

AN EVALUATION OF THE HISTOLOGICAL EFFECTS OF INTRA-ARTICULAR METHADONE IN THE CANINE MODEL

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Methadone hydrochloride is an opiate that has pharmacodynamic and pharmacokinetic properties that suggest it may provide longer analgesia than morphine when administered via the intra-articular route. However, no studies to date have been conducted examining the effects of intra-articular methadone hydrochloride on local tissues. Therefore, the purpose of this study was to determine the histopathologic effects of intra-articular methadone hydrochloride on local tissues in the canine knee.

Nine canines, 1 to 4 years old, weighing between 20 kg and 23 kg were used. All canines had their knees randomized to receive either bupivacaine, 0.5% with epinephrine 1:200,000 (4.5 mL), and 5 mg methadone hydrochloride (0.5 mL) for the study knee, or bupivacaine, 0.5% with epi-

nephrine 1:200,000 (4.5 mL), and 0.5 mL normal saline for the control knee. Serum methadone hydrochloride levels were obtained on all canines at 6 and 24 hours. Canines were randomly assigned to 1 of 3 groups to be euthanized at either 24 hours, 14 days, or 28 days. Following euthanization and necropsy, synovial fluid levels and tissue samples were obtained and examined for histopathologic changes.

Synovial fluid samples noted a few white blood cells at 24 hours and none at 14 and 28 days. Tissue samples showed no histopathologic changes, and serum concentration levels of methadone hydrochloride were negligible.

Key words: Euthanasia, histopathological, intra-articular, methadone hydrochloride, necropsy.

The Agency for Health Care Policy and Research (AHCPR) resolves that uncontrolled postoperative pain causes unnecessary suffering, delays recovery, prolongs hospitalization, and increases medical bills in 50% of the 23 million Americans who undergo surgery each year.¹ Based largely on the findings from the AHCPR, the Joint Commission on Accreditation of Healthcare Organizations has mandated that the effectiveness of postoperative analgesia be evaluated as an outcome measure of quality care of the surgical patient.

Innovative and novel techniques are continually sought to improve acute postoperative pain management. Numerous human and animal investigations have explored the antinociceptive effects of intra-articular (IA) local anesthetics and opioids. Existing data support the presence of a binding site with characteristics of the μ -opioid receptor on peripheral sensory nerves.² Recent studies have demonstrated

antinociceptive effects of opioid agonists mediated by peripherally located opioid receptors. These peripherally located opioid receptors become more receptive to opioid agonists in the presence of inflammatory mediators. Additionally, there is a direct correlation between the degree of receptor responsiveness and the amount of inflammation.³⁻⁷ Stein et al⁸ and Cepeda et al⁹ also were able to demonstrate peripheral opioid action at sites of inflammation.

The assertion that analgesia can be derived from peripherally located opioid receptors is further substantiated by the fact that IA cartilaginous structures are relatively avascular in adults. This relative avascularity accounts for the negligible serum opioid levels obtained following IA injection. Ironically, clinically significant analgesia was obtained in the absence of serum opioid concentrations required for postoperative pain relief.⁵

Studies seeking to compare the analgesic effects of

IA bupivacaine and morphine sulfate after arthroscopic surgery have been well documented. There is strong scientific evidence to suggest that morphine sulfate administered intra-articularly can provide sustained analgesia without causing histologic changes in any of the joint space tissues.¹⁰⁻¹³ The histologic effects of IA saline and bupivacaine also have been examined in several studies, and data has repeatedly demonstrated no histologic changes in the articular surfaces.¹⁴⁻¹⁶

The pharmacodynamic and pharmacokinetic properties of methadone hydrochloride suggest extended analgesia may be observed from the IA administration of this opioid. Methadone hydrochloride is a synthetic opioid agonist with pharmacologic properties similar to the opioid agonist morphine sulfate. Both methadone hydrochloride and morphine sulfate demonstrate equianalgesic dosing but differ significantly regarding their elimination half-lives. The half-life of methadone hydrochloride is approximately 35 plus or minus 12 hours compared to 1.9 plus or minus 0.5 hours for morphine sulfate. This prolonged elimination half-life is secondary to methadone hydrochloride's high degree of protein binding and low lipid solubility.^{17,18}

Methadone hydrochloride has the potential to provide patients with a clinically significant, longer period of postoperative analgesia; however, no study to date has established the safety of methadone hydrochloride administered intra-articularly in humans. Therefore, it was imperative to establish the safety of IA methadone hydrochloride in an animal model prior to conducting a clinical trial in humans. Additionally, the US Food and Drug Administration (FDA) mandates that an investigational new drug application be filed when a drug is administered via an unconventional or new route. The purpose of this study was to determine the histopathologic effects of methadone hydrochloride on various tissue types in the canine knee.

Materials and methods

Nine random source canines were obtained from a US Department of Agriculture approved vendor. The animals were sexually mature (1.5-4 years of age) and weighed 20 kg to 23 kg. Microchip transponders were injected subcutaneously (dorsoscapular) into the animals, and a scanner was used for individual identification. Animals were singly housed in environmentally controlled rooms (21°C, 12/12 hour light cycle). Fixed formula, extruded maintenance dog chow (Teklad Global 215 Protein Dog Diet, Harlan Tedlad, Madison, Wis) and tap water were available *ad libi-*

tum. The canines were given a 2-week period to acclimate to their environment.

This study was performed in a facility with an animal care and use program accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International and in full compliance with the *Guide for the Care and Use of Laboratory Animals*.¹⁹ The Institutional Animal Care and Use Committee at Naval Medical Center, Portsmouth, Va, approved all animal procedures.

On the day of testing, the canines were removed from their individual cages and sedated by veterinary staff with acepromazine (0.05-0.01 mg/kg) subcutaneously. Additional sedation was available in the event any of the canines responded to the procedure. The injection site on both knees was shaved and prepared with a Betadine solution. A 20-gauge, 1.5-inch needle was used for IA injection. A study design was used so the canines could serve as their own control. A randomly selected knee was chosen for injection of the study drug, while the other knee was designated as control. The study medication of bupivacaine, 0.5% with epinephrine 1:200,000 (4.5 mL) with 5 mg methadone hydrochloride (0.5 mL), was injected by staff orthopedic surgeons using aseptic technique into the knee joint, while the control knee received bupivacaine, 0.5% with epinephrine 1:200,000 (4.5 mL), with 0.5 mL normal saline. The attending veterinarian, veterinary pathologist, and veterinary staff were blind as to which knee received the study injectate or the control injectate.

Following the IA injection, veterinary staff observed the canines for signs or symptoms of discomfort and/or attention to the limb. A staff veterinarian was always available to evaluate the dogs if any problems were identified. Based on the staff veterinarian's examination and recommendation, buprenorphine, 0.3 mg subcutaneously, was available to treat postinjection pain. Additionally, any canine could be withdrawn from the study at any time based on recommendations of the attending veterinarian.

Veterinary staff obtained venous blood samples from each canine at 6 and 24 hours after IA injection. The serum samples were analyzed for methadone hydrochloride levels by MedTox Laboratories, St. Paul, Minn. Three canines were randomly assigned to 1 of 3 groups and euthanized at 24 hours, 14 days, and 28 days following IA injection. Euthanasia was performed by the attending veterinarian with the intravenous administration of Beuthanasia-D (1 mL/22 kg). Following euthanasia, immediate necropsy was performed by investigators and the following tissue types were obtained: synovial fluid, cranial cruciate liga-

ment, joint capsule synovium, lateral and medial meniscus, and femoral articular surface. Samples were obtained bilaterally from each canine. All tissues were preserved in formalin and immediately sent to the laboratory for slide preparation and histologic evaluation. A veterinary pathologist completed analysis of complete blood counts of the synovial fluid and examination of tissue slide preparations.

Results

Supplemental sedation was not required during any of the initial IA injections. Additionally, at no time was supplemental analgesia required in any of the canines following IA injection.

The synovial fluid analysis failed to demonstrate evidence of significant joint fluid abnormalities. Synovial fluid samples taken after 24 hours were reported by the veterinary pathologist as “no cells” to “a few” white blood cells (WBCs) noted on gram stain and demonstrated 500 to 4,250 WBCs/cm³. On day 14, synovial fluid samples demonstrated 147 to 1,107 WBCs/cm³, and on day 28 demonstrated 376 to 970 WBCs/cm³ with reports of “no” or “rare” WBCs noted on veterinary gram stain. The veterinary pathologist attributed the 24-hour synovial fluid samples with “a few” WBCs to the minor inflammatory processes initiated by the mechanics of the IA injection. Serum concentrations of methadone hydrochloride were below 10 ng/mL throughout the sampling period and consistent with similar studies performed using IA morphine sulfate.

In reviewing the tissue slide preparations, the veterinary pathologist was unable to note any histologic differences or signs of toxicity attributable to the IA injection of methadone hydrochloride between the study knee and the control knee in any of the canines. All tissues were determined to be within histologically normal limits without evidence of inflammation or degeneration.

Discussion

Canines, aged 1.5 to 4 years, weighing 20 to 23 kg were used for this study because they mimic the age at which anterior cruciate ligament reconstruction is generally done in humans and because the knee joint space can accommodate a 5-mL injectate. Additionally, the canines' immune mechanisms and biochemical pathways may resemble those of man more closely than do the changes in smaller laboratory animals.²⁰

Numerous studies have demonstrated the safety of morphine sulfate injected into the IA joint space following arthroscopic knee surgery. Although methadone hydrochloride remotely shares the same

chemical structure as morphine, its efficacy and safety could not be assumed when injected into the IA joint space. Serum results yielded plasma concentrations of methadone hydrochloride less than 10 ng/dL. Serum levels obtained were similar to those derived from the IA injection of morphine sulfate. Systemic pain relief necessitates a serum methadone hydrochloride therapeutic range of 100 to 400 ng/mL.¹⁸

Serum methadone levels were obtained at 6 and 24 hours for a number of reasons. First, serum levels were obtained in several studies examining the effects of IA morphine sulfate at these times.^{5,7,10} Second, initial specimens were obtained at 6 hours because even though the cartilaginous structures in the knee are relatively avascular, the synovial membrane is highly vascular. The time is arbitrary and selected on the basis that serum concentration of methadone peaks in 4 hours after an oral dose. It is presumed the uptake from the IA joint space is slower. Specimens were obtained at 24 hours based on the half-life of methadone hydrochloride in serum of 35 plus or minus 12 hours.

Times for tissue acquisition were determined in consultation with the FDA.²¹ The 24-hour time was selected to demonstrate any acute histopathologic tissue changes. Synovial membranes are highly vascular tissues that respond readily to inflammatory stimuli and trauma. Articular surfaces, ligaments, and cartilage are poorly supplied with blood vessels and have limited expression of inflammatory phenomena and capacity for repair. Accordingly, 28 days allowed these structures adequate time to manifest any histologic changes. Accordingly, special attention was given to examination of the synovium in each of these canines. The 24-hour synovial fluid samples with “a few” WBCs noted were attributed to the minor inflammatory processes initiated by the mechanics of the IA injection. Early changes identified in synovium exposed to inflammatory stimuli may include edema, vascular engorgement, variable inflammatory infiltrates, followed by stromal and synoviocyte proliferation. These histological lesions were not detected in either knee of any of the canines, suggesting a lack of significant inflammatory stimulus from the injection of the IA compound.

Animal studies have historically been used to generalize results across species. Canines of specific age and weight were used in this study because their immune mechanisms resemble those of man more closely than smaller laboratory animals. The sample size of 9 canines was recommended by the FDA and felt adequate to demonstrate a level of confidence and safety.

Based on results obtained from this study, the FDA has approved an investigational new drug application

to examine the antinociceptive effects of IA methadone hydrochloride in humans. Additionally, future studies may focus on evaluating the antinociceptive effects of varying doses of methadone hydrochloride injected into the intra-articular joint space.

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