.

The medications investigated in this study are commonly utilized for postoperative analgesia. Potential applications for this vehicle include post-operative hemilaminectomy and other spinal surgery, limb or digit amputation, radical reconstructive procedures (mandibulectomy, hemipelvectomy) and many more. In addition, delivered medications would not be limited to analgesia alone. Impregnating a gelatin sponge with antibiotics may be possible for placement within an infected wound environment or a location where systemically administered antibiotics may not penetrate well.

One of the potential limitations with this study was the use of phosphate buffered saline instead of bovine serum, which would more closely imitate physiologic conditions. We believe that elution was unlikely to be affected by using PBS, and many drug elution studies have utilized PBS as the eluent[2,4,9-12,17]. Differing drug protein-binding affinities would be apparent when using serum, but this variable is more likely to affect distribution and elimination *in vivo*, and unlikely to affect drug elution *in vitro*. During phase two, several dessicated sponges broke into several pieces when transferred from the evaporating test tube to the elution medium. This structural change and increase in sponge surface area could have affected drug elution, although did not appear to have this effect in the present study. In addition, variable amounts of

crystallized medication were present on the surface of each sponge, potentially altering the resulting concentrations. Although unlikely, it is possible that further medication was eluted beyond 24 hours, which would not have been recognized with our study design and clinically insignificant due to lack of sustained elution throughout the initial 24 hours. During sponge preparation, it was noted that gross structural inconsistencies existed within as well as between each sponge. Because of this, we decided to utilize mass which would more accurately reflect the quantity of microscopic interstices for each sponge to minimize variation. Exact geometric dimensions were not calculated, although each sponge was prepared in approximately rectangular cuboid fashion.

This study confirms that the absorbable gelatin sponge has potential for use as a delivery vehicle for analgesic medications. The relatively low cost of these commercially-available products makes them attractive compared with other expensive custom-made delivery matrices. However, the immediate release of these medications calls into question the practicality and duration of efficacy of this model in veterinary surgical patients. In addition, potentially toxic doses of analgesic medications could be eluted in an *in vivo* situation. Without further modification of the sponge to reliably extend drug release, we cannot recommend clinical use at this time. Further studies are necessary to determine if other alterations of the sponge-drug preparation technique will appropriately prolong drug elution while maintaining the practicality and cost effectiveness desired in veterinary surgery. In addition, prospective clinical trials are needed to ascertain analgesic efficacy and establish safe dosing regimes.

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Figures

Figure 1. Phase 1 results.

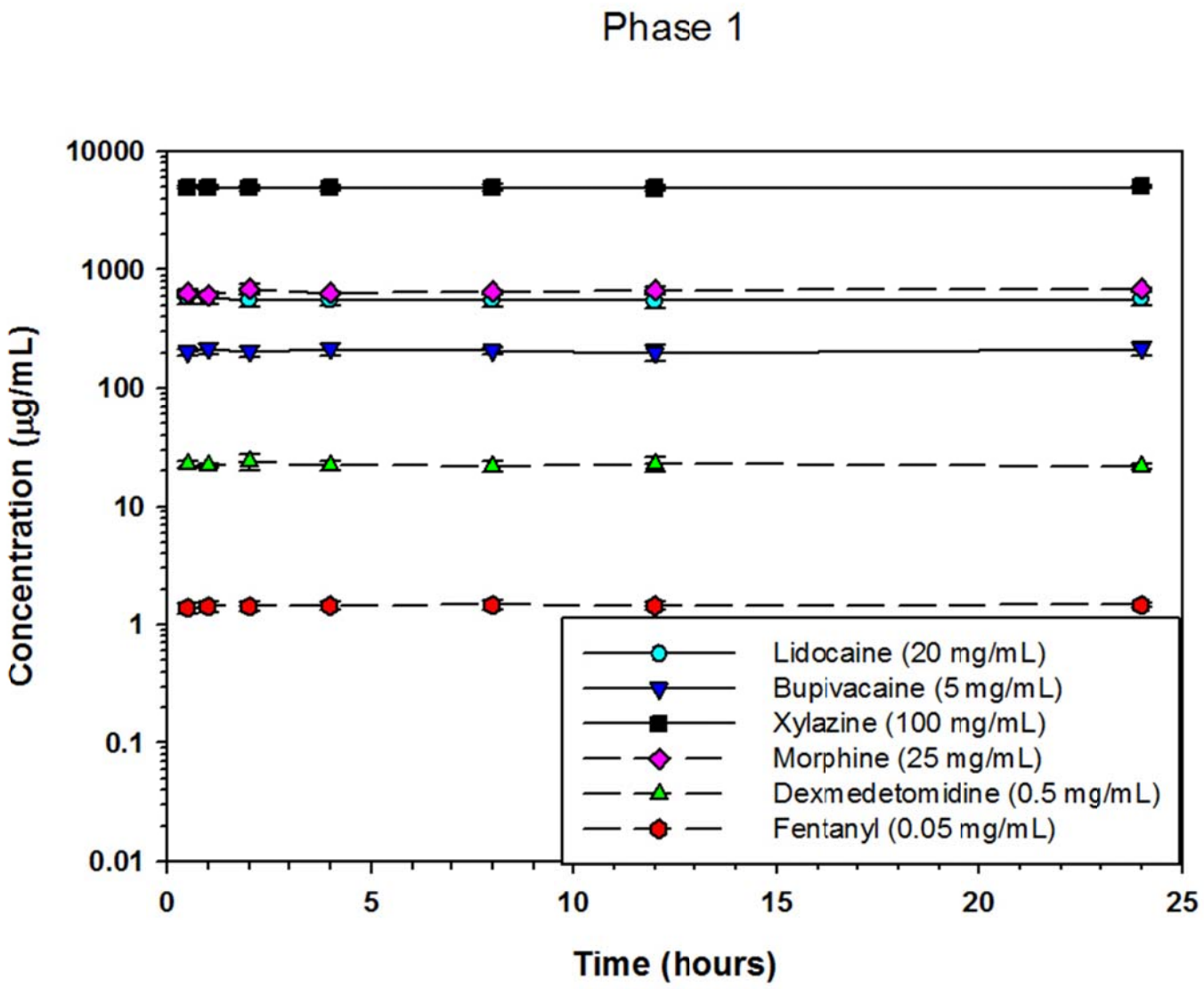


Figure 2. Phase 2 results.

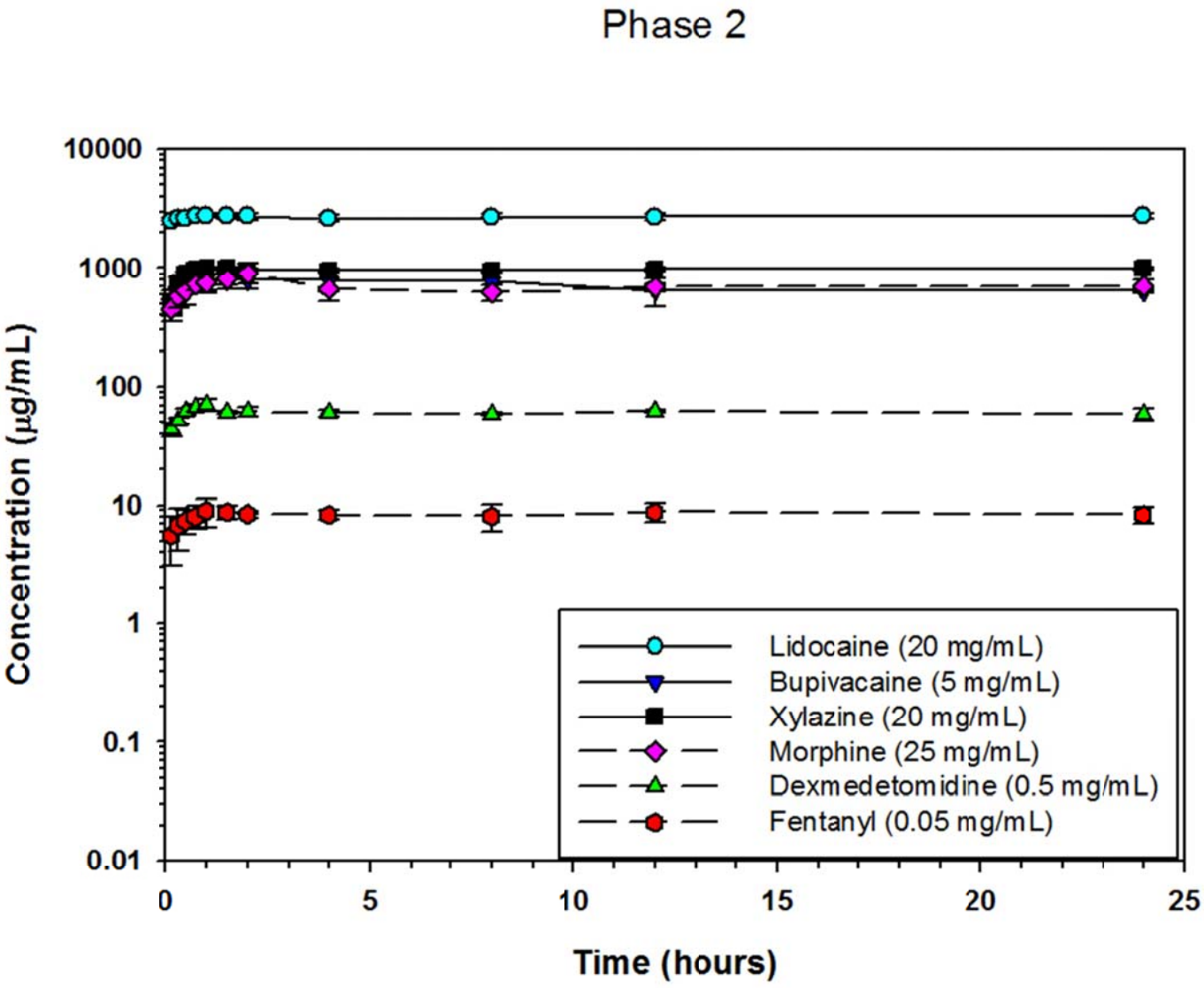
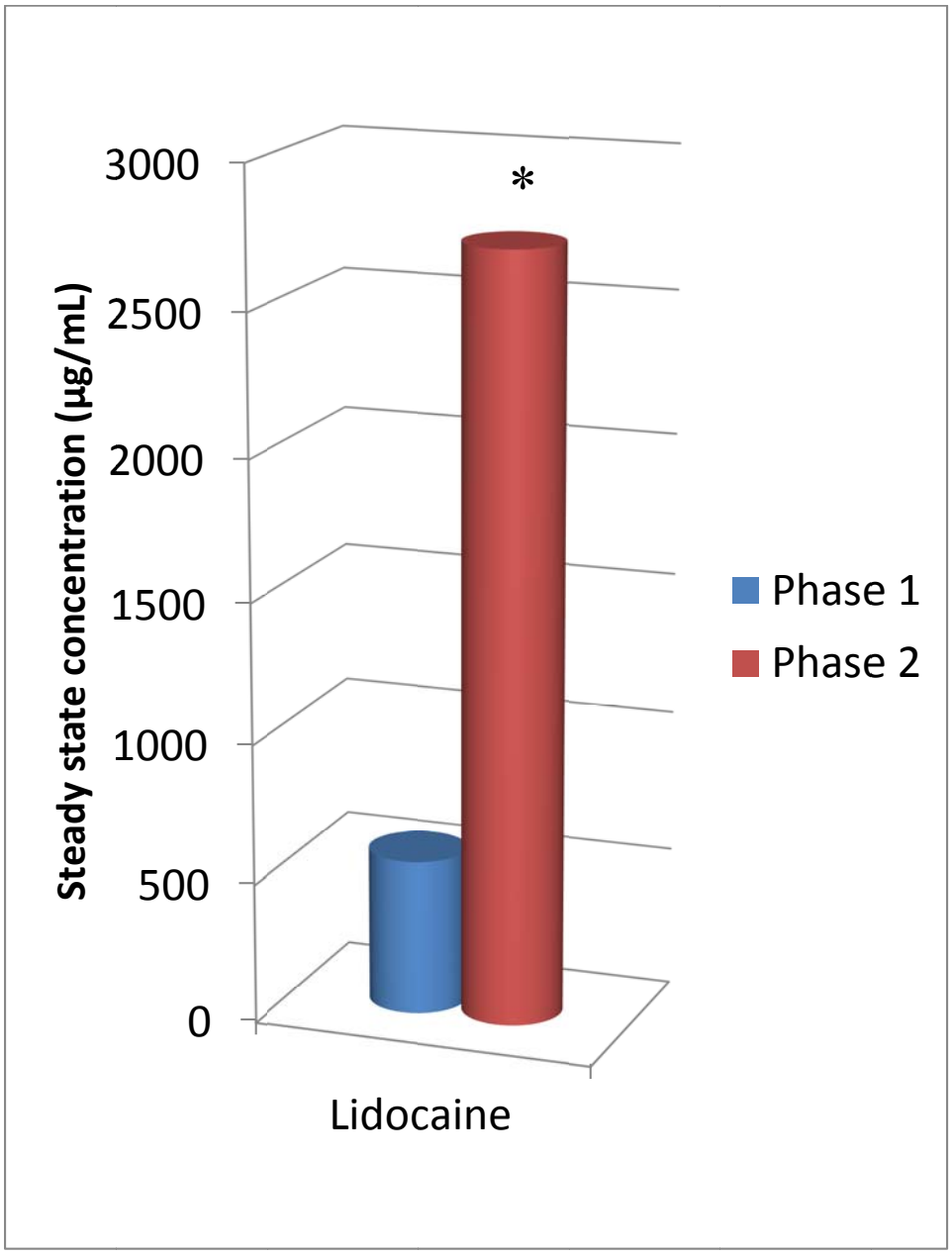
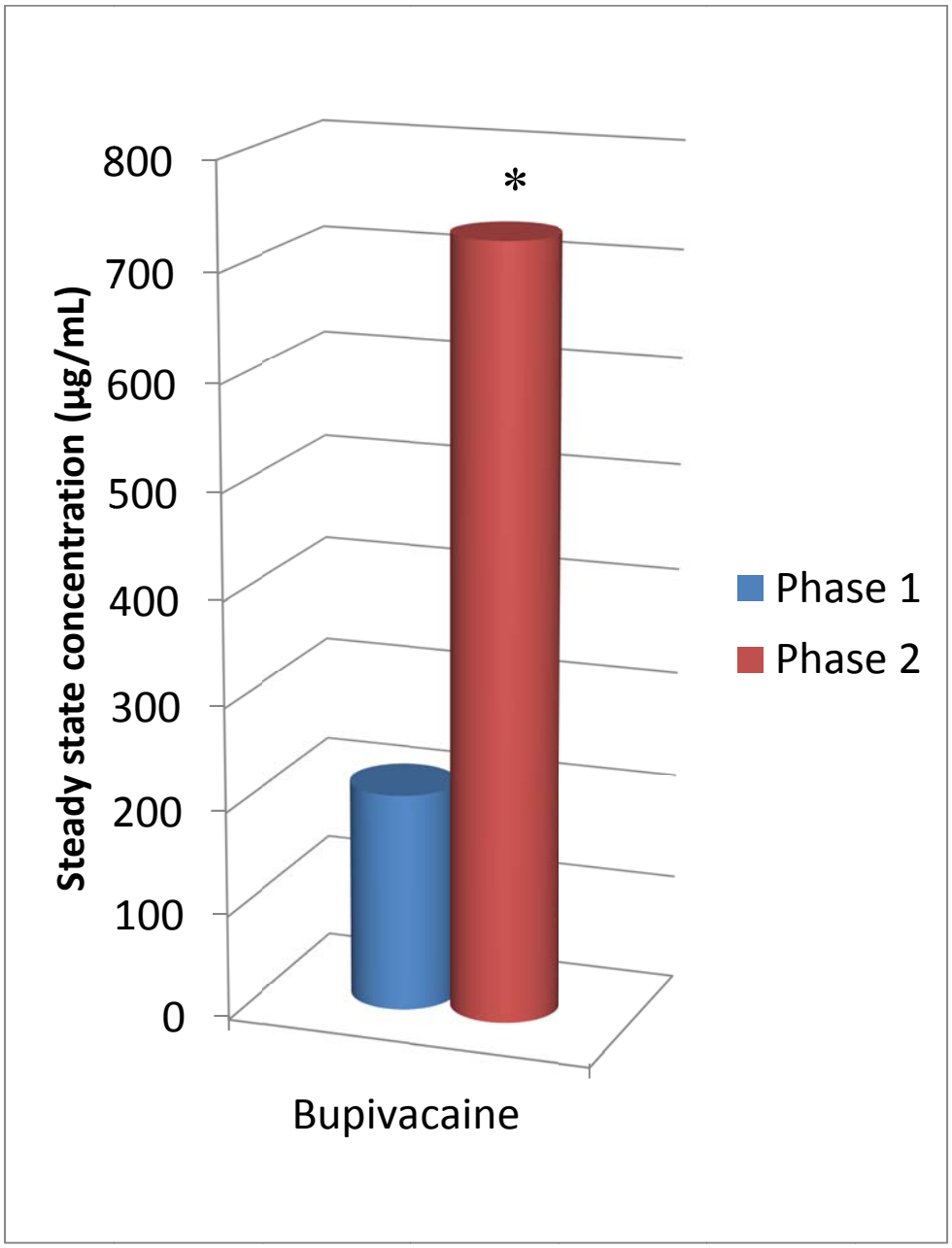


Figure 3. Comparison of steady state concentration of lidocaine between phase 1 and 2.



$P < 0.05$

Figure 4. Comparison of steady state concentration of bupivacaine between phase 1 and 2.



$P < 0.05$

Figure 5. Comparison of steady state concentration of morphine between phase 1 and 2.

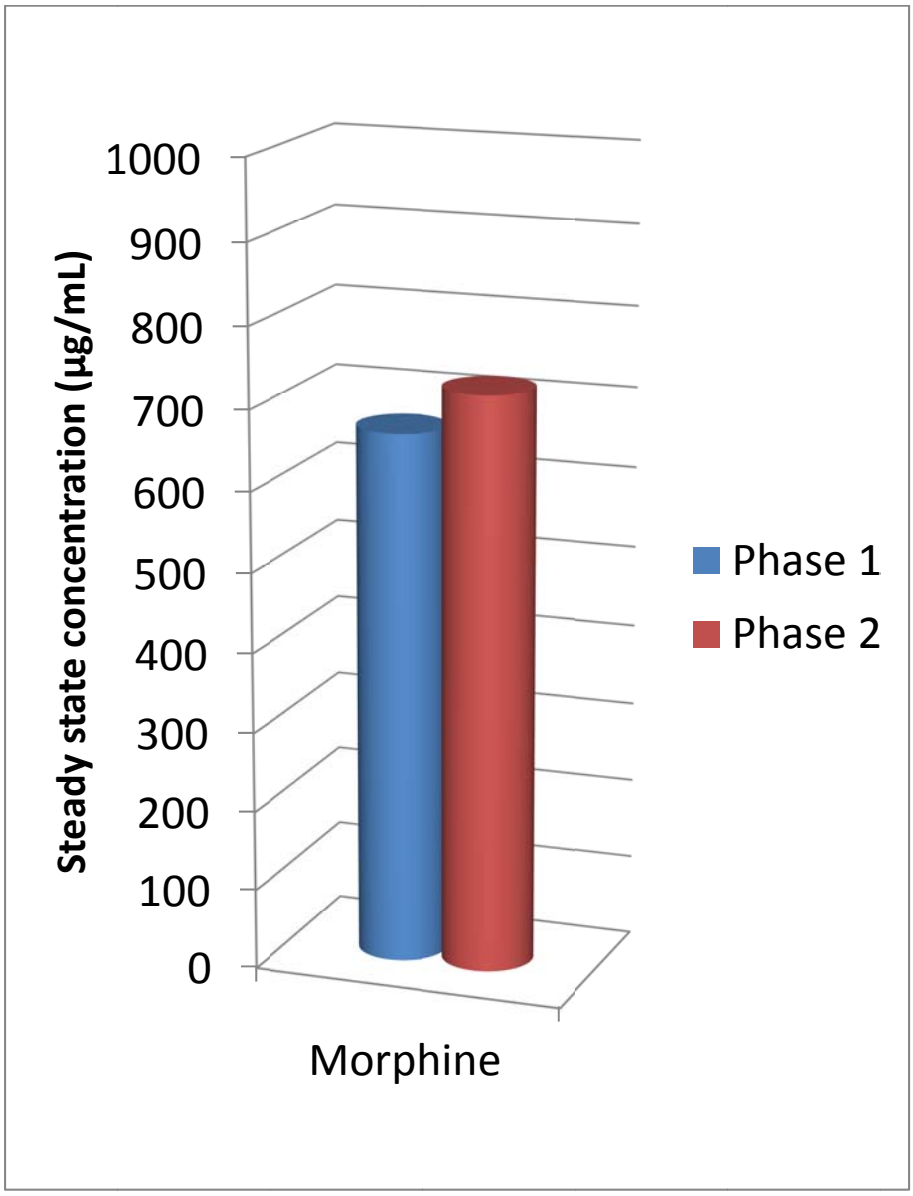
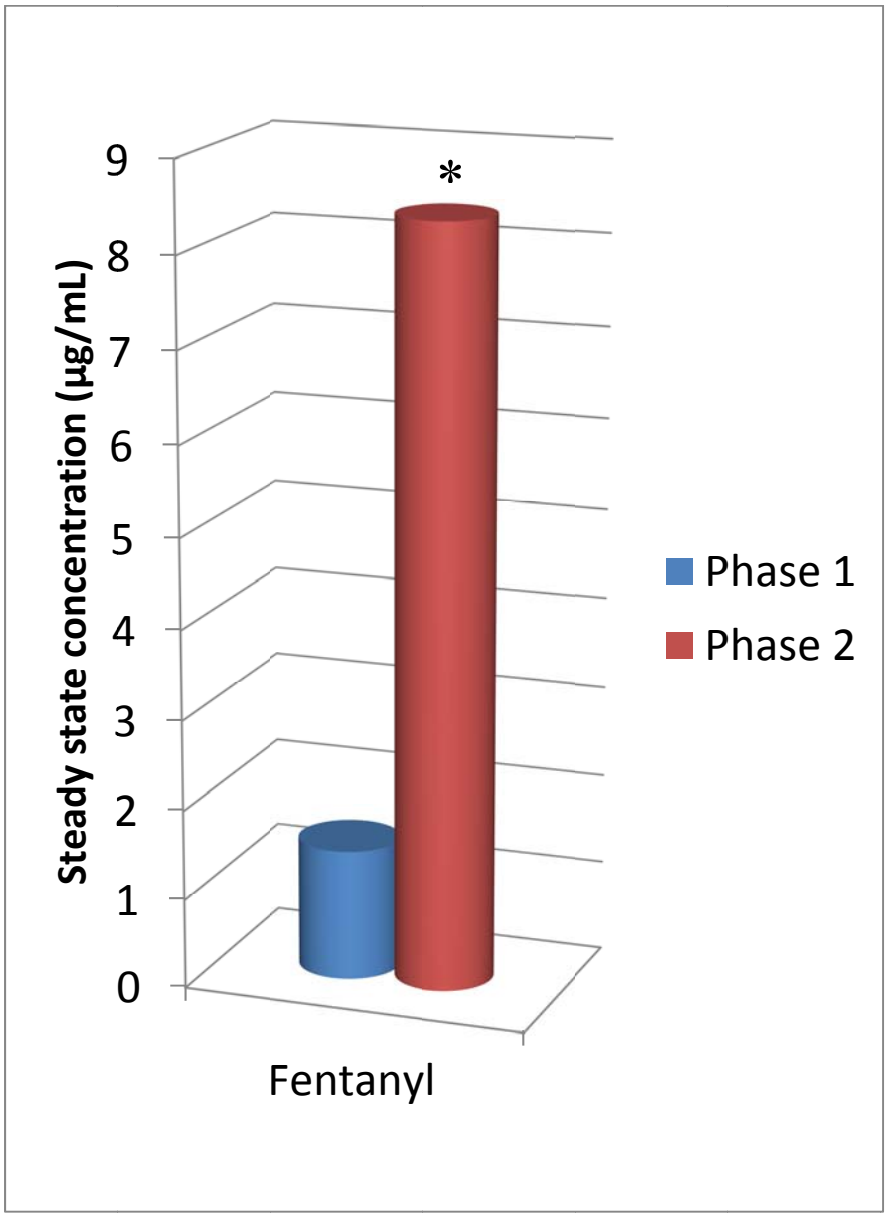
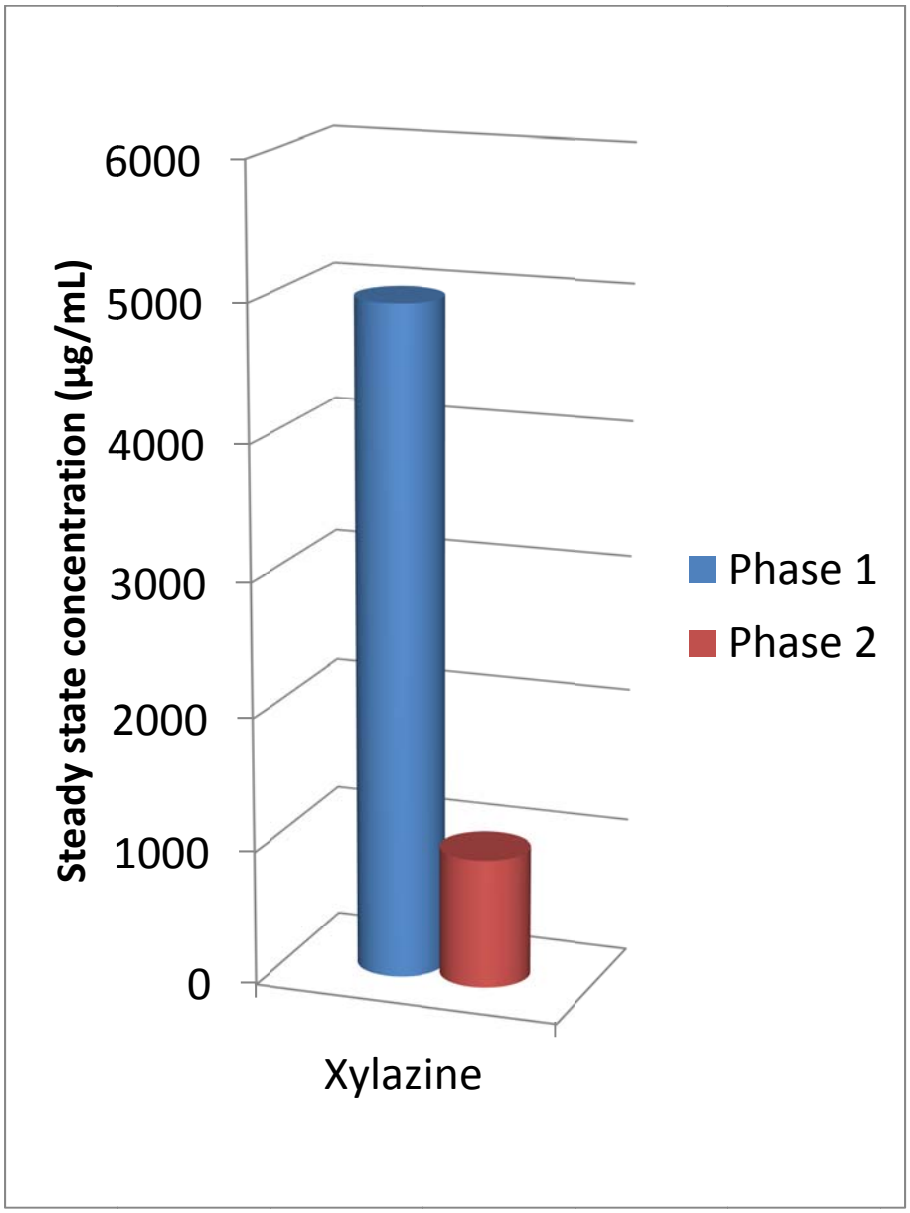


Figure 6. Comparison of steady state concentration of fentanyl between phase 1 and 2.



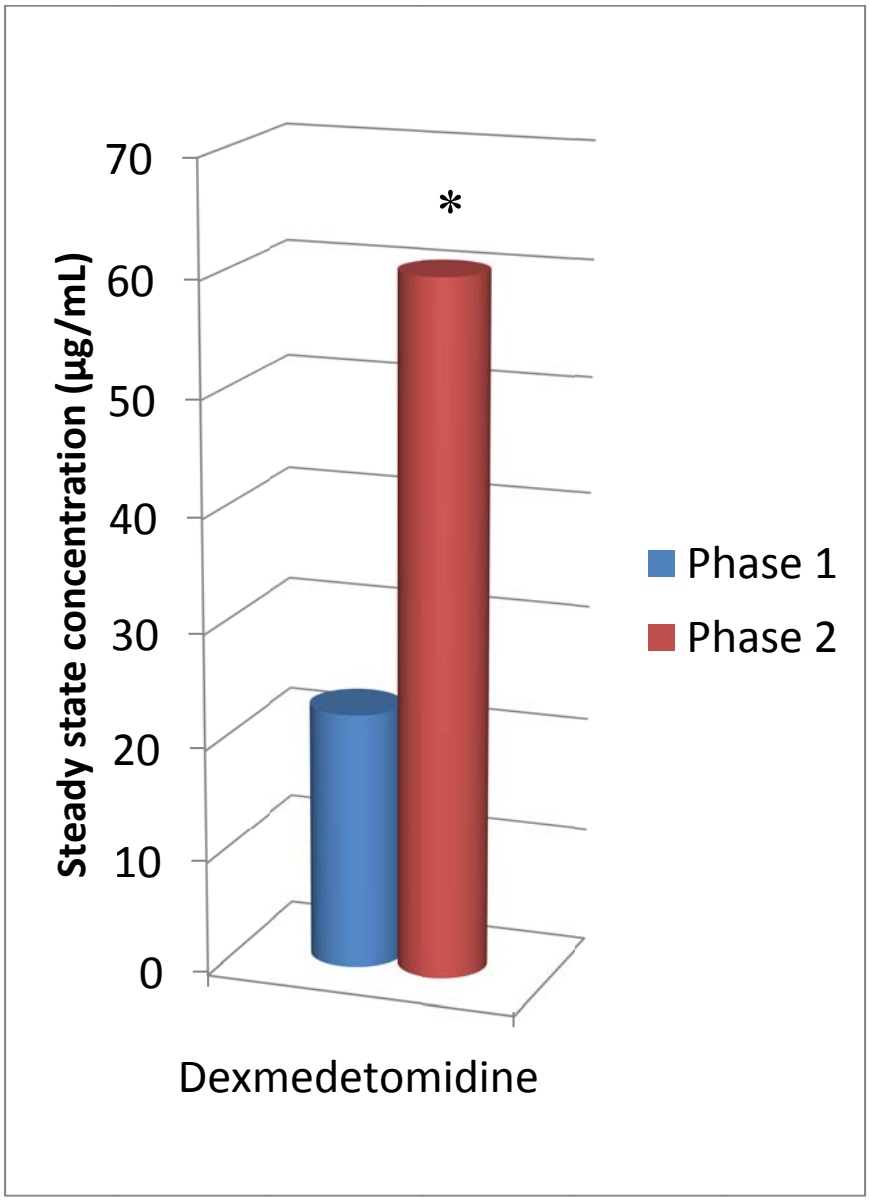
$P < 0.05$

Figure 7. Comparison of steady state concentration of xylazine between phase 1 and 2.



$P < 0.05$

Figure 8. Comparison of steady state concentration of dexmedetomidine between phase 1 and 2.



$P < 0.05$