THE EFFECTS OF MORPHINE SULFATE ON AGGLUTINATION, CLOT FORMATION AND HEMOLYSIS IN PACKED RED BLOOD CELLS

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ABSTRACT

Morphine sulfate is an opium alkaloid narcotic frequently used on patients suffering from acute and chronic disease processes. Often patients receiving either acute or long-term pain therapy with morphine require concomitant blood transfusion therapy. Based on current American Association of Blood Banks guidelines the addition of any medication other than normal saline to **packed red blood cells** is strictly prohibited. This restriction can lead to unwanted delays in medication schemes when patients with limited intravenous access are in need of both therapies at the same time. Few studies exist that have investigated the effects of morphine sulfate on packed red blood cells. Most of these have, in a limited manner, presented contrasting evidence on the efficacy of this practice. The aim of this study was to measure the in-vitro effects of morphine sulfate on packed red blood cells, while imitating the modern clinical infusion system. The effects of morphine sulfate on packed red blood cells was studied by measuring clot formation, **agglutination**, and **hemolysis** using relative semi-quantitative visual assay. Morphine sulfate did not cause time or temperature dependent clot formation or agglutination in the samples studied. This may be due to the fact that clotting was not possible due to removal of clotting factors during processing of the PRBCs. Grossly evident hemolysis occurred in both the control and test groups, most likely due to secondary sources. Findings from this study do not support compatibility between Morphine sulfate and Packed red blood cells. Recommendations for practice remain in accord with current practice standards.

Key Words: **morphine sulfate**, **packed red blood cells**, **agglutination**, **hemolysis.**

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PREFACE

This research was conducted to provide knowledge on the effects of co-administering morphine sulfate and packed red blood cells during transfusion therapy. It was designed in an effort to provide information that will be helpful in the revision of current standards of practice.

DEDICATION

I dedicate this work to the two most important women in my life: my wife, Susan, and my daughter, Taryn. Both have sacrificed and endured through the trying times in this program. Without their love and support, I would not have been able to complete this program, nor this thesis. To my parents I dedicate this time in my life, you have taught me strength and perseverance. Thank you for never letting me quit. For the true friends I have made during this journey, I am forever thankful. May our friendships continue to grow with our new-found professions.

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CHAPTER I: INTRODUCTION

Background

The Certified Registered Nurse Anesthetist (CRNA) is often required to provide pain management and hemotherapy. Presently, all accrediting and regulating agencies prohibit the simultaneous infusion of blood and medications in the same intravenous line. Many clinical settings can be envisioned in which the concomitant administration of the analgesic morphine sulfate and packed red blood cells would be advantageous. The treatment and prevention of pain is a primary concern of the CRNA. Several different intravenous narcotic analgesic agents are available for this purpose. Having a large selection of drugs to choose from enables the CRNA to meet specific needs individual patients present with. The responsibility of the CRNA to learn and keep updated on the pharmacokinetics, pharmacodynamics, untoward reactions, side effects, and incompatibilities of all these drugs can be at the least, very challenging. A thorough pharmacological understanding of each of these drugs provides the CRNA with the knowledge necessary to provide safe, beneficial anesthesia to all patients receiving intravenous narcotic analgesics. The narcotic analgesic morphine sulfate (MSO4), an opium alkaloid, is considered the standard opiate agonist commonly used for the treatment of severe acute and chronic pain. Morphine sulfate is commercially available in various concentrations ranging from 1 to 25 mg/ml and it is reputed to be compatible with all primary intravenous solutions. It can be administered by subcutaneous, intramuscular, and intravenous injection or infusion routes (Johnson, Bhatt-Mehta, Mancari, & McKown, 1994). IV administration of MSO4 remains the primary route of choice for the control of severe and refractory pain (Morgan & Mikhail, 1996).

Underlying medical problems that require intensive medical intervention are often the cause of acute and chronic pain symptoms. Cancer and trauma are two disease processes that most commonly fall into this categorization. Patients afflicted with these

disorders require extensive medical care in addition to management of pain. Among the numerous and dynamic treatments required to medically treat and support various critical physiologic processes are blood transfusions (Klein, 1994).

Because the CRNA is frequently faced with the administration of both intravenous MSO4 and blood, it is important to be aware of potential contraindications associated with their combined intravenous administration. "The American Association of Blood Banks [AABB], the Joint Commission of the American Hospital Association, the College of American Pathologists, and the Office of Biologics of the Food and Drug Administration [FDA] are all involved in accrediting and regulating the collection, handling, and the use of blood" (Monaghan, 1983, p.234). The standards committee of the American Association of Blood Banks has prepared the practice standards for blood banks and transfusion services used throughout the United States. The standards provide strict guidelines for the administration of blood and blood components as follows:

> Drugs or medications, including those intended for intravenous use, must not be added to blood or components. O.9% sodium chloride injection, USP, may be added to blood or components. Other solutions intended for intravenous use may be used in an administration set or added to blood or components under either of the following conditions: 1) They have been approved for this use by the FDA. 2) There is documentation available to show that addition to the component is safe and efficacious (American Association of Blood Banks [AABB], 1994, p.32).

Under the approval of the Center for Biologics Evaluation and Research of the Food and Drug Administration, the AABB, the American Red Cross, and the Council of Community Blood Centers jointly prepared the Circular of Information for the Use of Human Blood and Blood Components. The Circular is summarized along with the AABB standards in the Technical Manual of the AABB (1996) stating that medications must not be added to blood or components:

> Solutions not approved for addition to blood components or for simultaneous administration via the same intravenous line include Lactated Ringers solution, 5% dextrose in water, and hypotonic sodium chloride solution. Dextrose solution may cause red cells to clump in the tubing and, more importantly, to swell and hemolyze as dextrose and associated water diffuses from the medium into the cells. Lactated Ringer's (LR) solution contains enough ionized calcium (3 mEq/L) to overcome the chelating agents in anticoagulant, preservative or additive solutions and allow small clots to develop (pp. 452-453).

Statement of the Problem

As stated above, in some clinical conditions, patients require acute and long-term intravenous (IV) narcotic analgesics as well as frequent blood component transfusions. There is occasion where providing combined intravenous infusion of morphine sulfate solution (MSO4) and normal packed red blood cells (PRBC) would be necessary. The combined use of these clinical and pharmacological procedures could prove to be quite useful in the clinical setting. However, according to the standards of the American Association of Blood Banks (AABB,1995), drugs or medications must not be added to blood or blood components. Rigid enforcement of this all-encompassing regulation can, in patients with limited intravenous catheter access, lead to unwanted delays in medication schemes.

Significance of the Problem

Since the administration of all IV narcotic analgesics and blood products requires IV access, it is obvious that IV cannulation is a prerequisite prior to their provision. Intravenous cannulation of patients who are suffering from disease processes similar to those discussed above can be difficult or even impossible. Limited IV access poses many practical problems in the clinical infusion setting. When providing IV therapy to patients who are limited to one IV access site and/or are in need of many IV pharmaceutical

products simultaneously, it is only natural for the clinician to want to administer those products together. However, presently, in the case of MSO4 and packed red blood cells this practical combination is strictly prohibited.

Blood bank recommendations specify that co-administration of medications should be avoided while transfusing blood. However, few studies evaluate or quantify incompatibilities that may occur during infusion of IV medications and PRBCs. Studies on the co-infusion of any intravenous solution and packed red blood cells are very limited. Specific studies on the compatibility of MSO4 and packed red blood cells are almost non-existent. To date, most investigations that have studied the compatibility of blood products and intravenous medications have used static models, where the blood sample was incubated for some amount of time with a drug solution, and potential damage to the red blood cells was subsequently analyzed. While in vitro static studies reveal much about what might happen to red blood cells when mixed and incubated with MSO4 over a prolonged period of time. They do little to suggest what might happen in the clinical infusion setting where contact time between blood and medication would be very brief.

In 1975, Ryden and Oberman conducted such a study on the compatibility between different pharmaceutical solutions and citrate-phosphate-dextrose anti-coagulated whole blood. In 1989 Strautz, Nelson, Meyer, and Shulman conducted a study that partially replicated Ryden's and Oberman's work by measuring the compatibility of adenine-saline-dextrose solution (ADSOL) stored red blood cells with various pharmaceutical solutions. Both of these static model studies revealed fibrin clots that formed at blood to lactated ringer's solution ratios of 10:1 or lower.

The apparent dangers to patient well-being revealed by these studies have definitely impacted the decision of the AABB to prohibit the administration of blood products with medications. However, it is interesting to note that a growing body of

literature suggests it may be safe to administer LR with packed red blood cells.

Recent studies, such as the two conducted by Lorenzo et al (1998) and Helpling (1998), utilize a dynamic and clinically relevant methodology to reveal convincing evidence that LR solution can be administered with packed red blood cells safely at high rates of infusion.

The effects of medications on erythrocytes have also been studied using static in vitro models as well as dynamic in vivo models. Two in vitro studies by Seeman, Kwant, and Sauks in 1969, and Seeman in 1974, investigated membrane expansion of intact erythrocytes by anesthetics and tranquilizers, membrane expansion of erythrocyte ghosts by tranquilizers and anesthetics, and ultrastructure of erythrocyte membrane lesions in drug induced lysis, respectively. In all cases, the investigators found that erythrocytes exhibited structural changes that acted to protect them from osmotic lysis when exposed to different medications at sub-lytic doses.

An in vivo animal study performed by Vadas and Hosien (1978) on the alteration of the erythrocyte ultrastructure and blood viscosity by morphine, revealed that morphine sulfate can indeed affect the shape of the erythrocyte. In accordance with the findings of Seeman, Kwant, and Sauks (1969), this could suggest that MSO4 actually acts to stabilize the red blood cell against hypotonic hemolysis. The study also revealed that MSO4 actually decreased blood viscosity.

Review of the literature will examine the above studies in depth. Nonetheless, this brief overview of some of the relevant literature reveals lack of convincing evidence on the issue of compatibility. Furthermore, although these studies provide important information, their static methodologies are not applicable to the real-world clinical infusion setting. Therefore, valid generalization of their findings to the clinical setting is not possible. Examining the compatibility of MSO4 on PRBCs in the clinical transfusion setting will require more research to accurately ascertain whether or not morphine sulfate

and packed red blood cells can be used in combination to prevent practical complications and unwanted delays in medication schemes.

Purpose of the Study

The purpose of this quantitative research project was to measure the dynamic effects of morphine sulfate on agglutination, clot formation and hemolysis in packed red blood cells, by imitating the concentration admixtures and transfusions practices commonly utilized in the clinical infusion system. The aim of this study was to analyze and provide documentation that measures the effects of morphine sulfate on packed red blood cells during co-administration in the clinical infusion setting, and to provide a beginning body of empirical evidence that can be used by regulating agencies when determining blood transfusion practice standards.

Research Questions

1. Is morphine sulfate 10mg/ml associated with agglutination, clot formation, or hemolysis in fresh packed red blood cells when co-infused in the clinical transfusion system?

2. During transfusion therapy what is the observed effect of mixing morphine sulfate solution with normal packed red blood cells at both room temperature and 37°C.

Conceptual/Theoretical Framework

Basic to any professional discipline is the development of a body of knowledge that can be applied to its practice. As an emerging profession, nursing continues to be deeply involved in identifying it's own knowledge base. In identifying this base of knowledge, various concepts, models, and theories specific to nursing are constantly being recognized, defined, and developed.

Because this study is rooted in the basic biological sciences the theoretical

framework that will guide it needs to focus on directing nursing education in the biological sciences. A perfect fit for this was derived from Akinsanya's (as cited in Casey, 1996) theoretical model of bionursing. "This innovative model, designed to direct nursing education in the biological sciences, explores the link between nursing and these sciences and attempts to disassociate nursing from the dominance of the biomedical model" (p.1065). Bionursing is a framework for the delivery of biological science knowledge to students of nursing.

Akinsanya's (1987) model assumes that nurses require knowledge of the biological sciences for practice, and that nursing is a task-based profession. Akinsanya defines three main concepts in his model: bionursing, four-level task performance, and care.

The first concept is called bionursing due to Akinsanya's belief that nursing education and practice are rooted in the biological sciences. As such, Akinsanya (as cited in Casey 1996) proposes the label "bionursing" like other disciplines that utilize knowledge from the biological sciences.

The second concept, the four different levels of task performance, is proposed to guide nursing students' progress during their education as his/her professional responsibility and scope of practice develops. Level one, termed "task operational," depicts the basic tasks of nursing that are shared by everyone in the public and, therefore, do not require special skills or knowledge to carry out. Level two, termed "task specific," is the level where understanding of basic biological life science knowledge is necessary in order to carry out specific physiologic observations or tasks. Level three, termed "task contextual," is the level where specific and detailed knowledge of the life sciences is necessary to make informed professional decisions. Finally, level four is that of the personal and professional development that is obtained when the student reaches registration or certification (Casey, 1996).

Akinsanya divides care, the third and final concept, into three different interrelated elements: (a) "Routine tasks involving skills and techniques rooted in the life sciences", (b) "Decision making within the limits of professional responsibility, which is crucially dependent on a full understanding of the reason for every action or non-action, also essentially rooted in the life sciences", and (c) "Interpersonal relationships, the understanding of which lies mainly with the behavioral and social sciences" (Casey, 1996, p. 1066).

Clearly, bionursing provides a structural theoretical framework that can guide the nurse researcher through a quantitative study. Bionursing suggests a link between the biological sciences and bionursing, in which the development of knowledge in the biosciences impacts upon bionursing. Bionursing provides a rationale for the tasks performed by the nurse and it enables nurses to make sound and informed decisions that are based upon scientific evidence.

This theory enables the practitioner, including the CRNA, the freedom to debate the standards of care and to take a role in policy and decision making in what is still a very scientifically based clinical practice arena.

Definitions

1. Nurse anesthetist - An advanced practice nurse who has completed an accredited program that provides advanced education in the field of anesthesia. In order to be legally qualified to practice anesthesia, the graduate nurse anesthetist must pass a national board certification exam that affords him/her the title certified registered nurse anesthetist (CRNA). The terms certified registered nurse anesthetist (CRNA) and nurse anesthetist are used interchangeably.

2. Narcotic - This term is derived from the Greek word for stupor and has traditionally been used to refer to the morphine-like analgesic medications with the potential to relieve pain.

3. Analgesic - Denotes a medication that reduces or eliminates pain.

4. Compatibility - For the purposes of this research will be defined as the capability of two agents to maintain orderly, efficient integration and operation with each other while combined together as one in a system. Absence of detrimental cellular lysis and agglutination is the desired measurable entity.

5. Morphine Sulfate - Is an opium alkaloid based drug that is the prototype drug to which all other opioid analgesics are compared. In humans, morphine produces analgesia, euphoria, and sedation.

6. Whole Blood - A unit of whole blood derived from a donor that contains red blood cells and plasma components. These constitute approximately 450 ml of blood and contain about 65ml of anti-coagulant preservative solution. Whole blood can be a valuable volume expander as well as a major source of proteins, which are responsible for certain oncotic and coagulation properties. White blood cells and platelets are usually rendered ineffective during the storage process. Today the use of whole blood is generally reserved for catastrophic medical or surgical bleeding.

7. Packed red blood cells - Prepared by centrifuging whole blood and expressing the plasma into a satellite bag. Red blood cells are usually mixed with an anticoagulant and concentrated to a hematocrit of 80% or more equaling an approximate volume of 250ml. This remaining component is the red blood cell concentrate of choice for all patients in need of restoration of oxygen carrying capacity. Packed red blood cells are considered biological agents regulated by the Food and Drug Administration.

8. Plasma - Straw colored liquid consisting of water, dissolved solutes, and various proteins. It provides the medium of transport for many necessary components of physiologic homeostasis.

9. Coagulation - The process of clot formation in response to an internal or external stimulus. In this study, a trained laboratory technician will evaluate clot formation.

Determination of clot formation will be made through the observance of visible fibrin strands on orange wood sticks.

10. Supernatant - The clear liquid that separates from an insoluble substance in solution.

11. Agglutination - A clumping of red blood cells formed as a result of specific chemical interaction between surface antigens and antibodies.

Assumptions

Two main assumptions in this study are: (a) knowledge of the biological sciences is necessary for the nurse to make informed decisions about practice, and (b) patients with limited intravenous access, in need of pain control and blood transfusion at the same time, may have delayed medication schemes unless these two agents are administered concomitantly.

Limitations

The samples of blood used may not have been completely free of spontaneous clotting and lysis independent of exposure to morphine sulfate. This potential problem was difficult to quantify the accurate level of damage occurring as a direct result of exposure to morphine. A second limitation in this study was that sample collections were taken from one unit of packed red blood cells only. A third limitation to this study was the fact that the MSO4 to be used in this study was commercially prepared. The content in the commercial admixture contains 1 mg of edetate sodium and 5 mg chlorobutanol as preservative additives which were not be controlled for due to limited resources. Results of this study may lead to further, more comprehensive, studies including multiple units of PRBCs, commercial preservatives, and perhaps even in-vivo assessments in the future.

CHAPTER II: REVIEW OF LITERATURE

Introduction

Upon conducting an exhaustive literature review, it was noted that there is a significant lack of scientific data available for reference regarding the compatibility of medications and packed red blood cells. Studies that have specifically investigated the combination of packed red blood cells and morphine sulfate were not found.

The lack of published scientific data regarding this topic led to examination of the studies to follow. These studies identify relevant data collected on the mixture of pharmaceutical products, specifically crystalloid intravenous solutions and packed red blood cells in the clinical infusion setting. Other studies reviewed reveal significant information about the cellular changes that occur when morphine sulfate and red blood cells are mixed and incubated together for long periods of time. All of the studies reviewed failed to address the specific mixture of morphine sulfate and packed red blood in the clinical infusion setting, exposing a significant knowledge gap in the literature.

Studies that investigated the compatibility of various crystalloid solutions with anti-coagulated blood products do exist. Review of these pertinent studies will include those scientific reports that investigated the in-vitro effects of various medications on human red blood cells, as well as one study that examined the in-vivo effects of morphine sulfate on animal red blood cells.

Compatibility Studies

Ryden and Oberman conducted a study in 1975 that was later incorporated into the AABB standards for the use of blood and blood products. This study had a significant influence on the decision of the AABB to prohibit the concomitant use of medications and crystalloid solutions, other than 0.9% normal saline, with blood product transfusions.

Under normal circumstances only normal saline may be administered with blood components (AABB, 1994). Storage of blood requires the addition of citrate-phosphate-

dextrose in order to prevent clotting. This is effective in preventing clot formation by binding the calcium ion in the blood and disrupting the coagulation cascade. It has commonly been theorized that when administered together, the calcium contained in lactated ringer's solution will overcome the citrate anticoagulant in stored blood and cause clotting. This theory was the guiding base of the Ryden and Oberman study.

Ryden and Oberman (1975) evaluated the compatibility of lactated ringer's solution, 5% dextrose solution, 5% dextrose in 0.225% saline solution, 5% dextrose in 0.9% saline solution, and 0.9% saline solution with citrate-phosphate-dextrose anti-coagulated blood, at varying admixture and hematocrit concentrations. The mixtures were divided into separate sample groups (per solution type) and incubated at room temperature and 37°C for different time periods. Clots formed in the blood-lactated ringer’s mixture after five minutes at a citrate:calcium molar ratio of 4:1 or lower. Outcomes also revealed immediate agglutination and significant cellular clumping with the aqueous dextrose-blood mixture, and hemolysis of blood mixed with 5% dextrose and 0.225% saline solution. No hemolysis or agglutination in blood mixed with 5% dextrose in 0.9% saline solution or in blood mixed with 0.9% saline solution was detected.

Ryden and Oberman's work (1975) also included starting simulated transfusions through intravenous tubing already primed with lactated ringer's solution and 5% dextose in 0.225% saline solution. The simulated transfusions were performed at a slow rate of 60 drops per minute. The time required to clear the infusion tubing of the solution was measured at approximately 30 - 40 minutes after initiation of transfusion. This portion of their experiment provided an estimate of the amount of time the blood and high concentrations of priming solution would be in contact upon initiating a transfusion. An unquantified amount of clumped red blood cells were observed in the intravenous tubing, but were not discussed.

Ryden and Oberman (1975) reported "the danger of using lactated ringer's

solution for blood transfusions is assumed to be small, since no adverse results have been reported in years of clinical use"(pp. 254-255). Nevertheless, based upon their findings, they suggested that 5% dextrose with 0.225% saline, and lactated ringer's solutions should not be administered concomitantly with blood.

Their study revealed significant evidence that lactated ringer's solution rapidly produced clots when mixed with citrate-phosphate-dextrose anti-coagulated blood, and that crystalloid solutions like lactated ringer's can remain in intravenous tubing for extended periods of time. The suggestion that mixing the two in the clinical infusion setting poses little danger may be presumptive.

Two major problems with this study were: (a) Ryden and Oberman's study (1975) was performed with CPD anti-coagulated whole blood, which is rarely used today in the modern clinical setting. The content of calcium in whole blood compared to packed red blood cells is much higher. The higher levels of calcium in the whole blood increase the probability of citrate-phosphate-dextrose anti-coagulated whole blood binding with the calcium contained in lactated ringer's solution, creating clots, and (b) the study failed to examine the mixture of blood and crystalloid solutions in the clinical transfusion setting that required a high rate of flow through the transfusion administration tubing, as would be the case in emergency trauma and surgery situations where blood transfusion would be needed rapidly.

Although the in-vitro results of this study are undoubtedly alarming and correct, it is unclear why there have been no adverse reports from the use of Lactated Ringer's with blood in the clinical infusion setting. This fact, as well as others presented above, raise significant doubt that the findings of this study can be accurately generalized to the modern, real-world, clinical infusion setting. This study depicts a definitive need for replication utilizing a methodology that reflects current transfusion practice.

In 1989, Strautz, Nelson, and Shulman conducted a study that partially replicated

the work of Ryden and Oberman (1975)by measuring the compatibility of ADSOL-stored packed red blood cells with 5% dextrose solution and lactated ringer's solution. Gross clots were seen immediately after contact was made with lactated ringer's solution. Clumping and hemolysis were seen upon mixture of 5 percent dextrose and adenine-saline-dextrose solution (ADSOL) -stored red blood cells. Strautz et al. concluded that 5 percent dextrose in water and lactated ringer's solutions are incompatible with ADSOL-stored packed red blood cells. Furthermore, they suggested that mixing ADSOL stored RBCs with the above solutions was unsafe practice.

Strautz et al.(1989) failed to conduct this experiment utilizing a methodology that completely simulates modern clinical blood transfusion practice. This study failed to demonstrate convincing evidence that clotting and lysis will occur in the dynamic clinical infusion setting, as it did in the static test-tube environment, again leaving significant doubt that these findings are adequate enough to assume that incompatibility will occur in the dynamic clinical infusion setting.

Helpling (1998) replicated a portion of the Ryden and Oberman study utilizing a methodology that included modern transfusion products and techniques. He mixed citrate-phosphate-dextrose preserved packed red blood cells with lactated ringers solution and normal saline. Samples were collected in glass centrifuge tubes at concentration dilution ratios of 10:1, 5:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:5, and 1:10 (packed red blood cells to cystalloid) and incubated each at room temperature and 37° centigrade. The samples were then examined for clot formation at 5, 10, 20, 40, 60, and 120 minute intervals. Helpling detected clot formation at dilution ratio values of one and lower.

Further experimentation in Helpling's (1998) study examined 24 units of CPD anti-coagulated packed red blood cells diluted with both lactated ringer's solution and normal saline, to hematocrit concentration levels of 35, 45, 55, and 65 percent. The units were then mixed and administered through filtered transfusion tubing via gravity, at

wide-open flow rates. Total flow time for each unit was then measured and compared in order to determine if clot formation had occurred and effected flow. Samples of each simulated transfusion were then collected, incubated, and examined for clots. Interestingly, no statistically significant differences were noted in the flow rates of the two different solutions. Nor were clots observed in any of the collected samples.

Helpling's study (1998) in part validates Ryden and Oberman's work in that, at certain concentration ratios, packed red blood cells will definitely clot when mixed with lactated ringer's solution for extended time. However, it also provided convincing evidence that at high flow rates where contact time is brief, packed red blood cells mixed with lactated ringers solution may not clot.

In a study conducted in 1989, Lorenzo et al. hypothesized "that there is no difference in clot formation between normal saline (NS) and lactated ringer's solution (LR) while transfusing blood at a rapid infusion rate" (p. 308). They tested this hypothesis by evaluating clot formation, filter weights, and infusion times using normal saline, lactated ringer's (LR), and lactated ringer's solutions containing supplemental amounts of calcium chloride. This study was designed to simulate the emergency trauma/surgery setting where rapid "wide open" transfusion rates are necessary. Concentration ratios of PRBCs to crystalloid were 1:1.

No significant differences in infusion time or filter weight using whole blood and packed red blood cells with normal saline or lactated ringer's solution were noted. However, baseline filter weights were significantly elevated in the whole blood category when mixed with lactated ringers plus 1 gram of calcium chloride (LR-1) and lactated ringers plus 2 grams of calcium chloride (LR-2)

Statistically significant differences were also noted in the packed red blood cell group. Infusion time differences were seen between the units mixed with LR-2 and the units mixed with lactated ringers plus 5 grams of calcium chloride (LR-5).

This clinically relevant study demonstrated that lactated ringer's solution may be just as safe as the standards approved normal saline when mixed with packed red blood cells and transfused at high rates of flow. Further, it demonstrated that high levels of added supplemental calcium chloride were required, to create clinically significant clotting.

In-vitro Effects

As stated in the introduction, the second part of the literature review will include those scientific reports that investigated the in-vitro effects of various medications on human red blood cells, as well as one study that examined the in-vivo effects of morphine sulfate on animal red blood cells. While none of these studies directly examine the effects of morphine or other medications on packed red blood cells, they do provide insight on the cellular effects of drugs on the erythrocyte. Seeman, Kwant, Sauks and Argent (1969) investigated membrane expansion of intact human erythrocytes by anesthetics through highly sophisticated scientific and microscopic techniques. The drugs studied were pentanol, nonanol, benzyl alcohol, and chlorpromazine hydrochloride.

Seeman, Kwant, Sauks and Argent (1969) also found that many types of cells are protected or stabilized by a very wide variety of surface-active compounds, including tranquilizers, antihistamines, fatty acids, alcohol’s, anesthetics, and steroids. They observed that low doses of medications (.00000001M - .000001M) protect the cell from osmotic, mechanical, or acidic lysis, while higher doses (.0001M - .001M) are directly and immediately lytic to the cell membrane.

In the presence of a concentration of anesthetic, osmotic hemolysis was reduced by 50 percent. The mechanism of action was proposed to be that anesthetics increased the cellular volume, which reduced osmotic hemolysis. Although this study was unable to define a mechanism of action for this proposed protective effect, it was hypothesized that it works much the same way anesthesia does. In this study the hypothesis was supported

by the fact that all agents that produce this protective effect also produce anesthesia.

Another study conducted by Seeman, Kwant, and Sauks (1969) illustrated membrane expansion of erythrocyte ghosts by tranquilizers and anesthetics. This study like the one above found that certain drugs have the ability to provide cellular stability by penetrating, expanding, and modifying the properties of lipid, protein, and lipoprotein mono-layers of erythrocytes.

The main experimental finding in this study is that the erythrocyte ghost membrane area expands at anesthetic concentrations that are known to inhibit hypotonic hemolysis. These anesthetic concentrations are similar to those needed to anesthetize nerves.

Changes in erythrocyte geometry have been proven to have important consequence on blood rheology. Vadas and Hosien conducted a study in 1978 titled "Alteration of the Erythrocyte Ultrastructure and Blood Viscosity by Morphine". In this study the effects of morphine administration on intact in-vivo rabbit erythrocytes and on their flow properties was studied. Measurement consisted of mean cell volume, cell geometry, and whole blood and plasma viscosities.

Findings revealed that morphine sulfate caused only small 2-7% increases in mean cell volume. Cellular geometry was effected on a time dependent basis. Cellular geometry effects were found, using photomicrography, mainly in the bi-concave portions of the red blood cells. In all the samples collected from morphine treated rabbits, regardless of the dose or mode of administration, the cells were no longer concave. Morphine sulfate injected intravenously in doses of 2.5, 5, and 10 mg/kg resulted in significant decreases in whole blood viscosity.

A portion of a third study by Seeman in 1974 illustrated membrane lesions in drug induced lysis. This study highlighted drug-induced lysis based upon enzyme deficiencies.

A number of genetic disorders or enzyme defects confer to their carriers

modifications in response to drugs, without the biochemical defect being directly associated with the pharmacokinetics of the offending drug. Specifically, defects in erythrocyte glucose-6-phospahte dehydrogenase induce hemolysis after analgesics like morphine sulfate are administered. This important genetic enzyme deficiency disorder occurs in 10 percent of the world population, and is most frequent in tropical and subtropical countries.

Undetected in an asymptomatic blood donor this, deficiency could taint a unit of banked blood. Theoretically, should morphine sulfate be administered with such a unit of stored blood, the result would be frank hemolysis. This study brings to light a potentially detrimental risk of mixing packed red blood cells and morphine. However, the chances of this occurring are no greater than the chances of a patient afflicted with this disease actually receiving a dose of morphine.

Summary

The literature review provides an understanding of how the AABB came to recommend that medications and crystalloid solutions, other than normal saline, should not be added to blood and blood products during transfusion therapy. Findings have demonstrated that although incompatibilities have been shown to occur in the in-vitro static models, clinically relevant dynamic models have been unaffected.

Recent studies investigating the effects of medications on red blood cells have provided very promising evidence that it may be safe and even efficacious to mix certain medications and packed red blood cells when necessary. The combined infusion of morphine and packed red blood cells may be necessary in emergent situations. More research will need to be done before this practice is considered acceptable by the appropriate accrediting and regulatory agencies. Clearly, this topic invites more research to fully explore these potential benefits.

CHAPTER III: METHODS

Introduction

Various studies have been reported in the literature review that suggest it may be safe and efficacious to co-administer various pharmaceutical substances and packed red blood cells during transfusion therapy. For reasons described in Chapter One, these findings have encouraged me to pursue the assessment of compatibility of morphine and packed red blood cells. In this chapter the methods that were utilized to obtain data for this study, including research design, sampling and setting, and data analysis, are described.

Research Design

Experimental study designs are constructed to provide accurate information about causality by controlling extraneous variables. Burns & Grove (1993) state: "to study cause, one must eliminate all factors influencing the dependent variable other than the cause (independent variable)" (p.274). Experimental design was created to minimize the intrusion of extraneous variables, or the relationships among the variables the examiner wishes to examine (Burns & Grove, 1993, p. 251).

The post-test-only control group design is one type of experimental design utilized when the dependent variable cannot be measured before the treatment, as is the case in this study. This research was conducted utilizing this design. Experiments were conducted to determine if in-vitro agglutination or hemolysis occurs during or after co-administration of packed red blood cells and morphine sulfate solution in the same IV administration set. This study incorporated some of the materials & methods of earlier replications of Ryden and Oberman's original work by Cull, Lally & Murphy (1991) & Strautz et al.(1989).

This study was conducted in the pharmacology department laboratory at the Uniformed Services University. Permission was obtained from the University and the

pharmacology department for use of equipment and facilities prior to the gathering of data.

Data collection was carried out through careful laboratory experimentation, using fresh packed red blood cells collected from volunteer donors and containing a standard hematocrit between 70 to 80 mg/deciliter/units. Packed red blood cells were provided from the blood bank of The National Naval Medical Center (NNMC) in Bethesda, Maryland. Morphine Sulfate was obtained from the pharmacy department of the Uniformed Services University (USU). Crystalloid solutions, blood administration tubing, intravenous catheters, and other supplies were all obtained through standard central supply sources of Wright Patterson Air Force Base Medical Center. Both static and flow evaluations of blood compatibility was conducted.

Data collection began by observing universal precautions by hanging one unit of packed red blood cells at a standardized height of 70cm. The unit of PRBCs was accessed with Y set blood administration tubing. The opposite spike port contained as diluent, the AABB approved crystalloid 0.9% normal saline. The delivery end of the blood administration set was connected to one of three access ports on a standard intravenous in-line three-way stopcock. The second port on the intravenous in-line three way stopcock was connected to a 60 cm extension tubing set with an 18 gauge intravenous (IV) angio-catheter connected at its end. Contents were collected and quantified in a calibrated graduated cylinder. The third and final port of the intravenous in-line three-way stopcock contained an intravenous medication injection port for morphine sulfate injection.

Simulated blood transfusion began at a conservative rate of 100 cc/hr. Once the PRBCs reached the delivery end of the blood administration tubing, we injected 10mg/cc of morphine sulfate solution. Morphine sulfate was administered via syringe into the three-way stop cock allowing the PRBCs and morphine to co-mingle. Once mixing was

accomplished, the entire content of the pre-measured extension tubing was collected in a volumetric graduated cylinder. The contents were then divided into two equal amounts in different 12 x 75 mm glass centrifuge tubes. One sample was randomly placed in an international heat block and incubated at 37° centigrade, the other was randomly placed in a test tube rack and incubated at room temperature. Each sample was then placed into a laboratory centrifuge and spun at 3000 revolutions per minute for two minutes. Each aliquot was removed from the centrifuge. The aliquots were visually assessed macroscopically for hemolysis at five different time periods; 0, 5, 10, 20, and 30 minutes. Determination of clotting and agglutination was made at two different time periods; 0 and 30 minutes by the observance of visible fibrin strands adhering on to orange wood sticks. In accordance with Strautz et al. (1998) frank hemolysis was recorded on the data collection tool if the supernatant (plasma) developed a grossly evident pink-red tinge. The degree of hemolysis was quantified using a relative semi quantitative visual assay measurement tool known as the HAEMONETICS® color comparator. The HAEMONETICS® free hemoglobin comparator is designed for quick approximate visual determinations of free hemoglobin in plasma. This widely accepted laboratory measurement tool assigns a standard number 1 through 8 to a designated semitransparent color. Each color corresponds with value measured in milligrams percent of free hemoglobin 1 = 25mg%, 2 = 50mg%, 3 = 100mg%, 4 = 150mg%, 5 = 300mg%, 6 = 600mg%, 7 = 1000mg%, and 8 = 3000mg%. The color comparator was used to determine any hemolytic differences between the control and test groups.

As a control sample for this study, packed red blood cells were co-infused with normal saline in the same ratios, utilizing the identical procedure.

Sampling and Setting

Prior to data collection, approval from the Institutional Review Board (IRB) of the Uniformed Services University was obtained.

Forty, 5 milliliter (ml) samples were collected from one unit of packed red blood cells. Ten 5 ml samples were obtained within a two minute time frame. Each of the ten aliquots were then assigned a group according with which solution it was previously mixed. The aliquot was randomly assigned to either the room temperature group or the 37°C group. Incubation and observations was accomplished according to the above procedure. When experimentation was complete data was collected from four total groups: two morphine test groups, one at 37°C and one at room temperature, and 2 saline control groups, again one at 37°C and on at room temperature. Total sample collection time did not exceed two hours. Each aliquot was appropriately labeled for identification purposes.

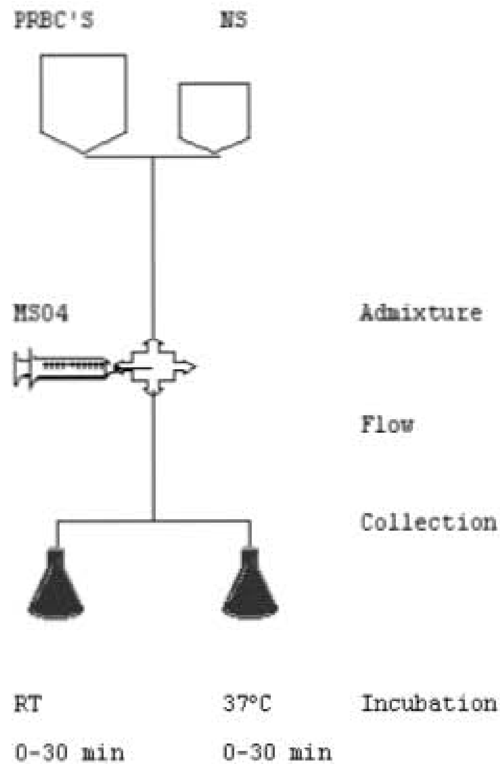

Figure 1. Diagram of Clinical Infusion Model.

Measurement Methods

The presence and absence of clots and agglutination was recorded according to the parameters specified by Strautz et al. (1989) in their examination of the compatibility of adenine-saline-dextrose stored red blood cells with intravenous solutions.

Clotting and agglutination was observed using macroscopic visual examination. Orange wood sticks were dipped into the PRBC morphine sulfate solution and normal saline solution, spun in a circular manner, removed, and visually examined for fibrin strands or clumping that may have formed but were not initially obvious in the solution. Observed clots and clumps were recorded on the data collection tool as "+" for presence of clots & clumps, or "-" for no clot or clumping presence. These values were categorized and recorded on the data collection tool according to which group they were observed in (see Appendices E, F, G, H).

Frank hemolysis was graded and recorded as described in the Strautz et al. (1989) study. Direct visual observation of the plasma was made to determine the levels of hemolysis. Visually apparent pink-red tinged supernatant (plasma) was determined using semi quantitative visual assay. Pink-red tinge in supernatant is indicative of the presence of frank hemoglobin and red blood cell hemolysis.

Grossly pink-red tinged supernatant was recorded on the data collection tool as H = hemolysis and 0 = no hemolysis, the degree of hemolysis in each was recorded using the HAEMONETICS® color comparator. The level of hemolysis occurring in each sample was assigned a number that correlated with the color scale on the color comparator. When a sample was determined to be positive for hemolysis an H was placed on the data collection sheet for that particular sample. Using the HAEMONETICS® color comparator the sample was then assigned a number (1-8) that correlated with color of the serum it contains. The number was placed next to the "H" on the data collection sheet.

Protection of Human Rights

The IRB committee from the Uniformed Services committee was contacted to determine the need for a consent form. Full informed consent was not indicated since no human or animal subjects were used.

Data Analysis

The data were recorded on the data collection from (Appendices A, B, C, D, E, F, G and H). Descriptive statistics were used to show differences in the test and control groups. The dependent variables, clotting, agglutination, and hemolysis were analyzed to determine measures of control tendency and variation. All findings are presented in Tables 1 through 8.

CHAPTER IV: Analysis of Data

Presentation

A simulated clinical infusion, as seen in Figure 1, was created. Packed red blood cells and the test solutions of morphine sulfate and normal saline were co-infused in an intravenous blood administration set. Morphine sulfate 10mg/ml, manufactured by Abbot laboratories, was the test solution. Fresh type A Rh positive packed red blood cells were used. Forty 5 milliliter aliquots of packed red blood cells--twenty each mixed with 1 milliliter of normal saline and twenty each mixed with 1 milliliter (ml) of morphine sulfate 10mg/ml--were collected in glass centrifuge tubes. The samples were randomly placed into 4 separate groups of 10 each. The morphine and saline aliquots were examined at room temperature and 37°C to test agglutination, clot formation, and hemolysis between the solutions during simulated clinical infusion therapy.

Analysis and Interpretation of data

When fresh packed red blood cells were mixed with normal saline, no agglutination or clot formation was observed at room temperature (20 degrees centigrade) or 37°C. Grossly evident pink-red tinged supernatent was observed in both temperature groups indicating that hemolysis occurred. The degree of hemolysis was quantified and varied between 300 - 1000 mg/%, the same range as the control samples. The reactions observed after a 30-minute incubation at room temperature and 37°C did not differ from those seen immediately after mixing. Tables 1-4 (see Appendices A-B-C-D) illustrate the agglutination and clotting profiles of the twenty samples examined.

Packed red blood cells were also mixed with 1 milliliter morphine sulfate 10mg/ml. Again, no agglutination or clotting was observed at room temperature or 37°C. However, grossly evident pink-red tinged supernatent was observed in all twenty of the samples examined. The degree of quantified hemolysis varied from 300 - 1000 mg/%. Reactions observed after 5, 10, 20 and 30 minutes incubation time did not differ form

those seen immediately after centrifugation. Tables 5-8 (see Appendices E,F,G,H) illustrate hemolysis profiles for all twenty samples examined at room temp and 37°C.

Flow rate for the simulated infusion was 100 milliliters /hour; therefore, contact time between the samples was constant for all admixtures.

CHAPTER V: Summary

Introduction

The purpose of this study was to determine the effect a clinically relevant dose of morphine sulfate (10mg/ml) has on agglutination, clot formation, and hemolysis in packed red blood cells, when both are combined and transfused through a clinical infusion system at a constant rate. Sodium chloride 0.9% solution is an established diluent that has been approved by the standards committee of the American Association of Blood Banks for simultaneous use with packed red blood cells during clinical infusion therapy. Thus, it served as the control for the test group to be measured against in this study. The study research questions were: (a) Is morphine sulfate compatible with fresh packed red blood cells? (b) What is the observed effect of mixing morphine sulfate 10mg/ml with fresh packed red blood cells at room temperature and 37°C, during clinical infusion therapy?

In all experiments fresh packed red blood cells were used, obtained from the blood bank of The National Naval Medical center. A modern clinical transfusion setup was designed (Figure 1). Morphine sulfate 10mg/ml was injected into packed red blood cells and collected in a 10ml glass centrifuge tube, each aliquot totaled 5ml. Each of the 40 aliquots were then placed in a centrifuge and spun at 1500g and 3000rpm for 3 minutes. The identical procedure was conducted using the AABB standards approved normal saline. 10 aliquots of each admixture was then incubated for up to 30 minutes at room temperature and the remaining 10 aliquots of each admixture was incubated for up to 30 minutes in a universal heat block at 37°C. The 40 aliquots were then examined at 0, 10, 20 & 30 minutes for evidence of clot formation, agglutination or hemolysis. Measurement consisted of visual examination for clot formation and agglutination. Dipping orangewood sticks into each sample and testing for adherence measured for visually unapparent clot formation and agglutination. The presence of hemolysis was

recorded if the supernatant developed a grossly evident pink-red tinge. Data from the two groups was compared to see if differences existed.

Conclusions

In this study packed red blood cells did not agglutinate and/or clot at room temperature or 37°C when mixed with morphine sulfate 10mg/ml and infused through a clinical infusion system. Packed red blood cells have 95 - 99% of all clotting factors removed during processing. This retrospective realization means that clotting in these samples may not have been possible. Many of the articles reviewed in the literature were studies that examined compatibility between packed red blood cells and citrate containing solutions. The citrate is responsible for binding Ca++ and activating the clotting cascade. The fact that Morphine sulfate contains no citrate may be the reason why no clotting or agglutination occurred in the test or control groups.

Analysis of all the samples demonstrated significant hemolysis. It is unclear why commensurate levels of hemolysis occurred in both the treatment groups and the control groups. It may be that with high concentrations of red blood cells (70 - 80% hematocrit), cellular lysis occurred spontaneously during handling, refrigeration, or centrifugation as a result of shearing forces between the erythrocytes. The fact that it occurred made it impossible to detect whether or not the morphine and/or the test control solution 0.9% sodium chloride was responsible for cellular lysis. Although this research was unable to verify the source of the cellular lysis, it occurred in commensurate levels in both the control group and the test group.

Absolute compatibility encompasses much more than the limited variables examined in this study. Due to time limitations, only the variables discussed above were examined. With the limited information collected and the questions posed above, it is not possible to conclude that it is safe to administer morphine sulfate 10mg/ml concomitantly with packed red blood cells during routine infusion therapy. Complete and accurate

determination of compatibility and safety in practice requires more research.

Recommendations

The findings in this study have produced as many questions as answers. This research was conducted in an attempt to provide beginning evidence that could be drawn upon by regulating agencies that determine practice standards for the safe administration of blood products to patients. It should be noted that the current standards prohibit the administration of all medication other than 0.9% normal saline with packed red blood cells. Recommendations for practice remain in accord with current practice standards.

Recommendations for future studies include conducting this study using photo-microscopy to better examine the cellular structure of the erythrocytes after collection. This would enable the researcher to better determine cellular condition in the test group vs. the control group. Other recommendations include quantifying hemolysis using specified laboratory equipment or spectrometry to more accurately determine hemoglobin levels in the collected samples. Predetermined levels of hemolysis in donated units of blood should be noted prior to laboratory experimentation commencement. Other variables that should be controlled for or tested include the possibility of chemical reactions, preservatives added to the morphine sulfate solution, and genetic abnormalities in donor units of blood.

With more research, thorough answers will be provided and the possibility of a standards change may become reality, benefiting both patient and provider alike.

This research project has provided education and insight in both the biological sciences and the research process. The information gained from this project has enhanced my biological understanding of cellular structure, pharmacology, and pathology. It has enabled me to see how truly important the link between clinical practice and research in the biological sciences is. Continued research in the biological sciences by nurse researchers will enhance clinical practice, by enabling standards compliance or reform in

the clinical practice arena. Without it the nursing practitioner, including the CRNA, will not fully enjoy the freedom to debate care standards or take part in policy and decision making in today's scientifically based clinical practice arena.

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APPENDICES

Appendix A: Presence of Clots in Packed Red Blood Cells Mixed with 10mg Morphine Sulfate Solution at 37°C (n = 10)

Appendix B: Presence of Clots in Packed Red Blood Cells Mixed with 10mg Morphine Sulfate Solution at Room Temp (RT) (n = 10)

Appendix C: Presence of Clots in Packed Red Blood Cells Mixed with Normal Saline Solution at 37°C (n = 10)

Appendix D: Presence of Clots in Packed Red Blood Cells Mixed with Normal Saline Solution at Room Temperature (RT) (n = 10)

Appendix E: Presence of Hemolysis in Packed Red Blood Cells Mixed With 10mg Morphine Sulfate Solution at 37°C (n = 10)

Appendix F: Presence of Hemolysis in Packed Red Blood Cells Mixed With 10mg Morphine Sulfate Solution at Room Temperature (n = 10)

Appendix G: Presence of Hemolysis in Packed Red Blood Cells Mixed With 1 milliliter Normal Saline Solution at 37°C (n = 10)

Appendix H: Presence of Hemolysis in Packed Red Blood Cells Mixed With 1 milliliter Normal Saline Solution at Room Temp (n = 10)

Appendix I: Task List and Time Line for Thesis Project

APPENDIX A

Table 1.

Presence of Clots in Packed Red Blood Cells Mixed with

10 mg Morphine Sulfate Solution (10mg/1ml) at 37°C (n = 10)

Solution Added Sample Number	Immediate 37°C	30min 37°C
1. Morphine	-	-
2. Morphine	-	-
3. Morphine	-	-
4. Morphine	-	-
5. Morphine	-	-
6. Morphine	-	-
7. Morphine	-	-
8. Morphine	-	-
9. Morphine	-	-
10.Morphine	-	-

- = NO CLOT OBSERVED; + = CLOT OBSERVED

APPENDIX B

Table 2.

Presence of Clots in Packed Red Blood Cells Mixed with

10mg Morphine Sulfate Solution (10mg/1ml) at Room Temperature (RT) (n = 10)

Solution Added	Immediate	30min
Sample Number	RT	RT
1. Morphine	-	-
2. Morphine	-	-
3. Morphine	-	-
4. Morphine	-	-
5. Morphine	-	-
6. Morphine	-	-
7. Morphine	-	-
8. Morphine	-	-
9. Morphine	-	-
10. Morphine	-	-

- = NO CLOT OBSERVED; + = CLOT OBSERVED

APPENDIX C

Table 3.

Presence of Clots in Packed Red Blood Cells Mixed with

1 Milliliter Normal Saline Solution at 37°C (n = 10)

Solution Added Sample Number		Immediate 37°C	30min 37°C
1.	NS	-	-
2.	NS	-	-
3.	NS	-	-
4.	NS	-	-
5.	NS	-	-
6.	NS	-	-
7.	NS	-	-
8.	NS	-	-
9.	NS	-	-
10.	NS	-	-

- = NO CLOT OBSERVED; + = CLOT OBSERVED

APPENDIX D

Table 4.

Presence of Clots in Packed Red Blood Cells Mixed with 1 milliliter Normal Saline Solution at Room Temperature (RT) (n = 10)

Solution Added Sample Number		Immediate RT	30 min RT
1.	NS	-	-
2.	NS	-	-
3.	NS	-	-
4.	NS	-	-
5.	NS	-	-
6.	NS	-	-
7.	NS	-	-
8.	NS	-	-
9.	NS	-	-
10.	NS	-	-

- = NO CLOT OBSERVED; + = CLOT OBSERVED

APPENDIX E

Table 5.

Presence of Hemolysis in Packed Red Blood Cells Mixed with

10mg Morphine Sulfate (10mg/ml) Solution at 37°C (n = 10)

Solution Added Sample Number	Immed 37°C	5min 37°C	10min 37°C	20min 37°C	30min 37°C
1. Morphine	H7	H7	H7	H7	H7
2. Morphine	H7	H7	H7	H7	H7
3. Morphine	H7	H7	H7	H7	H7
4. Morphine	H5	H5	H5	H5	H5
5. Morphine	H5	H5	H5	H5	H5
6. Morphine	H5	H5	H5	H5	H5
7. Morphine	H5	H5	H5	H5	H5
8. Morphine	H5	H5	H5	H5	H5
9. Morphine	H5	H5	H5	H5	H5
10.Morphine	H5	H5	H5	H5	H5

0 = NO HEMOLYSIS OBSERVED; H = HEMOLYSIS OBSERVED

APPENDIX F

Table 6.

Presence of Hemolysis in Packed Red Blood Cells Mixed with

10mg Morphine Sulfate (10mg/ml) Solution at Room Temperature (RT) (n = 10)

Solution Added Sample Number	Immed RT	5min RT	10min RT	20min RT	30min RT
1. Morphine	H7	H7	H7	H7	H7
2. Morphine	H6	H6	H6	H6	H6
3. Morphine	H5	H5	H5	H5	H5
4. Morphine	H6	H6	H6	H6	H6
5. Morphine	H7	H7	H7	H7	H7
6. Morphine	H6	H6	H6	H6	H6
7. Morphine	H5	H5	H5	H5	H5
8. Morphine	H6	H6	H6	H6	H6
9. Morphine	H6	H6	H6	H6	H6
10. Morphine	H5	H5	H5	H5	H5

0 = NO HEMOLYSIS OBSERVED; H = HEMOLYSIS OBSERVED

APPENDIX G

Table 7.

Presence of Hemolysis in Packed Red Blood Cells Mixed with

1 milliliter Normal Saline Solution at 37°C (n = 10)

Solution added Sample Number	Immed 37°C	5min 37°C	10min 37°C	20min 37°C	30min 37°C
1. NS	H5	H5	H5	H5	H5
2. NS	H5	H5	H5	H5	H5
3. NS	H5	H5	H5	H5	H5
4. NS	H5	H5	H5	H5	H5
5. NS	H5	H5	H5	H5	H5
6. NS	H5	H5	H5	H5	H5
7. NS	H7	H7	H7	H7	H7
8. NS	H5	H5	H5	H5	H5
9. NS	H7	H7	H7	H7	H7
10. NS	H5	H5	H5	H5	H5

0 = NO HEMOLYSIS OBSERVED; H = HEMOLYSIS OBSERVED

APPENDIX H

Table 8.

Presence of Hemolysis in Packed Red Blood Cells Mixed with

1 milliliter Normal Saline Solution at Room Temperature (RT) (n = 10)

Solution Added Sample Number	Immed RT	5min RT	10min RT	20min RT	30min RT
1. NS	H6	H6	H6	H6	H6
2. NS	H6	H6	H6	H6	H6
3. NS	H6	H6	H6	H6	H6
4. NS	H5	H5	H5	H5	H5
5. NS	H5	H5	H5	H5	H5
6. NS	H6	H6	H6	H6	H6
7. NS	H6	H6	H6	H6	H6
8. NS	H7	H7	H7	H7	H7
9. NS	H6	H6	H6	H6	H6
10. NS	H6	H6	H6	H6	H6

0 = NO HEMOLYSIS OBSERVED; H = HEMOLYSIS OBSERVED

APPENDIX I

Time Line

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Propasal Defense	■														
Obtain IRB Approval		■	■												
Collect Data				■	■	■									
Enter Data						■									
Analyze Data							■								
Prepare Draft Report							■	■	■						
Committee Review									■	■					
Prepare Final Draft										■	■	■			
Thesis Defense													■	■	
Make Revisions														■	
Obtain Signatures															■
Binding Thesis															■

Figure 2. Task List and Time Line for Thesis Project.