THE MYOTOXIC EFFECTS OF MICROENCAPSULATED NAPROXEN AND CARRIER POLYMER AFTER INTRAMUSCULAR INJECTION IN RATS

1996

BOHAN
THE MYOTOXIC EFFECTS OF
MICROENCAPSULATED NAPROXEN AND CARRIER POLYMER
AFTER INTRAMUSCULAR INJECTION IN RATS

A Masters Thesis
By
Kevin J. Bohan

Uniformed Services University of the Health Sciences
Graduate School of Nursing

APPROVED:

E. Jane McCarthy, Capt., PHS, CRNA, Ph.D., FAAN, Committee Chair

Ben H. Boedeker, Major, USA, DVM, M.D., Ph.D., Member

Eugene Levine, B.B.A., M.P.A., Ph.D., Member

Louis R. Cantu, Jr., M.D., Ph.D., Consultant

Faye G. Abdellah, Ed.D., Sc.D., RN, FAAN
Dean and Professor, Graduate School of Nursing

10/10/96
The author hereby certifies that the use of any copyrighted material in this thesis entitled:

“The Myotoxic Effects of Microencapsulated Naproxen and Carrier Polymer After Intramuscular Injection in Rats”

beyond brief excerpts is with the permission of the copyright owner, and will save and hold harmless the Uniformed Services University of the Health Sciences from any damage which may arise from such copyright violations.
Department of Defense

Disclaimer Statement

"This work was supported by the Uniformed Services University of the Health Sciences Protocol No. G175ED-01. The opinions or assertions contained herein are the private opinions of the author and are not to be construed as official or reflecting the views of the Department of Defense or the Uniformed Services University of the Health Sciences."
# Table of Contents

Chapter I Introduction ................................................................. 1  
  Background of the Problem .................................................. 1  
  Rationale and Significance of the Problem ............................... 2  
  Statement of Problem .......................................................... 8  
  Main Research Questions ...................................................... 8  
  Hypotheses .............................................................................. 8  
  Conceptual Framework .......................................................... 9  
  Definitions .............................................................................. 10  
  Assumptions ........................................................................... 11  
  Limitations ............................................................................. 11  
  Summary .................................................................................. 11  

Chapter II Review of the Literature .......................................... 12  
  Introduction ............................................................................. 12  
  Review of Literature ................................................................ 12  
  Summary .................................................................................. 15  

Chapter III Methodology .......................................................... 16  
  Introduction ............................................................................. 16  
  Drug Formulation .................................................................... 16  
  Experimental Animals ............................................................. 17  
  Research Design ...................................................................... 17
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instrumentation</td>
<td>19</td>
</tr>
<tr>
<td>Chapter IV Results</td>
<td>20</td>
</tr>
<tr>
<td>Presentation of Hypotheses</td>
<td>21</td>
</tr>
<tr>
<td>Summary</td>
<td>21</td>
</tr>
<tr>
<td>Chapter V Conclusion</td>
<td>33</td>
</tr>
<tr>
<td>Overview of the Study</td>
<td>34</td>
</tr>
<tr>
<td>Overview of the Results</td>
<td>34</td>
</tr>
<tr>
<td>Implications for Research and Practice</td>
<td>35</td>
</tr>
<tr>
<td>Summary</td>
<td>39</td>
</tr>
<tr>
<td>Appendix A</td>
<td>41</td>
</tr>
<tr>
<td>Appendix B</td>
<td>42</td>
</tr>
<tr>
<td>References</td>
<td>43</td>
</tr>
</tbody>
</table>
List of Tables

Table 1 ................................................................. 22
Table 2 ................................................................. 23
Table 3 ................................................................. 24
List of Figures

Figure 1 ................................................................. 4
Figure 2 ................................................................. 25
Figure 3 ................................................................. 26
Figure 4 ................................................................. 27
Figure 5 ................................................................. 29
Figure 6 ................................................................. 30
Figure 7 ................................................................. 31
Figure 8 ................................................................. 32
Figure 9 ................................................................. 36
Abstract

At the present time ketorolac is the only NSAID approved for intravenous and intramuscular use in humans. The half-life of naproxen is 12 - 15 hours compared to 4 - 6 hours for ketorolac, and naproxen has an antipyretic effect. A drug carrier system has been developed which allows for highly lipophilic compounds such as naproxen to be microencapsulated (MEC) for parenteral use. Intramuscular MEC naproxen could provide greater pain relief than ketorolac with a longer duration of action. MEC naproxen could also be given to reduce or replace opioid analgesics and reduce hyperthermia in patients unable to receive oral or rectal antipyretics. Since an assessment of the potential for local toxicity of a product intended for intramuscular injection is an important component of an initial drug development strategy, this research was conducted to determine the potential for myotoxicity from intramuscular MEC naproxen. Forty female Sprague Dawley rats were given intramuscular injections of 150mg/kg MEC naproxen into the right gastrocnemius muscle with an equal volume of carrier polymer alone injected into the left. Animals were placed into four groups of ten for sacrifice at 24 hours, 72 hours, one week, and one month post-injection. Multiple tissue sections were cut, stained with hematoxylin and eosin, and examined under light microscopy for histopathologic changes. All samples were blinded before grading by a pathologist using the following scoring system: 0 = no damage; 1 = sparsely scattered hyalinized fibers and a localized inflammatory reaction; 2 = definite fiber destruction with the presence of macrophage digestion of muscle fibers and a mixed cellular infiltrate in some but not all sections examined; 3 = widespread muscle damage throughout the original specimen.
Histopathologic scores were compared using Analysis of Variance (ANOVA) and paired t-tests with \( p < 0.05 \) considered statistically significant. Pathology was evident in each of the specimens examined. There were no statistically significant differences found between MEC naproxen and carrier polymer. The histopathology induced by MEC naproxen was significantly decreased by one month compared to 24 hour MEC naproxen. The histopathology induced by the carrier polymer alone was significantly decreased by one week compared to 24 hour carrier polymer. There were no scores of zero or three for any of the specimens. A power analysis revealed a level between 90% and 95%. MEC naproxen injected intramuscularly produces the same degree of histological damage to rat skeletal muscle as carrier polymer alone. A moderate level of histopathology occurred at 24 hours for both the MEC naproxen and carrier polymer. This was partially reversed by one month for the MEC naproxen and by one week for the carrier polymer. For further studies, a positive control using 10% formalin, negative controls of noninjected muscle, and muscle injected with 0.9% normal saline may be beneficial. These data do not suggest that myotoxicity from MEC Naproxen would be an obstacle to human trials.
CHAPTER I

Introduction

Background of the Problem

With the expanding awareness of the epidemiology and pathophysiology of pain, more attention is being paid to the management of postoperative pain in an effort to improve patient comfort, decrease perioperative morbidity, and decrease cost by shortening the time spent in postanesthesia care units, intensive care units, and hospital rooms (Yeager, Glass, & Neff, 1987). Pain is an unpleasant sensory and emotional experience arising from actual or potential tissue damage or described in terms of such damage (International Association for the Study of Pain, 1979).

All operations produce tissue trauma and release potent mediators of inflammation and pain (Hargreaves & Dionne, 1991). Without treatment, sensory input from injured tissue reaches spinal cord neurons and amplifies subsequent pain impulses. Pain receptors in the periphery also become more sensitive after injury. Recent studies have found long-lasting and permanent changes in cells within spinal cord pain pathways after brief painful stimulus (Bullit, 1989; Fitzgerald, 1990; Hunt, Pini, & Evan, 1987). The substances released from injured tissue evoke stress hormone responses in the patient. Such responses promote breakdown of body tissue; increase metabolic rate, blood clotting, water retention; impair immune function; and trigger a "fight or flight" reaction with autonomic features and negative emotions (Dinarello, 1984; Egdahl, 1959; Kehlet, 1982). Pain itself often leads to shallow breathing and cough suppression in an attempt to "splint"
the injured site, followed by retained pulmonary secretions and pneumonia (Marshall & Wyche, 1972; Sydow 1989). Unrelieved pain can also delay the return of normal bowel function in the postoperative patient (Watwill, 1989).

Patients with postoperative pain are often unable to receive oral analgesic agents. This may be due to the specific nature of their surgery or postoperative nausea and vomiting (PONV). Administration of oral analgesics may not be optimal for managing acute postoperative pain because of the prolonged time to peak effect and lack of titratability. Currently available parenteral analgesic agents possess one or both of the following drawbacks:

1. The potential for hazardous adverse effects such as respiratory depression, hypotension, and nausea and vomiting.

2. The requirement for either continuous intravenous (IV) or multiple IV or intramuscular (IM) injections.

The MEC naproxen used in this study has only recently been patented. Since no other parenteral formulation of naproxen is known to exist, no data on the potential myotoxic effect of parenteral naproxen is available.

Rationale and Significance of the Problem

Opioid analgesics are the cornerstone of pharmacological postoperative pain management. Other agents such as nonsteroidal anti-inflammatory drugs (NSAIDs) or local anesthetics also provide pain relief or reduce opioid requirements (Agency for Health Care Policy and Research, Guideline Report, 1992). The term "Opioid" refers to all
exogenous substances, natural and synthetic, that bind specifically to any of the several subpopulations of opioid receptors and produce at least some morphine-like effects (Stoelting, 1991). Intramuscular administration of analgesics is traditionally used for treating moderate to severe postoperative pain as they provide a more rapid onset and decreased time to peak effect than oral analgesics. However, plasma concentrations of opioids on a fixed intramuscular schedule (typically every three to four hours) often result in a cyclical period of sedation, analgesia, and finally, inadequate analgesia (Tuman, McCarthy, & Ivankovich, 1988). Consequently, as many as 75% of patients given intermittent intramuscular opioids remain in moderate to severe pain (Lubenow, & Ivankovich, 1991).

Delivery of opioids by patient controlled analgesia (PCA) circumvents many of the problems of intramuscular opioid administration. PCA provides better analgesia with less sedation, decreased total drug usage, and a more rapid return to physical activity (Egbert, 1990). Unfortunately, the potentially hazardous side effects of sedation, respiratory depression, hypotension, decreased peristalsis, nausea, and vomiting, still exist. Additionally, this method also requires prolonged IV access which may increase the patients' risk for nosocomial infection.

A recent alternative to opioids has been the injection of ketoralac, an NSAID with efficacy equal to that of moderate doses of opioids but lacking the deliterious effects of sedation, hypotension, and depression of ventilation (Stoelting, & Miller, 1994). NSAIDS inhibit the cyclooxygenase enzymes that convert arachidonic acid into thromboxane and prostaglandins (Figure 1). Thromboxane and prostaglandins are mediators that induce
Figure 1. Scheme for mediators derived from arachidonic acid with site of nonsteroidal antiinflammatory drug action.
inflammation and activate peripheral nociceptors. The primary mechanism for the analgesic effect of NSAID's is the inhibition of these inflammatory mediators generated at the site of tissue injury. (McCormack & Brune, 1991). Although it is likely that NSAIDs also act within the central nervous system, they do not, in contrast to opioids, cause respiratory depression or interfere with bowel or bladder function (Agency for Health Care Policy and Research, 1992).

Even when insufficient to control pain alone, NSAIDs have a significant opioid dose-sparing effect upon postoperative pain and can be useful in reducing opioid side effects (Hodsman, et al, 1987; Martens, 1982). At this time, ketoralac is the only NSAID approved by the Food and Drug Administration for parenteral use. In common with other NSAIDs, ketoralac inhibits platelet aggregation and increases bleeding time. This may be clinically significant, especially with preoperative or long term use. NSAID's can cause renal insufficiency, although the potential is slight when adequate fluid balance is maintained and function does not depend on renal prostaglandins (Stoelting, 1991). Since prostaglandins have a major role in maintenance of normal gastrointestinal physiology, it is not surprising that NSAID's interfere with normal gastrointestinal (GI) function (Brooks & Day, 1991). The GI effects of NSAID's include gastric erosion, peptic-ulcer formation and perforation (Henry, 1988), though these affects are typically associated with long term usage. Major benefits of ketoralac are that it does not depress ventilation, the cardiovascular system, or cause nausea and vomiting (Stoelting, 1991). Like parenteral opioids however, ketoralac dosing requires frequent IM or IV injections. This is primarily due to the short plasma half life of ketoralac, approximately four to six hours for both the
parenteral and oral form (Brocks & Jamali, 1992, Roche Laboratories, 1995).

A prolonged release drug carrier system has been developed by Haynes (1992). This technique involves microencapsulation with a polymer coating and allows for highly lipophilic compounds such as naproxen, a propionic acid derived NSAID, to be solubilized for parenteral use. In postoperative orthopedic, gynecological, and general surgery patients a single 550 mg oral dose of naproxen was shown to be as effective, after one hour, as a single 10mg intramuscular dose of morphine and to provide better analgesia from three to six hours than either morphine or placebo (Brown, Sevelius, & Wild, 1984; Martens, 1982). Naproxen in its oral form has a plasma half life from 12 to 15 hours (Brooks, & Day, 1991). A single intramuscular dose of MEC naproxen would have faster onset of action than oral naproxen and could provide prolonged analgesia equal to morphine without the potential for dangerous side effects associated with opioids. MEC naproxen may in many cases eliminate the need for continuous IV or frequent IM injections and save nursing time spent on the preparation and administration of analgesics.

In addition, NSAIDS are frequently used in the treatment of musculoskeletal pain from sprains and strains associated with sports injuries. These types of injuries are common during military training, field exercises, and on the battlefield, where a soldier may be required to carry out his or her duty regardless of injury. The austere environment may not be conducive to self administration of oral analgesics every three to six hours. An advantage of MEC naproxen relates to the potential for administration only once every 12 to 24 hours. This could offer greater convenience and compliance, especially in the military setting. For example, a soldier might have pain associated with a minor soft tissue
injury such as a strain or sprain. However, the injured person must carry the medication, remember, and have the time to take the pills. This could, for obvious reasons, be difficult if not impossible in a busy or hostile environment. An intramuscular injection of MEC naproxen given the morning or evening before duty by a nurse or medical technician in the field could provide the necessary pain relief to return a soldier to duty for extended periods of time without the sedative effects associated with opioids.

The potential benefits of MEC naproxen can also be applied to the surgical arena. A certified registered nurse anesthetist (CRNA), or anesthesiologist could administer a one time injection at the end of a surgical procedure to provide a patient with sustained analgesia without the detrimental effects associated with opioids. This could provide for earlier ambulation, return to activities of daily living (ADL’s), discharge, and return to duty, all of which can be translated into monetary savings. If the level of myotoxicity caused by MEC naproxen is determined to be acceptable for human use, patients could be provided with prolonged analgesia without dangerous side effects. Furthermore, the pain from multiple IM injections would be avoided and nursing time spent on medication administration would be decreased.

One of the desirable qualities which an intramuscularly injected drug formulation should possess is good local tolerance, i.e., lack of pain or discomfort associated with injection. Two types of pain/discomfort are of concern: acute pain on injection (occurring within seconds or minutes of injection), and delayed pain (associated with deep tissue trauma, most likely muscle damage, occurring within hours or even days after injection), (Chellman, Lollini, Dorr, & DePass, 1994). Since there is no data available on an
injectable form of naproxen, the potential for tissue toxicity must be ascertained using an animal model prior to clinical studies in humans. The purpose of this research is to determine the degree of myotoxicity resulting from the intramuscular injections of MEC naproxen and carrier polymer alone.

**Statement of Problem**

The degree of skeletal muscle damage caused by the intramuscular injection of MEC naproxen is unknown.

**Main Research Questions**

1. Is the degree of myotoxicity from MEC naproxen different than that caused by the carrier polymer at 24 hours, 72 hours, one week, and one month?

2. Is there a change in the degree of myotoxicity over time?

**Hypotheses**

1. There is no difference in the degree of myotoxicity caused by the injection of MEC naproxen and carrier polymer alone when compared at 24 hours, 72 hours, one week, and one month.

2. The degree of myotoxicity caused by the injection MEC naproxen does not change over time.

3. The degree of myotoxicity caused by the injection of carrier polymer alone does not change over time.
Conceptual Framework

Naproxen by mouth has been shown to have an analgesic effect similar to intramuscular morphine one to two hours after dosing (Brown, Sevelius, & Wild, 1984). A parenteral form of naproxen has recently been developed that would eliminate the lag time in analgesia and allow patients who are unable to take medication by mouth to receive naproxen. Naproxen is an analgesic with minimal side effects that is currently available in oral form without a prescription. This new formulation uses a microencapsulation technique that has been shown to have a sustained release effect on drug delivery leading to prolonged drug effects (Boedeker, Lojeski, Haynes 1992). The oral form of naproxen has been in use for an extensive period of time and the therapeutic dose and toxic effects have already been determined. One concern for this new parenteral form of naproxen is local tissue tolerability.

When testing drugs for human toxicity the best model would be the human one. The data derived could then be used in predicting the consequences of human exposure. However, actual toxicological experiments using human clinical trials are rarely allowed. The obvious benefit of using human data is that variations in absorption, excretion, metabolism and sensitivity on a cellular level between experimental animals and humans, do not become a problem for extrapolation (Meyer, 1993). Before it is legal to test any new chemical compound in humans, toxicological studies must be performed in laboratory animals. These studies are intended to illustrate adverse effects and confirm that the risks for humans is minimal or at least tolerable. A consequence of failing to conduct acceptable studies prior to exposure of the drug to humans can include dangerous adverse
reactions that could have been prevented. The principal assumption underlying toxicological studies is that the data acquired are predictive for effects that could be observed in humans (Spilker, 1991).

In general there are two main types of animal models: (1) a living organism in which deviations from the normal physical condition has been induced and (2) a living organism with a heritable or naturally acquired pathologic condition. A third type of model uses transgenic animals and involves the induction of changes in the genome of the animal cell (Meyer, 1993). This study incorporates the use of the first model by causing a deviation in the normal physical condition of skeletal muscle in rats secondary to the injection of MEC naproxen.

The rat is commonly used in pharmacokinetic studies due to its relatively large body size and ease of manipulation. Previous studies with similar methodologies used this animal for study. In order to evaluate the myotoxicity of MEC naproxen, intact animals are required, as cell culture models will not work to evaluate myotoxicity. The rat is the smallest animal model which provides both adequate size for injections and skeletal muscle similar to that of humans. The Food and Drug Administration (FDA) requires that testing in humans is begun after sufficient animal toxicity studies have been completed. This study is part of the preclinical studies necessary for the FDA approval of parenteral MEC naproxen.

Definitions

**Carrier Polymer**: A compound whose molecule is formed of a large number of simpler molecules of the same kind that is used to solubilize and coat another compound.
Microencapsulated (MEC) Naproxen: Microcrystals of naproxen which are coated by a polymer in order to provide a mechanism for parenteral drug delivery with the potential for a longer duration of action from sustained release of the drug.

Myotoxicity: Muscle injury resulting from direct intramuscular injection of a substance. Myotoxicity was measured using a scale ranging from zero to three.

Assumptions

1. The validity and reliability of the MEC naproxen formulation is accurate.
2. People desire the most effective analgesic agent with the least harmful side effects.

Limitations

1. Generalizability is somewhat limited since an animal model was used.

Summary

In an attempt to provide a parenteral, longer acting analgesic agent, without the dangerous side effects associated with the use of opioids, this study evaluates the degree of myotoxicity caused by the intramuscular injection of MEC naproxen in rats. In chapter one the background and significance of the problem, main research questions, hypotheses, conceptual framework, assumptions, and limitations for this study were presented. Chapter two provides a more specific review of the literature relative to the myotoxic effects of different drugs and carrier substances. The methodology will be presented in chapter three.
CHAPTER II
Review of the Literature

Introduction

Information on microencapsulation and prolonged release parenteral drug formulations is abundant in journal articles and textbooks. Research on myotoxicity from NSAID's other than naproxen was located. This chapter will focus on the significance of the related literature.

Review of Literature

In recent years a major approach to increasing the therapeutic efficiency of bioactive agents while decreasing their toxicity has involved their chemical attachment to naturally occurring or synthetic macromolecules (Harris, 1984). Microcapsules of various classes of drugs such as steroid hormones (Beck, Cowsar, Lewis, Gibson, Flowers, 1979), narcotic antagonists (Schowpe, Wise, & Hower, 1975), antimalarials (Tsakala, Gillard, Roland, Chabot, & Vert, 1988), anti-cancer agents (Bissery, Valeriote, & Thies, 1985), antibiotics (Lewis, Dappert, Meyers, Pritchett, & Suling, 1980), anti-inflammatory agents (Leelarassama, et al., 1986), analgesics (Wakiyama, Juni, & Nakano, 1982), and anesthetics (Haynes & Kirkpatrick, 1991), have been formulated. Thus various agents have been bound via degradable linkages to several different polymeric systems. The original rationale behind this was that systems could be developed that would undergo enzyme-catalized cleavages or hydrolysis when placed in the body. This would allow the
microencapsulated formulation to function as a depot that would release the agent at a predetermined rate for extended periods of time (Ranade, 1990). Microencapsulation also provides a mechanism by which highly lipophilic agents can be solubilized without the use of large amounts of diluent, thereby producing a formulation concentrated enough to attain therapeutic ranges when given in small volumes parenterally.

In their study on NSAID myotoxicity Chellman, et al., (1994) injected diclofenac, piroxicam, ketoprofen, metamizol magnesium, and ketorolac into skeletal muscle (hindlimb) of rats. All of the NSAIDs produced some degree of damage as measured histopathologically 24 hours post-injection. The ranking of the overall lesion severity and size from least to most severe was: 0.9% saline < ketorolac 10mg/ml < ketorolac 30mg/ml < ketoprofen 50mg/ml < formalin 10% < piroxicam 20mg/ml < diclofenac sodium 25mg/ml < metamizol magnesium 400mg/ml. Unfortunately, this study did not assess for myotoxicity beyond 24 hours and therefore may have failed to observe an increase or decrease in the severity of muscle damage over time. A progressive increase in the amount of destruction may have been evident at 72 hours. Also, the degree of regeneration or permanent damage that would have been observed by one week or one month was not determined. The tool used to determine the degree of muscle damage had eight variables, each one receiving a score from one to five depending on the severity, minimal to maximal. The data collection with this tool appeared overly subjective and the analysis became difficult to follow.

Local skeletal muscle irritation has typically been investigated by either histopathological examination, biochemical assay using creatinine kinase (CPK)
measurement, or both. Histological evaluation implies direct inspection of the muscle tissue using parameters such as, localized inflammation reaction, hyalinized fibers, fiber destruction, macrophage digestion, infiltration of polymorphonuclear cells, lymphocytes, and plasma cells to describe pathological changes from injection. Results obtained by the measurement of CPK levels provides information on the amount of muscle damage occurring immediately after the injection. Since the measurement of CPK level is costly, has no advantages in evaluating toxicity over time, would involve greater stress for the test animals (Surber & Sucker, 1987), and the results often show considerable variability (Kadir, Eling, Abrahams, Zuidema & Crommelin, 1992), histological examination was used to evaluate myotoxicity in this study.

The amount of tissue damage caused by a strongly irritating drug such as novaminsulfon has been shown to decrease if the drug is administered in an encapsulated formulation (Kadir, et al., 1992). It is reasonable to hypothesize that the encapsulation of NSAID's could decrease their toxicity. As stated previously, no studies assessing myotoxicity from a parenteral formulation of naproxen are available. This supports the determination that this research is not duplicative. A double-blind clinical comparison of patients receiving a sustained-release naproxen preparation by mouth compared to those recieving conventional naproxen found that the sustained release formulation caused a significantly lower incidence of gastrointestinal (GI) side effects (Kelly, Kinney, Devane, Mulligan, & Colgan, 1989). This suggests that a high, localized concentration of naproxen in the GI tract leads to an increased risk of localized lesions. Since a parenterally administered formulation such as MEC naproxen does not come into direct
contact with the GI mucosa, it is likely to produce an even lower incidence of GI complications than the sustained release formulation given by mouth.

**Summary**

Little research has been conducted on the myotoxic effects of parenteral NSAIDs, and no information was found pertaining to a parenteral naproxen formulation and myotoxicity. In chapter two, articles relative to this study were reviewed. Chapter three will discuss the methodology for this study.
CHAPTER III
Methodology

Introduction

This quantitative study used a blinded reading experimental research design to assess the myotoxic effect of MEC naproxen and the carrier polymer alone. Data were obtained from histological examination with the degree of muscle damage categorized into four groups by a pathologist who was unaware of the drug, dose, or time intervals. Blinded reading of tissue slides by the pathologist minimizes biases that can distort data analysis and interpretation (Spilker, 1991).

Drug Formulation. The method of particle size reduction used to prepare the naproxen nanosuspension (MEC naproxen) nanoparticle dispersions was microfluidization. The operating principle of microfluidization is as follows. A slurry consisting of the appropriate quantities of drug substance, stabilizer, and water is loaded into the microfluidizer inlet reservoir; this slurry constitutes the process stream. A hydraulic pump accelerates the process stream to extremely high velocities, on the order of hundreds of meters per second. This process stream then enters a processing zone called the interaction chamber. This chamber is the heart of the system and consists of well defined microchannels. Within the chamber, the process stream is separated into two streams, changes direction, and collides into a single stream again. The actual mechanisms of particle or droplet size reduction are the powerful forces of shear, impact, and cavitation.
occurring within the interaction chamber. Currently, a patent is pending on the formulation process and information on the validity and reliability is not available at this time.

Experimental Animals. Female Sprague Dawley rats were maintained and utilized according to public laws 89-544, 91-579 and 94-279, 99-198 (the Animal Welfare Act and amendments). Animals were maintained on a standard laboratory diet and under standard husbandry practices in accordance with The Guide for the Care and Use of Laboratory Animals. The Uniformed Services University of the Health Sciences (USUHS) Laboratory Animal Review Board (LARB) reviewed and approved the protocol on 22 March 1995 (Appendix A). The USUHS has an Animal Welfare Assurance Number (A3448-01) on file with the Office for Protection from Research Risks, National Institutes of Health.

Research Design. Forty female Sprague Dawley rats were used, providing 80 muscle specimens. Each rat was weighed and the cages were numbered. Animals were kept two per cage with the tail of each odd numbered rat marked with permanent ink. Sterile, one milliliter syringes with 23 gauge three-quarter inch needles capable of delivering .02 mls of injectate accurately were loaded with either .08 or .1 ml of 430.4 ± 0.5 mg/ml MEC Naproxen. The volume of injectate was determined by calculating the amount of MEC Naproxen in milliliters based on a dose of 150 milligrams per kilogram. These volumes, which ranged from .0849 to .1077 milliliters, were then rounded to .08 and .1 mls for
accurate syringe measurement. Volumes < .11 and ≥ .09 mls were rounded to .1 milliliters, and volumes < .09 mls were rounded to .08 milliliters. Individual animals were placed in a rat holder with the hind legs extended and immobilized by holding the feet. A single intramuscular injection of .08 or .1 cc of MECA naproxen was given into the right gastrocnemius muscle. An equal volume of carrier polymer was then injected into the left gastrocnemius muscle. Animals were returned to their cages and separated into four groups of 10. Rats 1-10 were sacrificed twenty-four hours after injection and rats 11-20 were sacrificed seventy-two hours after injection. Rats numbered 21-30 were sacrificed one week post injection and rats numbered 31-39 were sacrificed one month (30 days) after injection. One of the animals in the one month group, rat number 40, was found dead in its cage the morning after injection. A veterinary pathology necropsy was performed. The cause of death was reported as undetermined and possibly stress related (Appendix B).

At the appropriate time points individual animals were removed from their cages and placed in a glass container with dry ice under the floor and a lid was applied. Water was added to the bottom of the desiccator to speed the release of carbon dioxide gas to euthanize the animals. Each animal was removed after respiratory arrest was noted and the spinal cord was severed prior to harvesting. The skin was clipped with a scissors midway down the back and gently peeled down off the hind quarters to the feet. The feet were removed at the ankle and each hind quarter was separated at the hip. The individual hind leg specimens were placed in one hundred milliliter specimen cups filled with enough five percent buffered formalin solution to completely cover the sample (approximately 60
Specimens were labeled N1-6, N1-7, N2-6, N2-7 etc..., enabling the investigator to differentiate the samples and resulting histological scores from other NSAID myotoxicity studies being carried out during the same time period. The first number identified the individual rat and the second number identified the injected leg: six for right, seven for left. The specimens were held for a minimum of 24 hours and then delivered to a pathologist at the Naval Medical Research Institute, Bethesda, MD. The gastrocnemius muscles were removed by the pathologist, cut into four to five mm sections and processed in paraffin wax. Multiple seven micrometer thick tissue sections were cut, stained with hematoxylin and eosin, and examined under light microscopy. One veterinary pathologist then performed the histological evaluation of the muscle samples without prior knowledge of the treatment received.

Instrumentation. Specimens were graded histologically using the following scale developed by Yagiela, Benoit, Buoncristiani, Peters, and Fort (1981) to compare the myotoxic effects of lidocaine with epinephrine in rats and humans.

0 = no damage
1 = sparsely scattered hyalinized fibers and a localized inflammatory reaction;
2 = definite fiber destruction with the presence of macrophage digestion of muscle fibers and a mixed cellular infiltrate in some but not all sections examined;
3 = widespread muscle damage throughout the original specimen.

This scale was tested for reliability by Yagiela et al (1981), with agreement
between two evaluators' scores yielding an overall Kendall rank correlation coefficient of 0.90. The scale was also used in a recent study by Pere, Watenape, Pitkanen, Wahlstrom, and Rosenberg (1993) to determine the degree of myotoxicity caused by bupivacaine after continuous supraclavicular brachial plexus block in rabbits.

**Data Analysis.** The mean, standard deviation, and standard error of the histopathologic ratings for MEC naproxen and carrier polymer at each time point were determined. ANOVA and paired t-tests (two-tailed) were used to assess between group differences with \( p < 0.05 \) considered statistically significant. A power analysis conducted to verify the adequacy of the sample size of 40 revealed a power between 90% and 95%.

**Summary**

In chapter three, the drug formulation, research design, instrumentation, and data analysis were described. The chapters that follow describe the results, conclusions, and a discussion of the implications for further research.
Chapter IV

Results

Presentation of Hypotheses

Pathology was evident in each of the specimens examined and there were no scores of zero or three. Table 1 lists the means, standard deviations, and standard errors for all of the groups at each of the time points. The first null hypothesis was: There is no difference in the degree of myotoxicity caused by the injection of MEC naproxen and carrier polymer alone when compared at 24 hours, 72 hours, one week, and one month. This hypothesis was accepted since there was no statistically significant difference in histological scores between MEC naproxen and carrier polymer alone at the same time periods ($p > 0.05$, Table 2, Figure 2).

The second null hypothesis was: The degree of skeletal muscle damage caused by the injection of MEC naproxen does not change over time. This hypothesis was rejected because the histopathology induced by MEC naproxen injection was significantly decreased by one month compared to the 24 MEC naproxen group ($p < 0.05$, Table 3, Figure 3).

The third null hypothesis was: The degree of skeletal muscle damage caused by the injection of the carrier polymer alone does not change over time. This hypothesis was rejected because the histopathology induced by the carrier polymer alone was significantly decreased by one week and by one month when compared to the 24 hour carrier polymer alone ($p < 0.05$, Table 3, Figure 4).
Table 1.

Means, Standard Deviations and Standard Errors of Histopathology Scores.

<table>
<thead>
<tr>
<th>Group</th>
<th>Time</th>
<th>Mean of scores 0-3</th>
<th>Standard Deviation</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naproxen</td>
<td>24 hours</td>
<td>1.8</td>
<td>0.42</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>72 hours</td>
<td>1.9</td>
<td>0.32</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>1 week</td>
<td>1.6</td>
<td>0.52</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>1 month</td>
<td>1.33</td>
<td>0.5</td>
<td>0.16</td>
</tr>
<tr>
<td>Polymer</td>
<td>24 hours</td>
<td>1.8</td>
<td>0.42</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>72 hours</td>
<td>1.5</td>
<td>0.53</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>1 week</td>
<td>1.2</td>
<td>0.42</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>1 month</td>
<td>1.22</td>
<td>0.44</td>
<td>0.15</td>
</tr>
<tr>
<td>0.9% NaCl</td>
<td>24 hours</td>
<td>2</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>72 hours</td>
<td>1.2</td>
<td>0.45</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>1 week</td>
<td>1.8</td>
<td>0.45</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Note. Dashes indicate the standard deviation and standard error were not estimated since all scores = 2. MEC naproxen and carrier polymer alone at 24 hrs, 72 hrs, 1 week (N=10), and 1 month (N=9). NaCl (0.9%) data at 24 hrs, 72 hrs, and 1 week (N=5) was obtained from a concomitant study using a similar methodology.
Table 2.

Mean Difference and p values for t tests. Different compounds at same time periods.

<table>
<thead>
<tr>
<th>Comparison Group</th>
<th>Mean Difference</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hr Naproxen, 24 hr Polymer</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>72 hr Naproxen, 72 hr Polymer</td>
<td>0.4</td>
<td>0.0506</td>
</tr>
<tr>
<td>1 week Naproxen, 1 week Polymer</td>
<td>0.4</td>
<td>0.0506</td>
</tr>
<tr>
<td>1 month Naproxen, 1 month Polymer</td>
<td>0.111</td>
<td>0.6018</td>
</tr>
<tr>
<td>24 hr Naproxen, 24 hr NaCl</td>
<td>0.2</td>
<td>0.4072</td>
</tr>
<tr>
<td>72 hr NaCl, 72 hr Naproxen</td>
<td>-0.7</td>
<td>0.0046*</td>
</tr>
<tr>
<td>1 week NaCl, 1 week Naproxen</td>
<td>0.2</td>
<td>0.4072</td>
</tr>
<tr>
<td>24 hr Polymer, 24 hr NaCl</td>
<td>0.2</td>
<td>0.4072</td>
</tr>
<tr>
<td>72 hr Polymer, 72 hr NaCl</td>
<td>-0.3</td>
<td>0.215</td>
</tr>
<tr>
<td>1 week Polymer, 1 week NaCl</td>
<td>0.6</td>
<td>0.0144*</td>
</tr>
</tbody>
</table>

* p < 0.05 statistically significant. Dashes indicate no mean difference.
Table 3.

Mean Differences and $p$ values for $t$ test. Same compounds at different time periods.

<table>
<thead>
<tr>
<th>Comparison Groups</th>
<th>Mean Difference</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hr Naproxen, 72 hr Naproxen</td>
<td>-.100</td>
<td>0.6206</td>
</tr>
<tr>
<td>1 week Naproxen, 24 hr Naproxen</td>
<td>-.200</td>
<td>0.3234</td>
</tr>
<tr>
<td>1 month Naproxen, 24 hr Naproxen</td>
<td>-.467</td>
<td>0.0270 *</td>
</tr>
<tr>
<td>24 hr Polymer, 72 hr Polymer</td>
<td>0.3</td>
<td>0.1403</td>
</tr>
<tr>
<td>1 week Polymer, 24 hr Polymer</td>
<td>-.600</td>
<td>0.0039 *</td>
</tr>
<tr>
<td>1 month Polymer, 24 hr Polymer</td>
<td>-.578</td>
<td>0.0067 *</td>
</tr>
</tbody>
</table>

Note. * $p < 0.05$ statistically significant.
Figure 2. Mean Histopathology Scores for MEC Naproxen and Carrier Polymer
Figure 3. Histopathology for MEC Naproxen
Figure 4. Histopathology for Carrier Polymer
Figure 5 shows a tissue section of normal non-injected muscle which would have received a score of zero. Figure 6 shows a section of muscle that was injected with carrier polymer alone, harvested at 72 hours, and given a score of one by the pathologist. Figure 7 is a section of muscle that was injected with MEC naproxen, harvested at 72 hours, and given a score of two. Figure 8 is figure 7 at 50X, all other figures are at 10X.
Figure 5. Example of Histopathology score = 0, noninjected muscle 10X
Figure 6. Example of Histopathology score = 1, 72 hour polymer alone 10X
Figure 7. Example of Histopathology score = 2, 72 hours (MEC) Naproxen 10X
Figure 8. Example of histopathology score = 2, 72 hour (MEC) Naproxen 50X
Summary

In this chapter the hypotheses were restated and the data analysis was presented. Tables and graphs which contain histological comparisons were included to clarify the findings. Pictures of actual tissue sections with their corresponding myotoxicity scores were displayed. The results indicate that MEC naproxen injected intramuscularly produced moderate injury that was similar to carrier polymer alone. The histopathology induced by MEC naproxen injection was significantly decreased by one month compared to 24 hours, and the histopathology induced by the carrier polymer alone was significantly decreased by one week compared to 24 hours. Injury scores were maximal at or near the 24 hour time point suggesting that tissue injury was rapid and diminished over time. In chapter five, an overview of this research and the conclusions drawn from the results will be presented and compared to findings from other research on myotoxicity. A discussion of the implications for further research along with a final summary of this study will conclude the thesis.
CHAPTER V

Conclusion

Overview of the Study

At the present time ketorolac is the only NSAID approved for intravenous and intramuscular use in humans. The half-life of naproxen is 12 - 15 hours compared to 4 - 6 hours for ketoralac, and naproxen has an antipyretic effect. A drug carrier system has been developed which allows for highly lipophilic compounds such as naproxen to be microencapsulated (MEC) for parenteral use. Intramuscular MEC naproxen could provide greater pain relief than ketoralac with a longer duration of action. MEC naproxen could also be given to reduce or replace opioid analgesics and reduce hyperthermia in patients unable to receive oral or rectal antipyretics. Since an assessment of the potential for local toxicity of a product intended for intramuscular injection is an important component of an initial drug development strategy, this research was conducted to determine the potential myotoxicity of MEC naproxen.

Female Sprague Dawley rats (N=40), were given intramuscular injections of approximately 150mg/kg MEC naproxen into the right gastrocnemius muscle with an equal volume of carrier polymer injected into the left. This dosage is approximately 30 times the milligram per kilogram therapeutic dose in humans. Animals were placed into four groups of ten for sacrifice at 24 hours, 72 hours, one week, and one month post-injection. Multiple tissue sections were examined under light microscopy for histopathologic changes. All samples were blinded before grading by a pathologist.
Overview of the Results

Although MEC naproxen tended to produce slightly more damage than carrier polymer alone, there were no statistically significant differences found between MEC naproxen and carrier polymer when analyzed at the same time periods. The histopathology induced by MEC naproxen injection was significantly decreased by one month compared to 24 hours. The histopathology induced by the carrier polymer alone was significantly decreased by one week compared to 24 hours. There were no scores of zero or three for any of the specimens examined. With the moderate effect size that applies to this study (.50) and a two-tailed test at the 5% significance, a sample size of 40 has a power of between 90% and 95%, well above the recommended level of 80%.

Data from a concomitant study using 0.9% preservative free normal saline injections with the same methodology except for smaller groups (N=5), and the absence of a one month group were compared to MEC naproxen and the carrier polymer. The histopathology was significantly less for the 0.9% normal saline group than for the MEC naproxen group at the 72 hour time period \( p < 0.05 \), (Table 2, Figure 9). Also, the histopathology for the carrier polymer alone was statistically significantly less than for the normal saline injection group at the one week time period \( p < 0.05 \), (Table 2, Figure 9).

The fact that none of the injections caused widespread muscle damage throughout the original specimen is significant. In their study on the myotoxic effects of lidocaine with epinephrine, Yagiela and colleagues (1981) used a similar methodology with the same tool to describe their findings. They described a cellular infiltration of the muscle at 24 hours post-injection as having zones of mixed inflammatory cells with some specimens
Figure 9. Mean Histopathology Scores for MEC Naproxen, Carrier Polymer, and NaCl
exhibiting fiber destruction equating to histopathology scores of one or two. They also viewed the histological pattern at 48 hours and noted a progressive invasion of the necrotic muscle by inflammatory cells with the presence of macrophage digestion throughout most of the injured tissue which correlated to histopathology scores of two and three. These findings are similar to those found after MEC naproxen injection when compared at the 24 and 72 hour time points. The time points for tissue harvests were not identical and the Yagiela study did not examine myotoxicity beyond 48 hours. Although both studies used Sprague Dawley rats and the gastrocnemius muscle for injection, the slightly larger volume of injectate, 0.5 milliliters of 2% lidocaine with 1:100,000 epinephrine compared to 0.08 and 0.1 milliliters of MEC naproxen, may have accounted for the increased level of damage found in their study.

Local skeletal muscle damage has typically been investigated by either histopathological examination, biochemical assay such as creatinine kinase (CPK) measurement, or both. Because the measurement of CPK level has no advantage in evaluating toxicity over time and the results often show considerable variability (Kadir et al., 1992, Surber & Sucker, 1987), histological examination was used to evaluate myotoxicity in this study. Indeed, Yagiela and colleagues also measured CPK levels in their study and found that mean creatinine kinase activity was increased dramatically 8 hours after injection yet was back to within normal limits at 24 hours.

In their study of ketorolac and other NSAIDs on muscle damage in the rat, Chellman et al., (1994), injected 0.3 milliliters of 0.9% normal saline, 10% formalin, ketorolac 10 mg/ml, ketorolac 30 mg/ml, diclofenac sodium 25 mg/ml, piroxicam 20 mg/ml,
and ketoprofen 50mg/ml into the quadriceps femoris muscle group. All rats in their study were killed at 24 hours post-injection. The tissue samples were processed in an identical manor to this study, however, the tool used to assess histopathological changes was different. They described how each injection produced a well defined lesion, including those specimens that were injected with 0.9% normal saline. They found muscle degeneration with a linear focus and a small number of leukocytes within and adjacent to necrotic muscle. This would be equivalent to a score of one in this study, and a score of two for specimens that showed necrotic muscle. The characteristic lesions they found in rats injected with the NSAID formulations or with formalin were essentially the same, varying only in severity from moderate to marked and none were classified as severe. This increase in muscle degeneration and necrosis with leukocytic infiltrates in some but not all of the tissue sections examined correlates to a score of two in this study. These findings suggest similar degrees of myotoxicity at 24 hours post-injection for both studies. Since the longest post-injection period in the study by Yagiela et al., (1981) was 48 hours, and only 24 hours for the study by Chellman et al., (1994), the ability to compare long term damage and the potential for regeneration with these studies is not possible.

The results of this study demonstrate that the degree of histopathology decreased over time for both the MEC naproxen and carrier polymer alone groups. We were unable to assess this decrease for 0.9% normal saline injections since a one month group was not tested. In their study on local myotoxicity of bupivacaine in rabbits Pere, Watanare, Pitkanen, Wahlstrom, Rosenberg (1993) used the same tool as this study to assess histopathology and found that scores ranged from zero to two at 24 hours post-infusion,
and from zero to three at one week post-infusion. Although the test animals and methodologies are different it is important to note that even after severe damage (grade three) the Pere study found signs of regeneration four days after the cessation of bupivicaine infusion, with almost complete regeneration by four weeks. This finding is similar to that found with MEC naproxen injection by one month.

It is known that mammalian skeletal muscle fibers are capable of regeneration following partial destruction. If necrosis of a mass of muscle tissue is produced, myoblastic elements arise, by budding from the undamaged portions of the muscle fibers (Adams, Brown, & Pearson, 1962). Phagocytosis of necrotic tissue proceeds slowly from areas with an intact vascular supply at the periphery of the lesion. Regeneration elements follow the phagocytes and ultimately bridge the area of the lesion. The time course of regeneration can take weeks to months depending on the extent of the original lesion. Rapid regeneration of skeletal muscle has been observed previously in instances where the skeletal muscle was chemically injured with no damage to supporting tissues (Smith, Feagans, Belt, & Blair, 1969). The rapid recovery of the muscle following damage from both the intramuscular MEC naproxen and carrier polymer alone likely reflects the fact that the supporting tissues are intact, especially the vascular supply.

**Implications for Research and Practice**

The formulation of MEC naproxen used in this study caused a small degree of skeletal muscle damage that appears to be reversible over time. At times, it was difficult to maintain the position of the hind leg while being held for the injection. This may have
limited the reproducibility of injection location into the gastrocnemius muscle by causing injections to be off center. Since the rats were able to move slightly, motion during the time of injection could have caused excess damage to the muscle tissue. For this reason, having the animal anesthetized prior to injection may be helpful for further myotoxicity studies.

The loss of one of the animals in this study which the pathologist believed was probably due to stress from handling and the two injections, is of concern. If the animal suffered from an allergic or possibly toxic reaction one would expect a repeat of this in further studies, particularly in studies currently underway to assess the pharmacodynamic and pharmacokinetic properties of MEC naproxen after intravenous injection. If MEC naproxen is determined to be safe for parenteral use in humans, implications for further research are extensive. Research to assess the effectiveness of a parenteral naproxen formulation in regards to the analgesic, antiinflammatory, and antipyretic activity in the clinical setting would be paramount.

Another interesting area for research regarding this MEC naproxen formulation relates to the effect of general anesthesia on hypoxic pulmonary vasoconstriction. Potent inhaled agents, other drugs, and maneuvers used during anesthesia may have an inhibitory effect on regional or whole lung hypoxic pulmonary vasoconstriction (HPV). It has been demonstrated that prostaglandins play a role in hypoxic pulmonary vasoconstriction inhibition and therefore prostaglandin inhibitors have been investigated as potentiators of hypoxic pulmonary vasoconstriction (Conzen, et al., 1989). Ibuprofen, a cyclo-oxygenase inhibitor like naproxen has been found to potentiate hypoxic pulmonary vasoconstriction
and to reverse the inhibition of HPV caused by halothane. An injectable NSAID like MEC naproxen with its more rapid onset and longer duration of action may provide the anesthetist a valuable agent for the treatment of hypoxia often associated with one-lung anesthesia. The benefits of having a parenteral NSAID with the potential for greater analgesic properties and a longer duration of action than ketorolac are obvious. The antipyretic effects of naproxen would prove helpful to a patient unable to take oral or rectal antipyretics. The availability of a parenteral analgesic with prolonged duration and minimal cognitive side effects as compared to opioid analgesics would prove invaluable to a soldier suffering soft tissue injuries, such as a sprain or strain while in austere conditions.

Summary

Chapter five gives a general overview of the background, problem, and purpose for this research. Studies with similar methodologies and findings and others that used different methodologies were discussed. Implications for further research and clinical practice were presented. This study found that MEC naproxen injected intramuscularly produced similar injury to tissue as did the carrier polymer alone. The histopathology induced by MEC naproxen injection was significantly decreased by one month compared to 24 hours, and the histopathology induced by the carrier polymer alone was significantly decreased by one week compared to 24 hours. Injury scores were maximal at or near the 24 hour time point suggesting that tissue injury was rapid and diminished over time. These data do not suggest that myotoxicity from MEC naproxen would be an obstacle to human trials.
MEMORANDUM FOR LOUIS R. CANTILENA, JR., Ph.D., DEPARTMENT OF PHARMACOLOGY

SUBJECT: LARB Approval of Protocol

The following application was reviewed and approved by the Uniformed Services University of the Health Sciences (USUHS) Laboratory Animal Review Board (LARB) on 22 March 1995:

Title of Application: "Evaluation of Dermal/Myotoxicity from Injection of Lecithin-Coated NSID's (Rat)"

Protocol Number: G175ED-01

Name of Principal Investigator: Dr. Louis R. Cantilena

The USUHS has an Animal Welfare Assurance on file with the Office for Protection from Research Risks, National Institutes of Health. The Assurance Number is A3448-01. The LARB approved the above referenced application as submitted.

John H. Parrish, D.V.M., Ph.D.
LTC, VC, USA
Executive-Secretary, Laboratory Animal Review Board

cc:
Research Administration

Appendix A
REQUEST FOR VETERINARY PATHOLOGY SERVICES

VETERINARY PATHOLOGY NUMBER: USUHS 95-175: SLIDES A1 - A6
Necropsy Number: No necropsy request form supplied.
Necropsy Date: Unknown
Prossector: N/A
Routine
Contributor: Dr. Canaline
Species: Rat
Identification Number: Unknown
Spontaneous Death

HISTORY:

This rat, on a one month study was found dead in its cage. It was participating in a mild toxicity study which involved an injection in each leg.

GROSS OBSERVATIONS/DIAGNOSES:

No gross lesions were recorded.

MICROSCOPIC DIAGNOSES:

Lung: Congestion, acute, diffuse, mild, alveolar capillaries.
All other tissues (eye, brain, heart, liver, salivary gland, kidney, ovary and uterus): Within normal limits.

COMMENTS:

The mild acute congestion observed within the alveolar capillaries is considered to be a normal agonal change occurring at death. Most tissues exhibited only minimal post-mortem autolysis. There was no indication of either bacterial or viral involvement in any tissues or systems observed. Cause of death is undetermined but may be stress related.

Appendix B
References


anaesthesiologica Scandinavica, 74 (Suppl.), 173-175.


