PHARMACOKINETIC AND PHARMACODYNAMIC IMPLICATIONS OF INHALED ESTER-BASED ANESTHETIC AGENTS

by

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STATEMENT OF DISSERTATION APPROVAL

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ABSTRACT

The goal of this study was to investigate the bioavailability, efficacy, and safety of inhaled remifentanil, inhaled remimazolam, and combinations of both drugs in mouse, rat, and pig models. Anesthesiology could benefit from efficacious, noninvasively delivered, short acting, and thereby easily titratable analgesic/sedative agents. Remifentanil and remimazolam are potentially advantageous due to their esterase-based metabolism and rapid elimination profiles, particularly to high-risk populations such as obese, elderly, and pediatric populations. Dosing via spontaneous respiration can inherently and safely control the duration and level of sedation and analgesia via patient minute ventilation. There is no inhaled opioid or benzodiazepine currently available for clinical use as an anesthetic agent.

It was our hypothesis that remifentanil and remimazolam delivered by inhalation would be rapidly absorbed, pharmacologically active, rapidly cleared, and noninjurious to rodent airways and lungs. We also hypothesized that the pharmacokinetics of inhaled remifentanil in pigs would exhibit similar rapid onset and recovery.

Inhaled remifentanil in rats induced profound analgesia with rapid recovery. Inhaled remimazolam in mice produced sedation, while inhaled remimazolam in rats did not produce sedation at the maximum dose able to be achieved in aerosols. Remimazolam delivered in combination with remifentanil potentiated the analgesic response.

Pulmonary mechanics and histology showed no irritation or injury by either drug or the combination. Pharmacokinetic analysis of both drugs in rodents were consistent with the pharmacological effects and a study of inhaled remifentanil in pigs demonstrated rapid absorption and clearance of the drug consistent with those reported for intravenous dosing in humans and animals. We have shown that remifentanil and remimazolam, administered alone or in combination, can be a clinically relevant method of anesthesia. These fundamental experiments and results are critical for the future development of formulations for inhalation delivery of these drugs for clinical use. These inhaled drugs could eventually revolutionize the ease and practicality of administering inhaled anesthetic agents, both inside and outside of the operating room.

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CHAPTER 1

INTRODUCTION

Current potent inhaled anesthetics used for general anesthesia include isoflurane, sevoflurane, and desflurane. Although the exact mechanism of action of these agents is unknown, it is thought that inhalational agents interact with numerous ion channels present in the central and peripheral nervous system, such as activating gamma-aminobutyric acid (GABA) through chloride channel conductance.[1] These agents can cause varying degrees of risk to patients, including hemodynamic instability, nausea, and airway irritation. In addition, they are greenhouse gases, which are not currently regulated. There is also a risk of recall, or lack of amnesia, at lower doses, which can cause patients to have memories of painful or traumatic experiences. Inhaled anesthetics are desirable due to the ability to administer these anesthetics through a mask or secured airway. Dose adjustments can easily be made with each breath. There is currently no inhaled benzodiazepine or opioid available today, and development of these drugs for delivery via inhalation would be of clear clinical value to anesthesia practice to use in conjunction with, or possibly even in place of, the halogenated inhaled agents used today.

Remifentanil, an injectable, ultra-short acting μ-opioid receptor agonist, is FDA approved for use in human patients. Remimazolam, an analogue of midazolam, is a $GABA_A$ allosteric modulator and is a new benzodiazepine currently in Stage III clinical trials. Like remifentanil, it was created to improve pharmacokinetic properties by

introducing a metabolically labile and pharmacologically essential ester moiety that is readily susceptible to hydrolysis, and thus clearance, by esterases. This unique structural feature imparts remimazolam and remifentanil with more specific desirable properties, such as shorter duration of action and more easily titratable effects during anesthesia. These properties could improve ease of administration, titration, and recovery from anesthesia in medically complicated patients, such as obese patients and extremes in age. Clearance of remifentanil and remimazolam is body weight and ventilation independent, thereby ideal for obese patients. These drugs follow first order kinetics. However, if esterases were to become saturated, they would follow zero-order kinetics, but this is unlikely at recommended dosing. Most other anesthetic drugs are lipophilic and accumulate in fat, as manifested by prolonged half-lives and larger volume of distribution due to redistribution to lipid compartments. This results in delayed arousal from anesthesia and lingering anesthetic effects. Today's surgical population is becoming increasingly diverse, with complex medical issues. This population would benefit from the distinct clinical advantages of inhaled ester-based drugs, which include: easier titratability; IV-independent administration; facilitated sedation, analgesia, and amnesia for pediatric populations; body weight-independent clearance; a safety profile in which respiratory depression will limit uptake of the drug while metabolism continues, thereby preventing overdose; and drug reversibility via naloxone (remifentanil) or flumazenil (remimazolam). There are no currently available inhaled opioids or benzodiazepines for anesthetic use. As these drugs have only been studied via the traditional intravenous route, very little is known regarding the feasibility, efficacy, and pharmacokinetics when administered via inhalation.

Today's surgical population is complex, and high-risk patient procedures are on the rise, frequently requiring case-specific adjustments to anesthesia regimens. High-risk patients include obese patients with variable comorbidities, the young, and the elderly. This demographic change is due to both an increase in surgery rates worldwide, and advances in medicine, allowing medically complicated patients to live longer. The development of new, potent, highly titratable, short-acting anesthetic agents with greater safety margins across a diverse patient population could substantially improve the quality of care and reduce risks of surgical/anesthesia-related complications in high-risk patients; inhaled remimazolam and remifentanil represent such anesthetic agents.

Obesity-Associated Anesthesia Challenges

The World Health Organization has declared obesity a global epidemic, which has increasingly affected the management of anesthesia.[2] It is noted that 35.7% of the US population is obese.[3] Consequently, obesity-related diseases such as obstructive sleep apnea, hypertension, hyperlipidemia, type II diabetes, cardiovascular disease, orthopedic joint related derangements, and certain types of cancer are also on the rise.[4-6] These aforementioned diseases predispose patients to the need for surgery. In fact, outpatient surgery visits have increased over 300% between 1996-2006, with an estimated 57.1 million outpatient surgeries taking place in 2006.[7] Morbidly obese patients are at much higher risk for complications during surgery, including death.[6]

Obese patients also frequently present with obesity-related airway and pulmonary changes that include anatomically difficult upper airway access and Pickwickian syndrome (obesity hypoventilation syndrome).[8] Pickwickian syndrome has components

of both obstructive sleep apnea (OSA) and restrictive type breathing. Because many more general inhaled anesthesia cases will be performed on obese patients in the near future, more airway complications during induction and at the end of surgery are also expected to occur.[9] Additionally, once safely exiting the operative theatre, postoperative patients with obesity and obesity-related complications require careful monitoring in the postanesthesia care unit (PACU). Particularly, patients with OSA may require a longer stay as compared with non-OSA patients undergoing similar procedures.[10] This is due to their propensity to develop airway obstruction or central respiratory depression,[10] which is in part related to redistribution of lipophilic anesthetic agents back to the central compartment, in addition to pain medication requirements in the postoperative period. The unique pharmacological properties of remimazolam and remifentanil provide anesthetic choices that have rapid onset, short duration of action, and body-weight independent clearance, which could provide an increased safety margin during vulnerable operative times, such as induction, emergence, and recovery from anesthesia.

Age-Associated Anesthesia Challenges

Pediatric inpatient and outpatient surgery is also on the rise. Children less than eight years of age do not routinely have an intravenous (IV) line placed before surgery, thus necessitating anesthesia induction by intramuscular (IM) injection of ketamine and a benzodiazepine, such as midazolam, or mask inhalation with sevoflurane. However, these approaches are less than ideal. IM ketamine and midazolam can greatly prolong recovery from anesthesia, especially after short anesthetics. Further, ketamine/midazolam also has been shown to cause neurodegeneration in the brains of young mice.[11] In addition, mask

induction with sevoflurane can be complicated by breath holding and laryngospasm. An additional limitation of current practice is that oral benzodiazepines are also often given before surgery to facilitate amnesia. Benzodiazepine delivery can be complicated by problems with swallowing, the desire for an empty stomach before surgery, limited absorption, and slow onset after oral ingestion. Also, patients pretreated with oral benzodiazepines have delayed recovery, with an increased incidence of emergence agitation after receiving an inhaled anesthetic.[12] Midazolam has a half-life of 1.5-2.5 hours, which is significantly longer than the duration of most surgeries. The half-life of midazolam is increased in young children, and increased half-life paired with the active metabolite alpha-1-hydroxymidazolam can result in emergence agitation that can last up to 2 days in rare instances, and can be associated with prolonged postanesthesia care compared to nonagitated children.[13] Additionally, trauma to the child or to the site of surgery can occur in agitated patients.[12] The use of a rapid onset, rapid clearance, respirable anesthetic, such as remimazolam and/or remifentanil, could substantially decrease issues with placing IV lines and ingestion of medications, as well as possibly lower the risk of emergence delirium in children due to rapid elimination. Inhaled remifentanil could provide rapid analgesia with noninvasive delivery, an option that is not currently available. Specifically for remimazolam, pediatric patients may also benefit from amnesia without the long-term negative effects associated with currently available anesthetics.[14]

The elderly are yet another rapidly growing surgical population with unique anesthetic considerations. The population over age 85 is projected to increase 350% between the year 2000 and 2050, and the population over 65 is projected to increase

135%.[15] These patients require special anesthetic considerations as health conditions shift from acute to chronic conditions.[15] Many of these patients take multiple medications, increasing the possibility of drug interactions. The presence of sleep apnea also increases with age.[10] Geriatric patients more frequently display reduced rates of drug metabolism, thereby also increasing risk for emergence delirium and postoperative cognitive dysfunction after anesthesia with currently available inhaled anesthetics.[16] Elderly patients would benefit from an inhaled anesthetic exhibiting liver and kidney independent metabolism, predictable and rapid drug clearance, decreased risk for cognitive impairment postoperatively,[16] and the ability to fully reverse if necessary; no such anesthetic is currently available.

Inhaled Ester-Based Anesthetics-A New Paradigm for Anesthesia

One of the major benefits of ester-based compounds is the lack of uncertainty involved with renal and hepatic metabolism, which is affected by patient health status and extremes in age. Specifically, due to comorbidities frequently occurring in today's patients, variations in renal and hepatic status are not always unequivocally known before surgery. A patient's health status is often an educated guess based on a patient's medical history and physical appearance. Esterase-mediated metabolism and clearance of inhaled medications largely removes this metabolic uncertainty, and ester-based medications work through well-defined mechanisms. Ester-based medications are metabolized at a constant rate by plasma esterases, independent of body weight,[17] renal, and hepatic function. Ester-based medications also remove uncertainty associated with drug interactions. Midazolam, a commonly used IV benzodiazepine, is primarily metabolized

by hepatic cytochrome P450 3A4, which is susceptible to functional modulation by many drugs and dietary agents, is polymorphic, and is differentially expressed in patients, thus increasing the possibility for a patient to display variations in efficacy and safety.[18] The ester-based benzodiazepine remimazolam, and the ester-based opioid remifentanil present much lower risk for these types of adverse events, due to esterase metabolism in the blood, thereby circumventing metabolic differences seen with drugs, such as midazolam, that require liver metabolism. These would also be the only inhaled anesthetics that will be fully reversible by IV injection, thereby adding a layer of safety unavailable with any other inhaled anesthetics.

With inhaled remimazolam and remifentanil, dosage, in a nonconventional way, can be dynamically adjusted by the patient's depth and rate of ventilation. Patients experiencing pain or distress will reflexively hyperventilate, thereby allowing for delivery of proportionally more inhaled anesthetic. Alternatively, overdose can be attenuated, as a highly sedated patient will hypoventilate, thereby causing less medication to be delivered. Standard inhaled anesthetics require active pulmonary elimination before a patient will arouse. This not only is physiologically harder in sicker patients, but also translates to a much longer emergence time for patients who are at increased risks for complications. Costly complications due to residual effects of anesthesia could be reduced by the use of rapid acting and rapidly metabolized medications. Additionally, rapid plasma elimination of remimazolam and remifentanil facilitates titration as well as rapid patient arousal after surgery. These characteristics of inhaled remimazolam and remifentanil are an advancement and are diametrically different than standard inhaled anesthetics.[19]

There are currently no ester-based inhaled benzodiazepine or opioid available on the market today. Characteristics of an ideal anesthetic include ample potency, reliable amnesia, ability to titrate in high concentration oxygen, reliable smooth induction and maintenance of general anesthesia, odorless, safe for all ages, lack of injury to vital tissues, lack of propensity to cause seizures, lack of respiratory irritation or circulatory stimulation, little or no effect on the environment, and low acquisition cost.[20] Traditionally, lack of metabolism has been viewed as a desirable trait, as current inhaled anesthetics are exhaled unchanged. We theorize that complete metabolism would be more ideal, facilitating complete recovery independent of respiratory pattern. This would be a paradigm shift, suggesting a novel trait for ideal anesthetic agents. There is currently no ideal anesthetic agent available for use. Inhaled remimazolam and remifentanil have the characteristics of ideal anesthetics and could revolutionize the way anesthesia is performed in the future. We hypothesized that remifentanil and remimazolam (CNS 7056) would produce rapid onset of analgesia and sedation, followed by rapid recovery, while being noninjurious and nonirritating to lung tissues of rodents. These drugs would then be able to provide an alternative method of dosing with clear clinical advantages. Innovative and clinically beneficial components of this research are listed in Figure 1.1. An overview of each chapter is listed in Figure 1.2

1) Development of new inhaled analgesic and amnestic anesthesia agents that would increase safety for high-risk populations such as the obese, young, and elderly.
2) Dynamic respiratory dosing allowing ease of titration and an increased safety margin.
3) Respiration-independent clearance, allowing for rapid emergence from anesthesia.
4) Development of the only inhaled anesthetics that are not greenhouse gases.[21]
5) The ability to rapidly reverse via injection of flumazenil or naloxone.

Figure 1.1: Innovation and clinically beneficial components of this project.

Chapter 1: Introduction and overview of subsequent chapters

Chapter 2: Inhaled Remifentanil in Rodents

Chapter 3: Inhaled Remimazolam in Rodents

Chapter 4: Pharmacokinetics of Inhaled Remifentanil in a Porcine Model

Chapter 5: Determination of Remifentanil and Remimazolam in Blood by Liquid Chromatography-Mass Spectrometry

Chapter 6: Conclusion

Figure 1.2: Overview

Overview of Chapter 2: Inhaled Remifentanil in Rodents

The second chapter of this dissertation consists of a published manuscript about inhaled remifentanil in rodents. Although it goes into detail on the experiments and findings, what is missing is a description of some of the hurdles that were necessary to overcome in order to perform these experiments. As this drug delivered by inhalation had never been investigated in any model, animal or human, development of a delivery chamber, a method to reliably test analgesia, and an animal model would need to be developed or decided upon. First, a whole body exposure chamber was chosen, as the animal would need to be unanesthetized to test analgesic response. A whole body inhalation exposure system was constructed essentially as described by Schroder *et al*. (2009)[22] with some modifications, which are described in Chapter 2. A micromist nebulizer was chosen because it was something that has been used extensively in clinical practice, was readily available, and it was capable of producing an aerosol small enough to facilitate deep lung deposition (2.7 micrometers per the package insert). Previous research has found that particle size is key to deep lung deposition allowing for maximal absorption of the inhaled drug, with particle size <5 micrometers showing the best

absorption.[23] Rats were chosen because of extensive available research on pain measurements in rats, as well as cost and ease of use. Various ways of producing pain were attempted, such as tail rolling, paw pinching, electrical stimulation, and three different researchers monitored the rats for evidence of sedation, such as decreased movement, ataxia, laying down, and dropping their heads. Although the rats showed obvious signs of analgesia and sedation, it quickly became evident that whether or not a rat demonstrated pain when its paw was pinched was not easy to quantify. Therefore, Dr. Alan Light from the Department of Anesthesiology was consulted, who is an expert in rodent pain models. Dr. Light recommended the use of a tail flick meter to objectively measure analgesic response to painful stimuli. This meter electronically measures time to tail movement due to localized heat sensitivity, allowing for quantitative measurement of the analgesic effect in rats. He provided this machine for our use in these studies.

Next, a concentration of remifentanil to deliver by aerosol needed to be chosen. It was evident that a whole-body exposure chamber would require much more drug than intravenous or even intratracheal delivery. Therefore, starting at a very high concentration was decided, knowing there were many obstacles to overcome. It was unknown how much of the drug would be metabolized by pulmonary esterases before reaching the brain, or if exhaustion of pulmonary esterases would result in zero-order kinetics resulting in prolonged recovery. Also, rainout into the exposure chamber was a possibility. To limit rainout in the rodent's airway, 0.9% saline was used as a carrier solution, since remifentanil is readily soluble in saline and saline is an isotonic solution when compared to airway fluids. The first concentration of 2000 mcg/mL was delivered via aerosol to rats using a whole body exposure chamber over 5 minutes. Rats showed

profound analgesia when measured by tail flick meter. This provided initial proof of concept that inhaled remifentanil was bioavailable. Based on these results, the experiments outlined in Chapter 2 were performed.

Overview of Chapter 3: Inhaled Remimazolam and Remifentanil in Rodents

After many months of discussion with Paion Pharmaceuticals in Aachen, Germany, a shipment containing a vial of powdered remimazolam was received. Unlike remifentanil, which was readily water soluble, remimazolam created quite an obstacle when attempting to make a solution due to hydrophobicity. The lofty original goal was to aerosolize a liquid solution of 250-350 mg/mL, which was quickly recognized to be quite unrealistic. After several failed attempts, it was found that remimazolam could be solubilized at a concentration of 25 mg/mL when dissolved first in 100% dimethyl sulfoxide (DMSO), then diluted to 10% DMSO with 0.9% saline; this was a reasonable vehicle composition for aerosol generation and delivery. The chamber described above was subsequently modified to deliver inhaled remimazolam. It was found that the forced air nebulizer was not compatible with remimazolam. The saline would aerosolize, leaving a thick slurry of DMSO and remimazolam in the nebulizer. Contact was made with Aerogen Ltd. (Galway, Ireland). Aerogen's patented nebulizers use a palladium mesh that is perforated with 1,000 precision formed holes that vibrate at 128,000 times per second resulting in vaporization of the drug solution. After discussion with their research team, Aerogen generously donated an Aerogen lab vaporizer for this research. This was then fitted onto the exposure chamber. In intravenous rodent work on

remimazolam, Paion had used loss of righting reflex, the pinna reflex (ear flick in response to gentle touch of the auditory meatus), and the haffner reflex (response to paw pinch) to measure sedation in rats. With this information, modifications were made to the exposure chamber to allow for such testing during inhalation drug exposure. After spending most of the day at a local home improvement store, a toilet flange and a rubber glove were used to modify the chamber. These were secured to the top of the chamber, allowing a gloved hand to enter the exposure chamber to perform reflex testing. However, after exposing rats to inhaled remimazolam in this chamber, it was discovered that our drug concentration was not nearly high enough to cause measureable sedation in rats.

It was decided to change to a mouse model, as a 20 gram mouse was 1/10 the body weight of the 200+ gram rats, and the drug solution of 25 mg/mL was 1/10 of our desired drug solution of 250 mg/mL. It was found that after exposure to aersolized remimazolam, mice retained the pinna, haffner, and righting reflex, but they did appear sedated. The mice did not move for minutes or even hours, depending on the exposure/ dose, which was significantly different than control mice. We adapted the study to look at time to movement outside a 4-inch square perimeter as a measure of sedation following exposure to inhaled remimazolam for 10 minutes. The results of this pilot study are shown in Figure 1.3.

With evidence of the ability to elicit profound sedation in mice, ways to relate inhaled remimazolam to earlier research performed in rats on inhaled remifentanil were

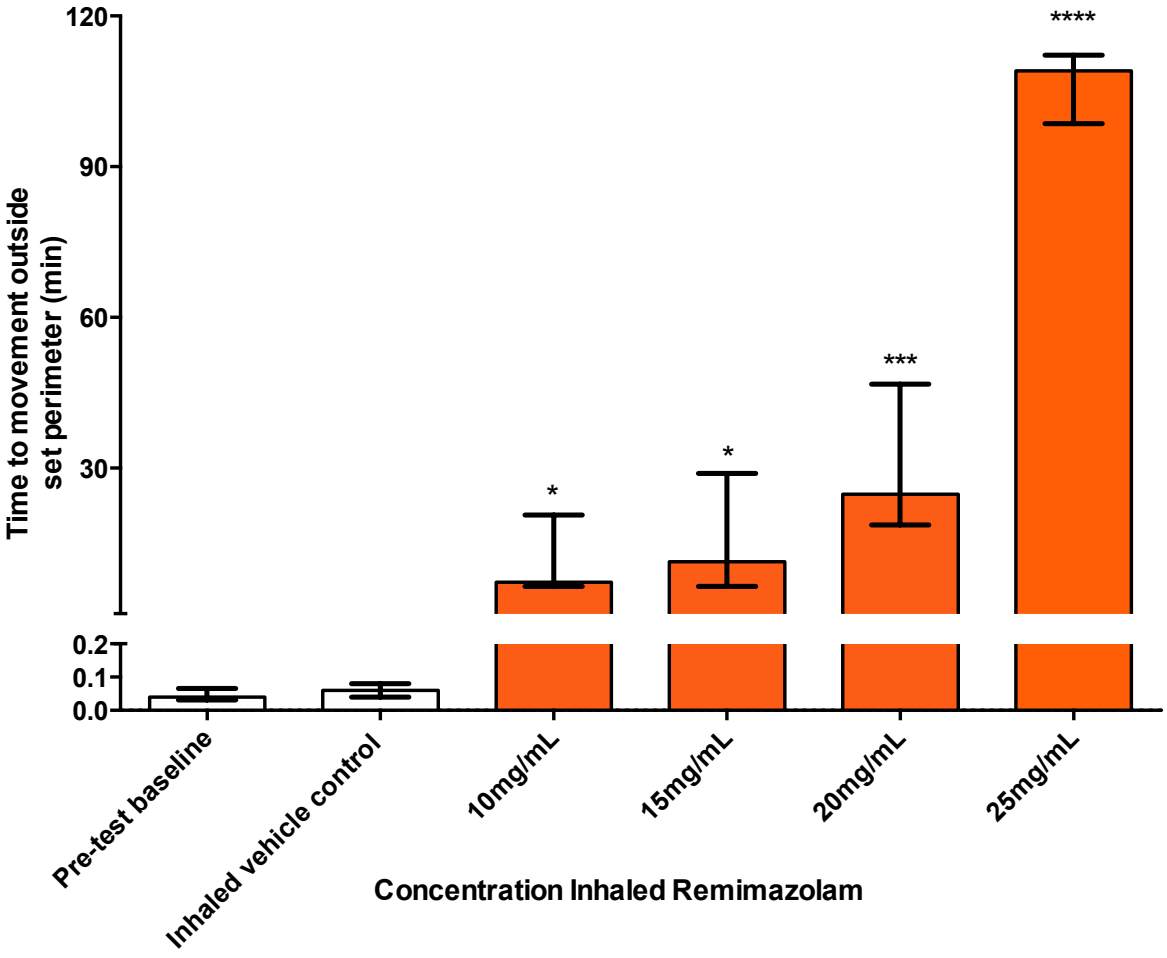

Figure 1.3: Sedative response to increasing concentrations of inhaled remimazolam (orange bars) following exposure for 10 minutes as measured by time to movement outside set perimeter.
*=statistically different than pretest baseline or vehicle control P=0.01
**=statistically different than pretest baseline or vehicle control P<0.0006
****=statistically different that all other groups P<0.0001
n=4

pursued. It was decided to give various concentrations of the combination of drugs to rats and evaluate for potentiated analgesic response, as opioids and benzodiazepines are frequently given in combination in operating rooms to complement each other. This research is outlined in Chapter 3: Inhaled Remimazolam in Rodents.

Overview of Chapter 4: Inhaled Remifentanil in a Porcine Model

Following favorable results when testing inhaled remifentanil in a rodent model, subsequent testing in a large animal model using a custom delivery system that was reasonably comparable to what could be used in humans was indicated. Through collaboration with the Department of Anesthesiology, a study on intubated Duroc pigs was initiated. A sensitive and selective liquid chromatography mass spectroscopy method was required for the pharmacokinetic analysis. Also, an appropriate dose and delivery method needed to be determined. Dr. Chris Stockmann (Dept. of Pediatrics) was consulted regarding the optimal sampling regime he had developed for inhaled remifentanil in rats. It was decided that this same pharmacokinetic model would work well for the pharmacokinetic study in pigs. A dose of 100 mcg/kg inhaled remifentanil was chosen. This relatively high dose was chosen to be sure that the drug could be detected, as we had concerns about variable pulmonary absorption. The first pig was scheduled. Following intramuscular sedation, two peripheral intravenous lines were placed in the ears, as well as an arterial line in the femoral artery. The pig was intubated and sedated by intravenous infusion to prevent interference with pulmonary absorption. Following administration of aerosolized remifentanil, samples were drawn for pharmacokinetics (PK) analysis. These samples were assayed using a validated liquid-chromatography-tandem mass spectrometry (LC-MS-MS) method and showed detectable levels of inhaled remifentanil. Details of this study and the development and validation of the LC-MS-MS methods are described in Chapter 4.

Overview of Chapter 5: Determination of Remifentanil and Remimazolam in Blood by Liquid Chromatography-Mass Spectrometry

Chapter 5 outlines the development and validation of an LC-MS-MS method for the analysis of the combination of remifentanil and remimazolam in blood.

Overview of Chapter 6: Conclusion

Chapter 6 is a summary of this research project with a detailed discussion of future studies.

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CHAPTER 2

INHALED REMIFENTANIL IN RODENTS

Inhaled Remifentanil in Rodents

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BACKGROUND: Remifentanil is an injectable opioid that is metabolized rapidly at a constant rate by plasma esterases. This supports its use as an analgesic for short-term, but painful, procedures in a wide range of patients. The aim of this study was to explore the feasibility and safety of administering remifentanil via inhalation. Our hypothesis was that inhaled remifentanil would be absorbed rapidly, pharmacologically active, rapidly cleared, and noninjurious to rodent airways and lungs.
METHODS: Rats were exposed to remifentanil aerosol (100–2000 μg/mL) for varying times (1–5 minutes). Analgesia was quantified as a function of dose and time by measuring time to tail flick in response to a painful stimulus. Remifentanil was measured in blood using liquid chromatography-tandem mass spectrometry. Pulmonary mechanics and histology were assessed in mice for the evidence of adverse effects after acute and repeated (subacute) dosing.
RESULTS: Exposure of rats to remifentanil aerosols produced dose-dependent analgesia within 2 minutes, which was sustained for the exposure period. Subsequently, the rats experienced rapid and complete recovery with a return to baseline tail flick response to a painful stimulus within 5 minutes. Analgesia mirrored the concentration profile of remifentanil in blood, and the animals were not affected adversely by repeated dosing. Pulmonary mechanics measurements in mice indicated that remifentanil was nonirritating and that the nasal and respiratory tissues of rats were free of significant morphological changes.
CONCLUSIONS: Remifentanil delivered by inhalation is rapidly absorbed, pharmacologically active, rapidly cleared, and noninjurious to respiratory tissues in rodents. (Anesth Analg 2016;XXX:00–00)

Remifentanil is a potent, injectable μ-agonist approved by the Food and Drug Administration that has only been studied via the traditional IV delivery. Because of its ester-based pharmacophore, it has a short, context-sensitive half-life, which is relatively independent of infusion duration and/or hepatic/renal function.[1,2]

The efficacy of inhaled remifentanil is unknown. Specifically, the abundance of pulmonary esterases may prevent remifentanil from attaining therapeutic levels in plasma via inhalation. In addition, the safety of exposure to inhaled remifentanil is unknown.

Clinically, inhaled opioids have been investigated for the treatment of pain and dyspnea in end-stage cancer and chronic lung disease.[3–10] The few pharmacokinetic studies that have been conducted on inhaled opioids investigated the pharmacokinetics of morphine and fentanyl.[11–14] In a study in which they evaluated inhaled fentanyl, the authors concluded that the pharmacokinetic profile of a single dose of inhaled fentanyl was comparable with IV administration,[15] demonstrating the feasibility and efficacy of inhaled opioids. No studies, however, have evaluated the safety of repeated administration of inhaled opioids or the pharmacokinetics and pharmacodynamics of the ultrashort-acting opioid, remifentanil.

The aim of this study was to demonstrate, in rodents, that remifentanil could produce analgesia via inhalation while being noninjurious to airway and lung tissue. Our hypothesis was that inhaled remifentanil would produce rapid onset of analgesia, followed by rapid recovery, while being noninjurious and nonirritating to lung tissues and nasal turbinates of rodents.

The rationale for this study was several-fold: the ability to noninvasively induce profound short or potentially even longer-term analgesia and sedation has many potential clinical uses; for example, to place an IV, change bandages, or insert/remove sutures. Inhaled remifentanil also would address several key issues associated with commonly used anesthetics and analgesics; chlorofluorocarbons and nitrous oxide are both greenhouse gases.[16] Remifentanil is not a known environmental hazard, and chlorofluorocarbons and nitrous oxide are eliminated only via exhalation. Because of the inertness of chlorofluorocarbons and nitrous oxide to biologic degradation, elimination also can become a safety issue in patients in whom spontaneous respiration is impeded because of residual sedation. Thus, inhaled remifentanil, used alone or in combination with a traditional volatile anesthetic, may decrease the amount of volatile anesthetic needed for a given effect[17] or perhaps replace such anesthetics in certain scenarios. In addition, development of a well-defined method to deliver inhaled remifentanil through an anesthesia machine could prevent drug dilution and drug pump errors associated with IV drug delivery and risks inherent to IV delivery of drugs. IV delivery of anesthetics such as remifentanil and other opioids inherently bypasses the body's ability to regulate and maintain

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spontaneous respiration, posing potential risks to patients. If remifentanil were administered via inhalation, the dose would be regulated, in part, via inherent respiratory drive: when the patient is more anxious or in pain, he or she is likely to breathe more; when the patient is more relaxed, he or she is likely to breathe less and inhale less drug, while actively eliminating the drug, independent of respiration, renal function, or hepatic function. Thus, we sought to provide the proof of concept that remifentanil is capable of inducing reversible and safe analgesia via inhalation.

METHODS

Animals

All the studies described in this report were approved by the Institutional Animal Use and Care Committee at the University of Utah. All animals were housed 2 per cage (rats) or 5 per cage (mice) in a fully staffed and Association for Assessment and Accreditation of Laboratory Animal Care-approved vivarium. The vivarium was maintained at 22°C to 26°C with a relative humidity of 40% to 50% under 12-/12-hour light/dark cycles. Animals were provided water and standard laboratory chow ad libitum. Dose-response, onset of action, and pharmacokinetic studies were performed with male Sprague–Dawley rats weighing between 200 and 300 g. Pulmonary mechanics measurements were performed with 6-week-old male C57Bl/6 mice weighing 19 to 23 g and a FlexiVent FX-1 instrument (Scireq, Montreal, Quebec, Canada).

Drugs and Reagents

Acetonitrile, methanol, formic acid, sodium thiopental, CalEx II decalcification solution, *n*-butyl chloride, and formaldehyde were purchased from Fisher Scientific (Fair Lawn, NJ). Ethanol was purchased from Decon Labs (Baltimore, MD). Ketamine/xylazine, methacholine, and meperidine were purchased from Sigma-Aldrich (St. Louis, MO). Remifentanil (Ultiva) was purchased from Mylan Inc (Canonsburg, PA). Vecuronium was purchased from Sun Pharmaceuticals (Mumbai, Maharashtra, India).

Inhalation Chamber

A whole-body small animal inhalation chamber was constructed essentially as described by Schroeder et al.,[18] with the following modifications: a 6.5-quart Hefty® bin was fitted with a low-volume MicroMist Nebulizer (Hudson RCI, Morrisville, NC). The forced air nebulizer was attached at one end, with air flowing through the vaporizer into the chamber. A vacuum system was not incorporated. A small hole was made at the opposite end of the bin to allow for pressure balance and access to the rat's tail for real-time analgesic testing during the exposure. Rats were restrained using a Broome style small rodent restrainer (Plas-labs, Lansing, MI) within the chamber with their noses located proximal to the nebulizer. Varying concentrations (100–2000 μg/mL) of remifentanil were diluted in 0.9% saline and administered through the nebulizer with filtered air as the carrier.

Analgesia Testing

Analgesia was assessed using an IITC Tail Flick Analgesia Meter, model 336G (IITC Life Science, Woodland Hills, CA). This is an established outcome measure to evaluate analgesia in rodents.[19] A 4 × 6 mm heat source generated a tail stimulus. A built-in sensor on the tail groove detected time to tail flick, with 0.01-second accuracy. Tails were tested 2 cm from the tip using 50% light intensity and a preprogrammed cutoff time of 20 seconds to prevent tissue damage/surface burn injury.

Dose–Response Study

Fifty-three rats (n = 5–11/group) were exposed to increasing concentrations of remifentanil aerosols or 0.9% saline (control) for 5 minutes. Analgesia testing was performed by the use of tail flick after 5 minutes of exposure to inhaled remifentanil. Time to tail flick in drug-exposed groups was compared with time to tail flick in pretest baseline (naive) and inhaled saline control groups. Each rat was tested once. Aerosols were generated from 0.9% saline and solutions containing remifentanil at concentrations of 0, 100, 250, 500, 750, 1000, and 2000 μg/mL. Concentrations of remifentanil were increased until the cutoff was achieved; specifically, a lack of tail flick within 20 seconds of heat exposure; 2000 μg/mL was tested first, as researchers were unsure of the concentration required to produce analgesia via inhalation in rats. Because of profound effect, the dose was dramatically lowered to 100 μg/mL and increased incrementally until 5 of 5 rats showed a time to tail flick of 20 seconds. A Student t test was performed to identify doses that elicited a statistically significant difference in the time to tail flick compared with the pretest baseline (naive) and inhaled saline control groups. Additional details are provided below in the Statistical Analysis section.

Onset of Action and Recovery Study

To measure the kinetics of analgesia, 50 rats (n = 4–9/group) were exposed to an aerosol from a 1000 μg/mL solution of remifentanil, which was the concentration that caused the maximal measurable level of analgesia in 5 minutes in the dose–response study. Rats were exposed for up to 5 minutes, and then the chamber was vented and opened to allow the rats to recover. Animals were tested for tail flick response at the following time points during (0, 1, 2, 3, 4, and 5 minutes) and following 5-minute exposure (6, 7, 8, 9, and 10 minutes), demonstrating both onset of and recovery from analgesia. Each rat was tested once.

Measurement of Remifentanil in Blood

Two milliliters of blood was collected per rat after decapitation at the following time points: 0, 1, 3, 5 (during exposure), 8, and 20 minutes (postexposure) after whole-body chamber exposure to 1000 μg/mL aerosolized remifentanil. Time points for blood collection were predetermined using a D-optimal sampling strategy (Supplemental Digital Content 1, http://links.lww.com/AA/B382). The blood was collected into 6 mL of *n*-butyl chloride to stop esterase-mediated degradation of remifentanil.[20] The organic fraction containing remifentanil was separated and dried, and the residues were stored at –80°C until analysis. A validated quantitative liquid chromatography-tandem mass spectroscopy assay was used to measure the amount of remifentanil in rat blood. The analysis was performed on a TSQ Quantum AM liquid chromatography-tandem mass spectroscopy

instrument using an XBridge C_8 50 × 2.1 column eluted at room temperature with 15% acetonitrile: 85% aqueous formic acid (0.1% v/v) at 0.25 mL/min to chromatographically separate the analytes. The quantitative range of this assay was 0.25 to 2500 ng/mL with intra-assay accuracy and precision within 15% of all target concentrations. Here, data are represented as the ratio of analyte to internal standard (meperidine, 50 ng/sample).[21] Absolute quantitative values were deemed unreliable because of the potential of contamination from the fur during the blood collection procedure, as well as the goal of relating blood concentrations to effect, versus establishing specific pharmacokinetic parameters for rats using this specific exposure system.

Safety Assessments

Histopathology

Three rats were exposed to 1000 µg/mL aerosolized remifentanil, a dose-eliciting maximal analgesia, for 5 minutes every other day for a total of 3 exposures, to evaluate the potential adverse effects of remifentanil on respiratory tissue after repeated exposure. Rats were killed by lethal injection of sodium thiopental. The lungs were fixed using 10% neutral-buffered formalin delivered through a tracheal cannula at a constant pressure of 25 cm H_2O for 30 minutes before excision, followed by fixation for an additional 5 days. Skulls were dissected and fixed in CalEx II fixative/decalcifier for 14 days to allow for sectioning and assessment of the nasal turbinates. Before processing, the tissues were placed in 70% ethanol. Serial sections of 5 µm were prepared and stained with eosin and hematoxylin by the University of Utah Research Histology core. Nasal turbinates were evaluated at 3 levels: level 1, immediately posterior to the upper incisor teeth; level 2, at the first and second palatal ridge; and level 3, at the first upper molar teeth, according to the National Institute of Environmental Health Sciences standards for histologic analysis of rat nasal turbinates. A board-certified veterinary pathologist evaluated all samples.

Pulmonary Mechanics

Twenty mice (n = 5/group) were used to assess pulmonary function after acute and repeated exposure to aerosolized remifentanil using a FlexiVent FX -1 small animal ventilator (Scireq). Forced expiratory maneuvers using FlexiVent often are used to assess airway responsiveness in mice.[22–26] Specifically, changes in lung resistance, airway resistance, tissue resistance, lung compliance, lung elastance, and tissue elastance were determined by the use of a constant-phase model that has been extensively and successfully used to assess lung mechanics in mice.[27,28] Mice were anesthetized with intraperitoneal ketamine/xylazine (50/8 mg/kg), tracheotomized with an 18-g metal cannula, and connected to the FlexiVent. Mouse default ventilation pattern was initiated (rate 150/min, tidal volume 10 mL/kg, 30 cm H_2O max, 3 cm H_2O positive end-expiratory pressure). Mice were then paralyzed with vecuronium (0.5 mg/kg, intraperitoneally). An electrocardiogram was monitored continuously to ensure proper anesthesia and viability throughout the procedures. Body temperature was monitored constantly and maintained by the use of a heat lamp. Normalization of lung volumes was then performed by initiating a large amplitude perturbation (deep inflation), as described previously.[25,26] After normalization, baseline measurements were assessed using broadband low-frequency forced oscillations. Pulmonary mechanics were then evaluated after exposure to an aerosol using an Aeroneb vaporizer (Aerogen Ltd, Galway, Ireland) that creates an aerosol of 2.5 to 4.0 µm particle volume mean diameter. This small particle size facilitates deep airway deposition. Lung mechanics were tested on each mouse 6 times. Control mice were exposed to aerosols of 0.9% saline for 5 treatments followed by a methacholine challenge of 25 mg/mL. Methacholine is a synthetic, nonselective, muscarinic receptor agonist that is used widely to evaluate for airway hyperresponsiveness. For remifentanil exposure, the aforementioned procedure was performed with 1 dose of saline followed by 4 treatments of increasing solution concentrations of remifentanil (25, 50, 100, and 200 µg/mL in saline), followed by a methacholine challenge (25 mg/mL), providing an assessment of any irritating acute effects of remifentanil. For repeated exposure, the mice were exposed to 1000 µg/mL aerosolized remifentanil or saline for 5 minutes every other day for 3 treatments via the whole-body exposure chamber; 1000 µg/mL was chosen, as it elicited the maximal measurable level of analgesia in the dose-response study and was used for the histopathology studies. Forty-eight hours after the third exposure, pulmonary mechanics measurements were performed as described previously for the acute exposures. Hereafter, these 4 exposures over 7 days will be referred to as "subacute administration."

Statistical Analysis

The experiments featured in this study were powered to achieve 80% power with 1-way analysis of variance and 2-sample t tests. The Shapiro-Wilk test was used to assess the normality of the data and/or the residuals before performing any statistical comparisons. Data are expressed as medians (interquartile range), and comparisons between groups at a single point in time were performed using the t test or the nonparametric Mann-Whitney U test, as appropriate.

For the dose–response and the onset of action tests, we expected a mean time to tail flick of 3 seconds in the control group and a mean of 20 seconds in the high-dose remifentanil groups with an SD of 4 seconds expected for each group. This yielded >99% and 87% power to detect a statistically significant difference using the Student t test for the dose–response and onset of action tests, respectively. The acute pulmonary mechanics experiments were performed using a 1-way analysis of variance with a mean lung elastance of 30 cm H_2O/mL expected in the saline control group. Expected lung elastances in the remifentanil group ranged from 30 to 45 cm H_2O/mL across the dose range. The SD was assumed to be 20 cm H_2O/mL for all groups. These effect size estimates yielded >99% power to detect a difference in lung elastance as a function of varying inhaled remifentanil doses for both the acute and the repeated exposure experiments. For all comparisons, $P < 0.001$ was considered to be statistically significant. All statistical comparisons were 2-sided. R 3.1.1 (R Foundation for Statistical Computing, Vienna, Austria) and GraphPad Prism (La Jolla, CA) were used to perform the power calculations and statistical analyses.

RESULTS

Dose-Response Studies

Rats exposed to increasing concentrations of remifentanil aerosol exhibited increasing depth of analgesia, as indicated by increased time to tail flick with significant analgesia measured at 5 minutes with doses ≥750 μg/mL compared with the pretest baseline (naive) ($P < 0.0001$) and inhaled saline ($P = 0.0002$) groups. The maximal measurable level of analgesia was achieved after 5 minutes using a 1000 μg/mL solution of remifentanil, as measured by time to tail flick ($P < 0.0001$), and no further increase in analgesia was detectable using 2000 μg/mL (Fig. 1). At greater doses (1000 and 2000 μg/mL), rats appeared sedated; the rats were not moving or grooming for 2 to 3 minutes after removal from the restrainer. However, there was no loss of consciousness (loss of righting reflex) or adverse events noted regardless of dose. Even the rats exposed to the highest concentration were visually normal/fully recovered within 2 to 3 minutes of cessation of remifentanil delivery.

Onset of Action and Recovery

Studies evaluating the time of onset of analgesia were performed using the 1000 μg/mL solution concentration. The onset of action for aerosolized remifentanil was rapid, reaching maximal measurable effect approximately 2 minutes after the initiation of exposure. This maximal measurable analgesic effect was maintained until cessation of administration at 5 minutes. After the 5-minute exposure to inhaled remifentanil, recovery was rapid and complete. Recovery was visually apparent at 3-minute postadministration, with animals moving around and engaging in grooming behaviors. In addition, baseline sensitivity to a painful stimulus was observed within 3 minutes of cessation of remifentanil delivery. Analgesic effect was significantly different than baseline (time 0) at time points 2 to 7 minutes ($P < 0.0001$). Time points 1 and 8 to 10 were not significantly different than baseline (Fig. 2).

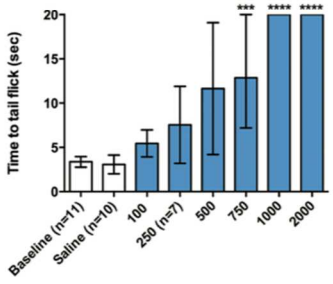

Figure 1. Analgesic response to increasing concentrations of inhaled remifentanil for 5 minutes as measured by time to tail flick. Maximum test duration 20 seconds. Baseline = pretest baseline. Saline = inhaled saline control. ***Significant difference from pretest baseline ($P < 0.0001$) and inhaled saline ($P = 0.0002$). ****Significant difference from baseline and saline control ($P < 0.0001$); 2000 μg/mL was the dose tested first, followed by 100, 250, 500, 750, then 1000 μg/mL. Dose tested was stopped at 1000 μg/mL when 5 of 5 rats had 20-second time to tail flick (maximum test duration). $n = 5$/group unless otherwise noted.

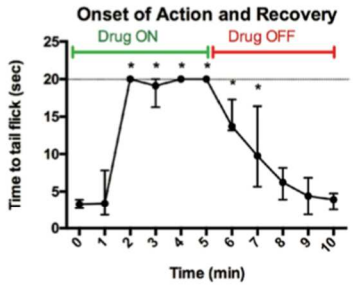

Figure 2. Onset of action and recovery times after exposure to 1000 μg/mL inhaled remifentanil via whole-body chamber for 5 minutes. *Significant difference from time 0, $P \leq 0.0001$. —Maximum time to tail flick = 20 seconds, $n = 4$/group unless otherwise noted.

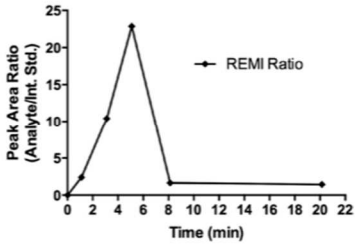

Figure 3. Liquid chromatography-tandem mass spectroscopy (LC/MS²) chromatographic peak ratio of remifentanil to internal standard in rat blood after pulmonary exposure for 5 minutes.

Remifentanil in Blood

Remifentanil was measured in blood, and its concentration increased over time with continuous inhalation exposure. At 5 minutes, exposure was discontinued and blood concentrations of remifentanil (Fig. 3) rapidly decreased to baseline levels within 3 minutes, essentially mirroring the analgesic effects (Fig. 2).

Safety Assessment

Pulmonary Mechanics

Acute intratracheal remifentanil exposure up to 200 μg/mL in mice did not significantly alter any of the pulmonary mechanics measurements compared with saline exposure. Lung parameter response to methacholine challenge in acute remifentanil-exposed mice was also no different than saline-exposed mice (lung elastance and other parameters shown, Supplemental Digital Content 2, Supplemental Figure 4A, http://links.lww.com/AA/B383). Animals subacutely exposed to inhaled remifentanil did not show any alterations in pulmonary mechanics. However, after exposure to inhaled remifentanil, mice subacutely exposed to a methacholine challenge showed a significantly diminished change in lung resistance ($P < 0.0001$), airway resistance ($P = 0.0001$), tissue resistance ($P < 0.0001$), and lung elastance ($P = 0.0013$) compared with animals subacutely exposed to inhaled saline, while lung compliance and tissue elastance were unchanged (lung elastance, Fig. 4; Supplemental Digital

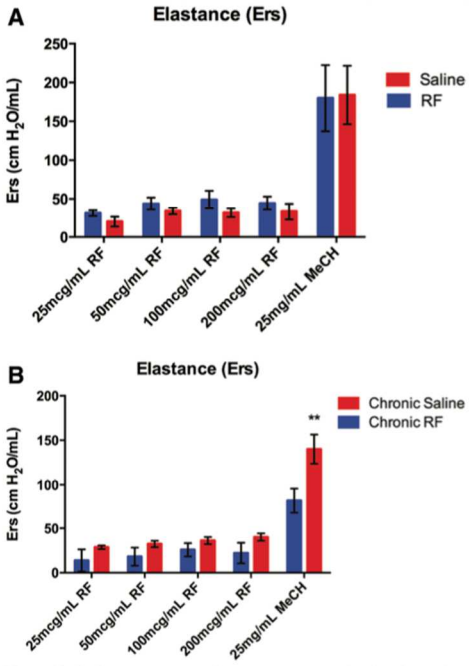

Figure 4. A, Acute exposure. Dose-response evaluating lung elastance (Ers) of C57Bl/6 mice to inhaled remifentanil (RF) compared with mice exposed to inhaled vehicle (saline), followed by methacholine challenge ($n = 5$). B, Repeated exposure. Dose-response evaluating Ers of C57Bl/6 mice to inhaled RF compared with mice exposed to inhaled vehicle, followed by methacholine challenge ($n = 5$). Two-way analysis of variance with Bonferroni post hoc analysis. $^{**}P = 0.0007$.

Content 2, Supplemental Figure 4B, http://links.lww.com/AA/B383). On the basis of these data, it was concluded that remifentanil aerosols did not cause lung irritation, bronchospasm, or other adverse pulmonary effects. Furthermore, a potential benefit of decreased irritant-induced bronchoconstriction was observed with repeated administration of inhaled remifentanil, evidenced by a decrease in methacholine-induced changes in lung resistance, lung elastance, airway resistance, and tissue damping.

Toxicity and Histopathology

Rats subacutely exposed to inhaled remifentanil had no obvious difference in behavior, eating habits, or weight gain compared with unexposed rats. Histopathologic examinations of rat lung and nasal turbinates by a veterinary pathologist revealed no evidence of inflammatory changes or tissue damage with subacute exposure to remifentanil aerosol. (Fig. 5A–F shows representative images in 4×–40×. More images are available.)

DISCUSSION

To our knowledge, this is the first study to evaluate the safety and efficacy of remifentanil inhalation. To that end, this study was designed to be a pilot proof of concept study to show feasibility and safety before progressing to greater fidelity pharmacokinetic/pharmacodynamic studies. Therefore, we used rodent models, because these animals are relatively easy to study but still allow pharmacodynamics to be crudely but effectively elucidated. There also are established models in mice to study pulmonary mechanics after pulmonary drug administration. Our results show that inhaled remifentanil is rapidly absorbed, pharmacologically active, rapidly cleared, and noninjurious.

In this initial study, the chamber design did not mitigate dosing variables, such as quantification of inhaled uptake of drug, rodent positioning in the chamber relative to aerosol introduction, and minute ventilation changes associated with the test procedures or drug effect. Although the actual inhaled dose of the drug by the rats was unknown, however, the dose/response study did show that rats exposed to ≥750 μg/mL concentration of remifentanil aerosol exhibited statistically significant analgesia in response to the tail flick test. Given the aforementioned deficits, it is predicted that the required concentration would be much lower for facemask or direct tracheal delivery because of the large amount of drug that is not inhaled when using a whole-body exposure chamber.

The onset of action from inhaled remifentanil occurred within 2 minutes, and recovery occurred within 3 minutes after cessation of drug administration. In addition, blood concentrations of remifentanil mirrored the analgesic effects, including a rapid spike after pulmonary exposure and a rapid decrease to baseline after cessation. Remifentanil's rapid onset of action suggests that, inhaled, it could be used to produce rapid analgesia to facilitate short-term clinical procedures, such as establishing IV access in pediatric or mentally delayed patients, whereas rapid drug clearance would limit the potential for airway compromise after drug administration.

Pulmonary mechanics were measured with a FlexiVent to assess acute pulmonary changes in mice. Subacute pathology also was assessed via histologic evaluation of pulmonary tissues in subacutely exposed rats. Significantly lower doses of inhaled remifentanil were used compared with the dose–response study in rats, as mice were used for this experiment, and direct delivery into the trachea via the breathing circuit of the mechanical ventilator rather than a whole-body exposure chamber. It is suspected that doses achieved in mice were comparable with or greater than those achieved in the whole-body exposures using rats. There was no difference between pulmonary mechanics measurements when we compared acute remifentanil with acute saline or when comparing subacute remifentanil and subacute saline. However, although it has not been reported by others using the FlexiVent system, it is possible that the use of ketamine as an anesthetic during these studies may have concealed subtle changes in pulmonary mechanics associated with remifentanil, although significant irritant-like responses occurred after stimulation by a moderate dose of methacholine (25 mg/mL) in mice. In addition, rat tissues were histologically normal.

From these data, we concluded that remifentanil inhalation was nonirritating and noninjurious, even after repeated exposure in both rats and mice. This study agrees

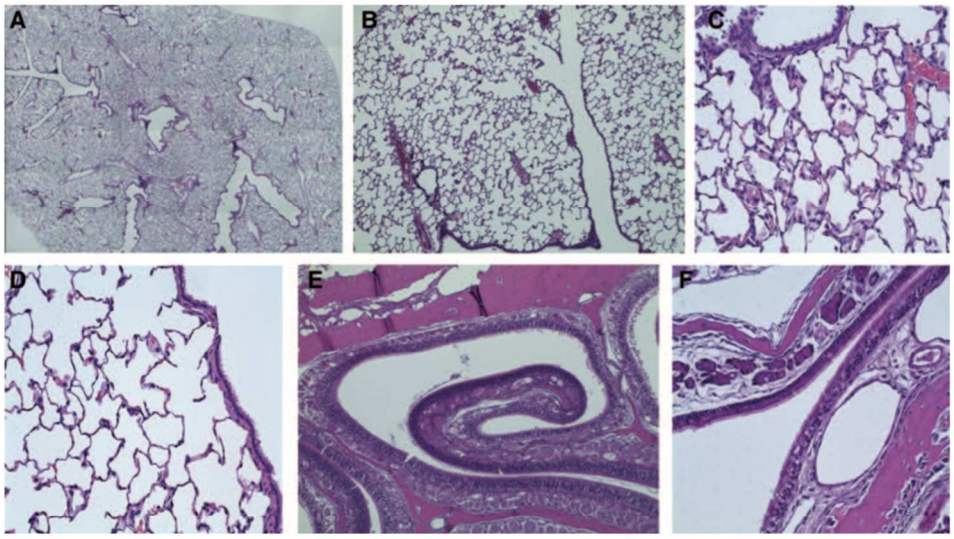

Figure 5. A, Right cranial lobe of male Sprague-Dawley rats after subacute exposure to inhaled remifentanil (4× magnification). B, 10× magnification of right cranial lobe lung tissue. Normal transition from respiratory bronchiole to alveoli without evidence of inflammation or tissue. Damage (10× of A). C and D, 40× magnification of right cranial lobe lung tissue (40× of A). E, Level III nasal turbinates of male Sprague-Dawley rat after subacute exposure to inhaled remifentanil (10× magnification). F, 40× magnification of level III nasal turbinates (40× of E).

with previous studies on inhaled morphine and fentanyl, which also showed no toxicity.[5] Regardless, future studies will test inhaled remifentanil in more suitable large animal models, as well as humans, including an assessment of the safety of longer-term and chronically administered inhaled remifentanil.

Repeated administration of inhaled remifentanil may attenuate respiratory hyperresponsiveness. After subacute exposure to inhaled remifentanil, resistance (airway, lung, and tissue) and elastance were less affected by exogenous methacholine challenge compared with subacute saline exposure. Previous studies have investigated the inhaled morphine and fentanyl for relief of dyspnea related to pulmonary disease.[5–10] One theory is that opioids mitigate dyspnea by reducing cholinergic responses secondary to the inhibitory action of opioid agonists on the release of acetylcholine in the airway.[6,9,10,29] Our findings on remifentanil are consistent with previous reports of a diminished cholinergic response after subacute exposure to inhaled remifentanil. Inhibition of acetylcholine release reduces acetylcholine-induced airway smooth muscle contraction and also reduces acetylcholine-induced increases in mucus secretions. This not only supports our hypothesis that inhaled remifentanil is nonirritating but also suggests that there may be benefit. Again, the safety of chronic dosing will also need to be evaluated.

This study does not exactly mimic potential human exposure scenarios that are envisioned for inhaled remifentanil; however, it does provide conclusive evidence that remifentanil can be delivered via inhalation to produce profound analgesia and limited sedation. Limitations to the current study include the use of a whole-body exposure chamber in which large amounts of drug were wasted and in which determining exact doses was not possible. We attempted to overcome this limitation by tracheal administration of aerosolized drug; however, this required delivery of the drug to an anesthetized animal, which prevented the assessment of remifentanil pharmacodynamics. In addition, the translatability of animal pain models and pharmacokinetics are inherently limited because of the relative perception of pain and large interspecies variability in airway and lung structures, aerosol deposition, and blood concentrations after pulmonary exposure. This study also is limited because of physiological volume limitations in drawing serial blood samples for pharmacokinetic analysis in mice and rats. A large animal study or human study would be required for a comprehensive population pharmacokinetic and pharmacodynamic study with clinically generalizable results. In addition, further studies are needed to fully determine the pharmacokinetic/pharmacodynamics relationship in humans because here there was an artificially induced plateau of analgesic effect (20 seconds) at concentrations of remifentanil aerosol of 1000 μg/mL. In general, increasing concentrations of remifentanil in the aerosols increased analgesia (Fig. 1), but because of the safety cutoff in this assay, it is unclear whether the level of analgesia would also continue to increase. We suspect the answer is no, given the profound analgesia and sedation observed under these experimental conditions at the highest doses. Pain testing beyond the 20-second cutoff in our initial studies caused burn injury, suggesting that the pain stimulus at 20 seconds was likely substantial and that the depth of analgesia had reached a near physiological maximum, regardless of the safety cutoff.

Future studies to develop inhaled remifentanil for human use also will need to address the delivery vehicle and its inherent fluid mechanics. First, unlike volatile-inhaled anesthetics, nebulized remifentanil is not an ideal gas. Therefore, gas laws such as partial pressure will not govern its uptake and distribution, as is the case for traditional inhaled anesthetics. Second, issues associated with laminar and turbulent flow may also affect drug uptake. And third, basic respiratory parameters such as spontaneous and controlled ventilation may complicate pharmacokinetic and pharmacodynamic assessments using inhaled remifentanil.

Finally, known side effects of opioid administration in humans also will need to be assessed. Bradycardia, nausea, and chest rigidity are of concern, particularly in patients who do not have IV access. It may also be possible to alter future formulations to include racemic epinephrine or concurrent inhaled benzodiazepine administration to minimize these risks.

CONCLUSIONS

The evidence described here supports our hypothesis that inhaled remifentanil would produce rapid onset of analgesia, followed by rapid recovery, while being nonirritating and noninjurious to the airways and lungs of rodents. To further elucidate the translatability of inhaled remifentanil, pharmacokinetic, pharmacodynamic, and safety studies will need to be performed in humans.

DISCLOSURES

Name: Tatjana Bevans, CRNA, MSN.
Contribution: This research is part of this author's PhD dissertation. This author was involved in study design, data collection, data analysis, and manuscript preparation.
Attestation: Tatjana Bevans is first author and wrote and approves the final manuscript. Tatjana attests to the integrity of the original data and analysis reported in this manuscript. Tatjana is the archival author.
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Attestation: Christopher Reilly approves the final manuscript. Dr. Reilly attests to the integrity of the original data and analysis reported in this manuscript.
Name: Derek J. Sakata, MD.
Contribution: This author contributed to study design and manuscript preparation.
Attestation: Derek J. Sakata approves the final manuscript.
This manuscript was handled by: Markus W. Hollmann, MD, PhD, DEAA.

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CHAPTER 3

INHALED REMIFENTANIL AND REMIMAZOLAM IN RODENTS

Abstract

Remimazolam is an ester-based short acting benzodiazepine in clinical trials. This study explored the feasibility of delivering remimazolam as an adjunct to remifentanil via inhalation. Rats were exposed to remimazolam and remifentanil aerosol alone and in combination. Analgesia was quantified by using a tail flick meter and pulmonary injury was assessed using mechanics measurements. Exposure of rats to inhaled remimazolam alone failed to produce sedation or analgesia following a 5-minute exposure. Rats exposed to both remimazolam and remifentanil exhibited a significant increase in analgesia as compared to the same doses of either agent alone. Remimazolam delivered alone or in combination with remifentanil was nonirritating to the respiratory tract of mice. Inhaled Remimazolam can significantly potentiate the analgesic effect of inhaled remifentanil when concurrently delivered.

Introduction

Remimazolam is an ester-based benzodiazepine in phase III clinical trials. Much like the opioid remifentanil, remimazolam is rapidly cleared by plasma esterases to a relatively inactive metabolite (CNS 7054). Like other benzodiazepines, remimazolam is a

positive allosteric modulator on the $GABA_A$ receptor. However, remimazolam differs from other benzodiazepines based on its ester-based pharmacophore, which allows for rapid metabolism independent of hepatic or renal function.[1-3]

Inhaled midazolam has been investigated for seizure protection in mice,[4] but the efficacy of inhaled remimazolam is unknown. Previous research on inhaled remifentanil has shown rapid onset of analgesia and rapid recovery, while being noninjurious and nonirritating in rodents.[5] Benzodiazepines are frequently used to augment opioid effect during anesthesia. The objective of this study was to demonstrate that inhaled remimazolam in conjunction with inhaled remifentanil would potentiate analgesia versus remifentanil alone,[5] while being nonirritating to the lungs.

Methods

Animals

All described studies were approved by the Institutional Animal Use and Care Committee (IACUC) at the University of Utah. Mice were housed 5/cage and rats were housed 2/cage in an AAALAC-approved vivariam maintained at 22-26℃ with relative humidity 40-50% under 12/12-h light/dark cycles. Water and standard lab chow was provided *ad libitum.* Time to tail flick study was performed using male Sprague-Dawley rats weighing between 200-300g. Pulmonary mechanics measurements were performed using 8-week-old male C57Bl/6 mice weighing 19-25 grams and a Flexivent FX-1 instrument (Scireq, Montreal, Qc, Canada).

Drugs and Reagents

Remimazolam was graciously provided by PAION pharmaceuticals (Aachen, Germany). Remifentanil was purchased from Mylan Inc. (Canonsburg, PA, USA). Ketamine/xylaxine and methacholine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dimethyl sulfoxide (DMSO) was purchased from Fisher Scientific (Pittsburgh, PA, USA). Vecuronium was purchased from Sun Pharmaceuticals (Mumbai, Maharashtra, India).

Inhalation Chamber

The whole body inhalation chamber used was as previously described in Bevans *et al*.,[5] which was adapted from a small animal inhalation chamber described by Schroderer *et al.* (RF 18). Essentially, a 6.5-quart Hefty® bin was fitted with a low-volume Micromist nebulizer (Hudson RCI, Morrisville, NC) and an Aerogen Lab ultrasonic nebulizer (Aerogen Ltd., Galway, Ireland). The Micromist nebulizer produced a particle size of approximately 2.7 micrometers through a forced air nebulizer and was used to nebulize remifentanil. The aerogen nebulizer produces 2.5-4.0 micrometer volume mean diameter aerosolized particles through a vibrating palladium mesh which vibrates 128,000 times per second and was used to nebulize remimazolam.

Analgesic Testing

Analgesia was assessed as described previously.[5] Breifly, analgesia was measured using a IITC Tail Flick Analgesia Meter (model 336G, IITC Life Science, Woodland Hills, CA). A 4 x 6 mm heat source generated a tail stimulus, and tail movement away from the stimulus was measured by a built-in sensor, with 0.01-second accuracy. Tails

were tested 2 cm from the tip using 50% light intensity and a preprogrammed cut-off time of 20 seconds to prevent tissue damage or suface burn injury to the rat.

Time to Tail Flick Study

This study of 25 rats was performed in addition to the dose response study of 53 rats already performed using inhaled remifentanil.[5] Time to tail flick in drug-exposed groups was compared to time to tail flick in pretest baseline and inhaled saline control groups. For this study, remimazolam was tested at 10 and 25 mg/mL, and in combination with remifentanil 100 mcg/mL or 250 mcg/mL.

Pulmonary Mechanics

30 mice (n=5/group) were used to assess pulmonary function following acute aerosolized remimazolam exposure and acute and repeated exposure to combined inhaled remimazolam and remifentnail using a *Flexi-Vent* FX-1 small animal ventilator (Scireq, Montreal, Qc, Canada). Specifically measured were changes in lung resistance (Rrs), airway resistance (Rn), tissue resistance (G), lung compliance (Crs), lung elastance (Ers) and tissue elastance (H). These were determined using a constant-phase model which has been extensively used to assess lung mechanics in mice.[6,7] Methods were also as previously described in Bevans *et al*.[5]

For acute remimazolam exposure, control mice were exposed to vehicle (10% DMSO/ 90% normal saline) for five treatments followed by a methacholine challenge of 25 mg/mL. Treatment mice were exposed to one dose of vehicle followed by four treatments of increasing concentrations of remimazolam (5, 10, 15, 20 mg/mL), followed by a methacholine challenge (25 mg/mL). For combination exposures, mice were

exposed to vehicle control, followed by four treatments of 200 mcg/mL remifentanil combined with 20 mg/mL remimazolam, followed by a methacholine challenge (25 mg/mL). These mice were compared to mice exposed 5 times to vehicle followed by methacholine. For repeated sub-acute exposure, mice were exposed to a combination of 250 mcg/mL remifentanil and 20 mg/mL remimazolam every other day for 3 treatments via the whole body exposure chamber. Forty-eight hours following the third exposure, pulmonary mechanics were measured as above.

Statistical Analysis

Statistical analysis was performed as in Bevans *et al*.[5] The experiments featured in this study were powered to achieve 80% power with one-way ANOVA and two-sample t-tests. The Shapiro-Wilk test was used to assess the normality of the data and/or the residuals prior to performing any statistical comparisons. Data are expressed as medians (interquartile range [IQR]) and comparisons between groups at a single point in time were performed using the t test or the nonparametric Mann-Whitney U test, as appropriate.

The time to tail flick test was performed using a student's t test. The acute pulmonary mechanics experiments were performed using a one-way ANOVA. For all comparisons, $P<0.001$ was considered to be statistically significant. All statistical comparisons were two-sided. R 3.1.1 (R Foundation for Statistical Computing, Vienna, Austria) and Graphpad Prism (La Jolla, CA, USA) were used to perform the power calculations and statistical analyses.

Results

Time to Tail Flick

Inhalation of remimazolam alone failed to produce analgesia. Concentrations >25 mg/mL could not be tested due to lack of solubility in a reasonable vehicle. When remimazolam (10 or 25 mg/mL) was administered in combination with 250 mcg/mL remifentanil there was a significant difference in time to tail flick (P<0.0001), comparable to analgesia achieved using 1000 mcg/mL remifentanil alone (P<0.0001). See Figure 3.1.

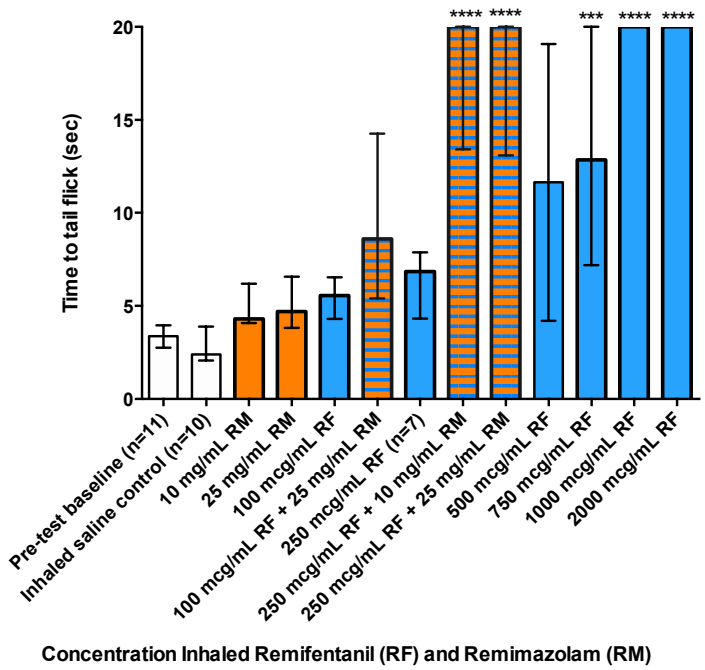


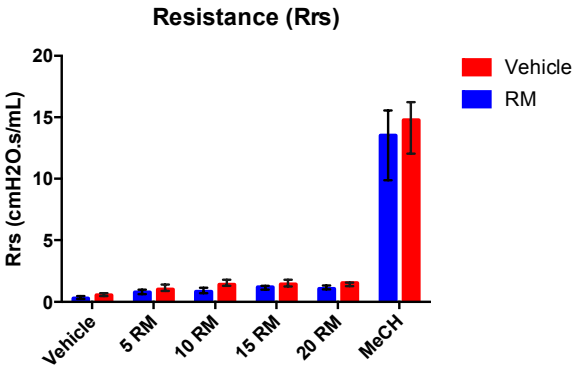
Figure 3.1. Analgesic response to increasing concentrations of inhaled remifentanil and/or remimazolam for 5 minutes as measured by time to tail flick. Maximum test duration 20 seconds.
***Significant difference from pretest baseline (P<0.0001) and inhaled saline (P=0.0002)
****Indicates significant difference from baseline and saline control (P<00001)
n=5/group unless otherwise noted. Shown as mean with interquartile range

Pulmonary Mechanics

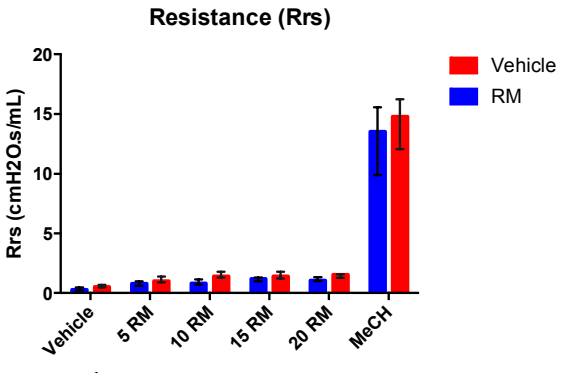
Acute inhalation delivery of remimazolam up to 20 mg/mL did not alter the pulmonary mechanics of mice (Figure 3.2). Likewise, mice acutely (Figure 3.3) or sub-acutely (Figure 3.4) exposed to a combination of remifentanil and remimazolam showed no alterations to pulmonary mechanics, except when comparing the methacholine challenge for airway resistance, where sub-acutely exposed mice showed diminished changes in lung resistance compared to vehicle exposed mice ($P<0.0007$). These data show that remimazolam alone or in combination with remifentanil does not cause lung irritation, bronchospasm, or other adverse pulmonary events. As previously reported,[5] the decrease in lung resistance is attributable to remifentanil.

Discussion

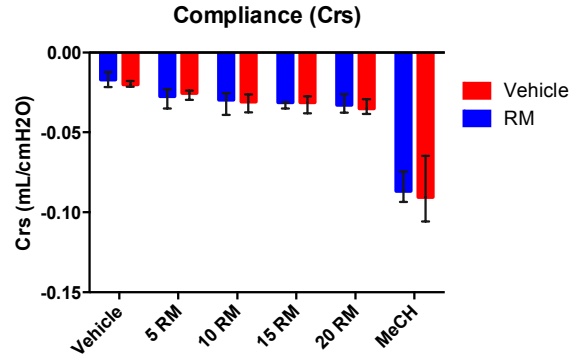
This study advances previous research on inhaled remifentanil,[5] which concluded that inhaled remifentanil was rapidly absorbed, pharmacologically active, rapidly cleared, and noninjurious. These additional experiments show that remimazolam, when administered in conjunction with remifentanil, has a synergistic affect on analgesia while also sharing the desired pharmacokinetic, pharmacodynamic, and safety profile of ester-based, short acting agents. Although it would be ideal to test higher concentrations of inhaled remimazolam alone, this was not possible due to limitations in solubility. As such, the clinical utility of remimazolam as a single inhaled sedative is low compared to remifentanil, but its use as a potentiating agent for inhaled remifentanil is a realistic possibility for application where brief, but deep analgesia is required.



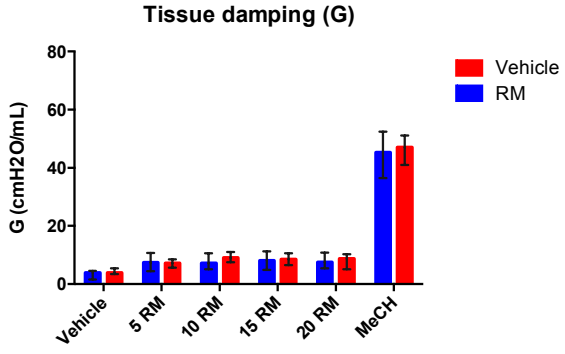
Dose response of lung resistance of C57Bl/6 mice acutely exposed to increasing concentrations of inhaled remimazolam (mg/mL RM) as compared to inhaled vehicle (10% DMSO/ 90% saline) exposure, followed by 25 mg/mL methacholine (MeCH) challenge. n=5



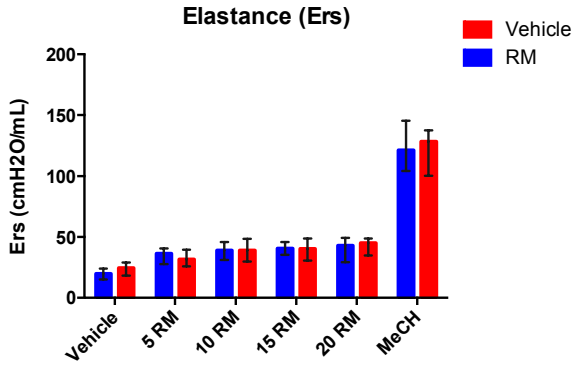
Dose response of airway resistance (Rn) of C57Bl/6 mice acutely exposed to increasing concentrations of inhaled remimazolam (mg/mL RM) as compared to inhaled vehicle (10% DMSO/ 90% saline) exposure, followed by 25 mg/mL methacholine (MeCH) challenge. n=5



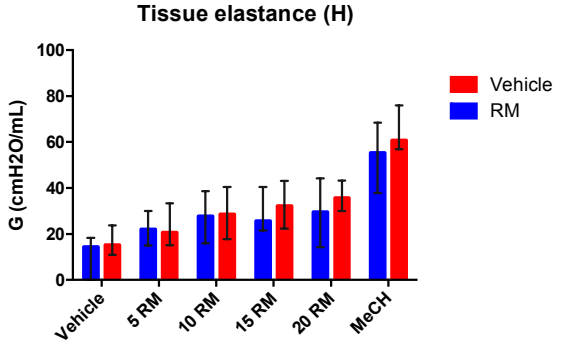
Dose response of lung compliance of C57Bl/6 mice acutely exposed to increasing concentrations of inhaled remimazolam (mg/mL RM) as compared to inhaled vehicle (10% DMSO/ 90% saline) exposure, followed by 25 mg/mL methacholine (MeCH) challenge. n=5



Dose response of tissue damping or resistance (G) of C57Bl/6 mice acutely exposed to increasing concentrations of inhaled remimazolam (mg/mL RM) as compared to inhaled vehicle (10% DMSO/ 90% saline) exposure, followed by 25 mg/mL methacholine (MeCH) challenge. n=5

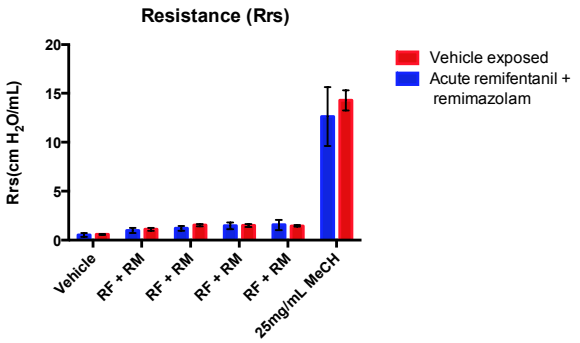


Dose response of lung elastance of C57Bl/6 mice acutely exposed to increasing concentrations of inhaled remimazolam (mg/mL RM) as compared to inhaled vehicle (10% DMSO/ 90% saline) exposure, followed by 25 mg/mL methacholine (MeCH) challenge. n=5

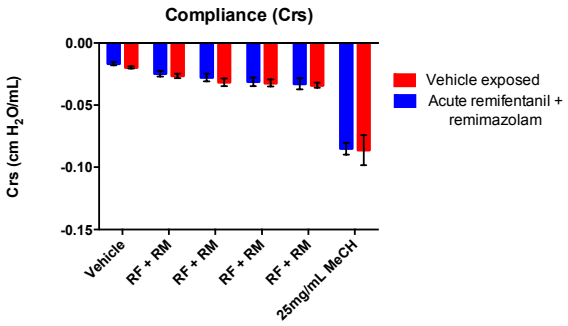


Dose response of tissue elastance of C57Bl/6 mice acutely exposed to increasing concentrations of inhaled remimazolam (mg/mL RM) as compared to inhaled vehicle (10% DMSO/ 90% saline) exposure, followed by 25 mg/mL methacholine (MeCH) challenge. n=5

Figure.3.2. Pulmonary mechanic measurements after exposure to increasing concentrations of inhaled remimazolam followed by methacholine challenge.



Lung resistance of C57Bl/6 mice repeatedly exposed to 200 mcg/mL remifentanil (RF) combined with 20 mg/mL remimazolam (RM) by inhalation as compared to inhaled vehicle (10% DMSO/ 90% saline) exposure, followed by methacholine (MeCH) challenge. n=5



Lung compliance (Crs) of C57Bl/6 mice repeatedly exposed to 200 mcg/mL remifentanil (RF) combined with 20 mg/mL remimazolam (RM) by inhalation as compared to inhaled vehicle (10% DMSO/ 90% saline) exposure, followed by methacholine (MeCH) challenge. n=5

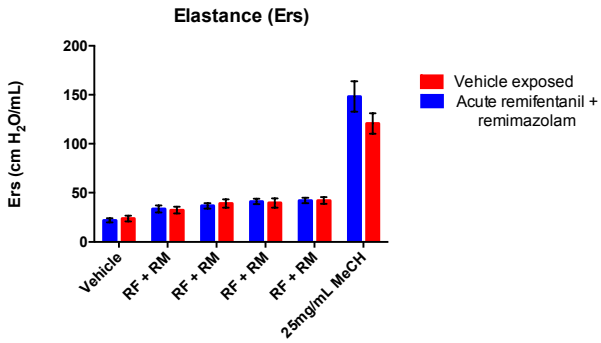
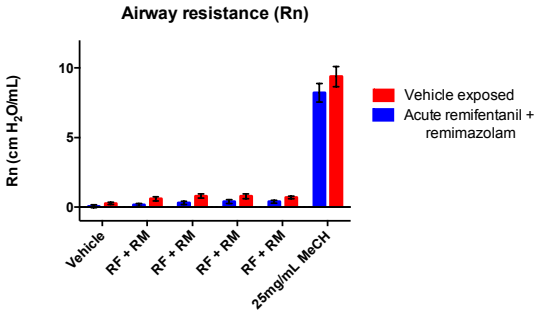
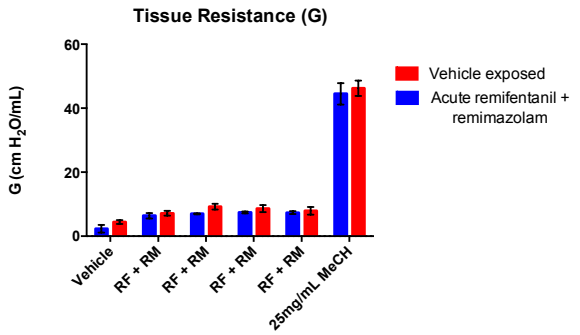


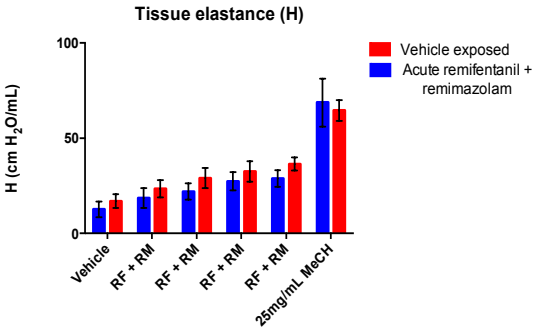
Figure 4A: Acute exposure. Lung elastance (Ers) of C57Bl/6 mice repeatedly exposed to 200 mcg/mL remifentanil (RF) combined with 20 mg/mL remimazolam (RM) by inhalation as compared to inhaled vehicle (10% DMSO/ 90% saline) exposure, followed by methacholine (MeCH) challenge. n=5



Airway resistance (Rn) of C57Bl/6 mice repeatedly exposed to 200 mcg/mL remifentanil (RF) combined with 20 mg/mL remimazolam (RM) by inhalation as compared to inhaled vehicle (10% DMSO/ 90% saline) exposure, followed by methacholine (MeCH) challenge. n=5

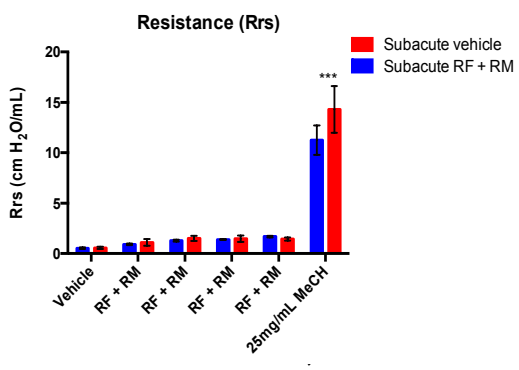


Tissue damping or resistance (G) of C57Bl/6 mice repeatedly exposed to 200 mcg/mL remifentanil (RF) combined with 20 mg/mL remimazolam (RM) by inhalation as compared to inhaled vehicle (10% DMSO/ 90% saline) exposure, followed by methacholine (MeCH) challenge. n=5

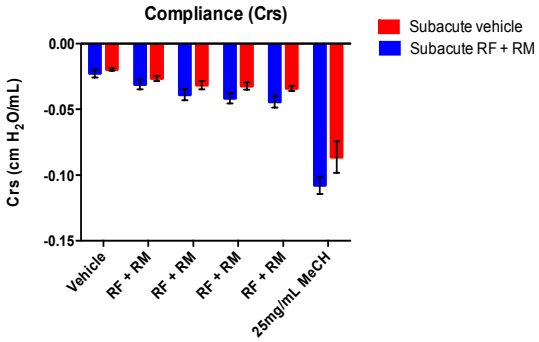


Tissue elastance (H) of C57Bl/6 mice repeatedly exposed to 200 mcg/mL remifentanil (RF) combined with 20 mg/mL remimazolam (RM) by inhalation as compared to inhaled vehicle (10% DMSO/ 90% saline) exposure, followed by methacholine (MeCH) challenge. n=5

Figure 3.3. Pulmonary mechanics measurements after acute exposure to a combination of inhaled remimazolam and remifentanil followed by methacholine challenge



Lung resistance (Rrs) of C57Bl/6 mice after repeated pulmonary exposure to 200 mcg/mL remifentanil (RF) and 20 mg/mL remimazolam (RM) with repeat prior exposure to inhaled RF and RM as compared to mice with repeated exposure to inhaled vehicle, followed by methacholine challenge. n=5, ***=P<0.0007



Lung compliance (Crs) of C57Bl/6 mice after repeated pulmonary exposure to 200 mcg/mL remifentanil (RF) and 20 mg/mL remimazolam (RM) with repeat prior exposure to inhaled RF and RM as compared to mice with repeated exposure to inhaled vehicle, followed by methacholine challenge. n=5

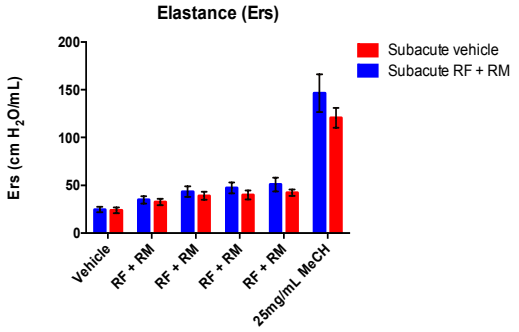
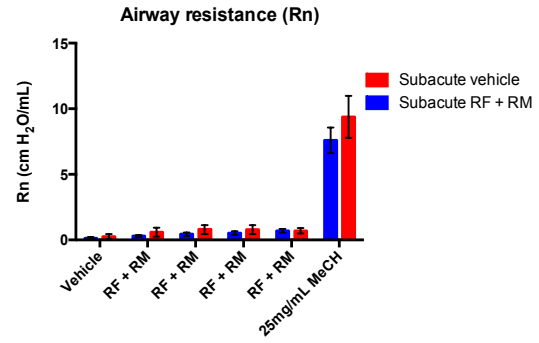
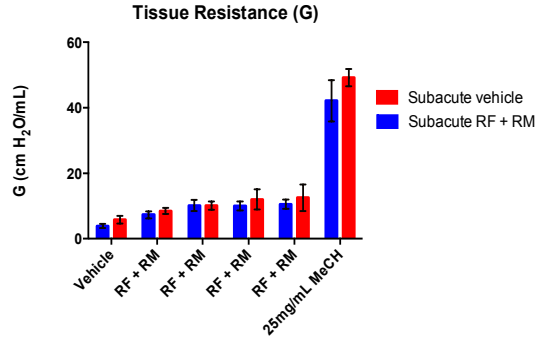


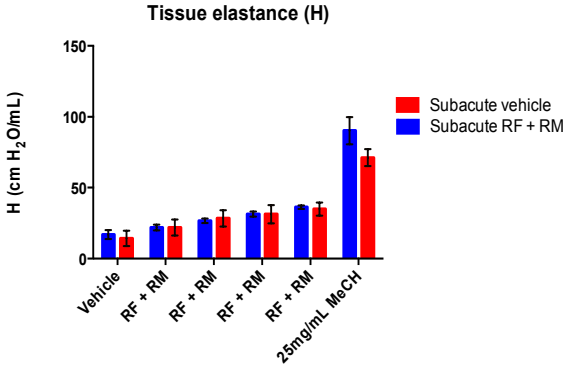
Figure 4B: Subacute exposure. Lung elastance (Ers) of C57Bl/6 mice after repeated pulmonary exposure to 200 mcg/mL remifentanil (RF) and 20 mg/mL remimazolam (RM) with repeat prior exposure to inhaled RF and RM as compared to mice with repeated exposure to inhaled vehicle, followed by methacholine challenge. n=5



Airway resistance (Rn) of C57Bl/6 mice after repeated pulmonary exposure to 200 mcg/mL remifentanil (RF) and 20 mg/mL remimazolam (RM) with repeat prior exposure to inhaled RF and RM as compared to mice with repeated exposure to inhaled vehicle, followed by methacholine challenge. n=5



Tissue damping or resistance (G) of C57Bl/6 mice after repeated pulmonary exposure to 200 mcg/mL remifentanil (RF) and 20 mg/mL remimazolam (RM) with repeat prior exposure to inhaled RF and RM as compared to mice with repeated exposure to inhaled vehicle, followed by methacholine challenge. n=5



Tissue elastance (H) of C57Bl/6 mice after repeated pulmonary exposure to 200 mcg/mL remifentanil (RF) and 20 mg/mL remimazolam (RM) with repeat prior exposure to inhaled RF and RM as compared to mice with repeated exposure to inhaled vehicle, followed by methacholine challenge. n=5

Figure 3.4. Pulmonary mechanics measurements following exposure to sub-acute combination of inhaled remimazolam and remifentanil followed by methacholine challenge.

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CHAPTER 4

PHARMACOKINETICS OF INHALED REMINFENTANIL IN A PORCINE MODEL

Abstract

Remifentanil is an ester-based μ-opioid receptor agonist. Little is known about the pharmacokinetics of inhaled remifentanil. The object of this study was to study the population pharmacokinetics of inhaled remifentanil in an intubated porcine model. A sensitive and specific liquid chromatography-tandem mass spectrometry (LC-MS-MS) method for the determination of remifentanil was developed and validated. Arterial blood samples of 1 mL were collected at various time points from an anesthetized intubated pig following administration of 100 μg/kg inhaled remifentanil. Population pharmacokinetic modeling was performed using a nonparametric adaptive grid (NPAG) using a one-compartment model (NONMEM, ICON plc, Dublin, Ireland). The population pharmacokinetic model using a single compartment model show a plasma clearance rate (CL) of 0.41 ± 0.18 with the volume of the central compartment being 0.03 ± 0.02. The predicted model adequately reflects the observed model (R^2=0.884)

Introduction

Remifentanil is a potent μ-opioid receptor agonist FDA approved for intravenous use in humans. A major benefit of remifentanil is its ester-based pharmacophore, making

it susceptible to ester hydrolysis by blood and tissue esterases allowing for an ultra-short duration of action. This rapid metabolism is relatively independent of infusion duration, and/or hepatic/renal function.[1,2] The carboxylic acid metabolite (GI-90291) and the N-dealkylated metabolite (GI-94219) have less than 1% of the potency of the parent compound and are thereby considered essentially inactive[1] (Figure 4.1).

The pharmacokinetic properties of inhaled remifentanil have not been previously described in humans or large animals. The aim of this study was to characterize the pharmacokinetics of inhaled remifentanil in anesthetized, intubated pigs, using frequent blood sampling and computer-assisted pharmacokinetic modeling techniques. The intent

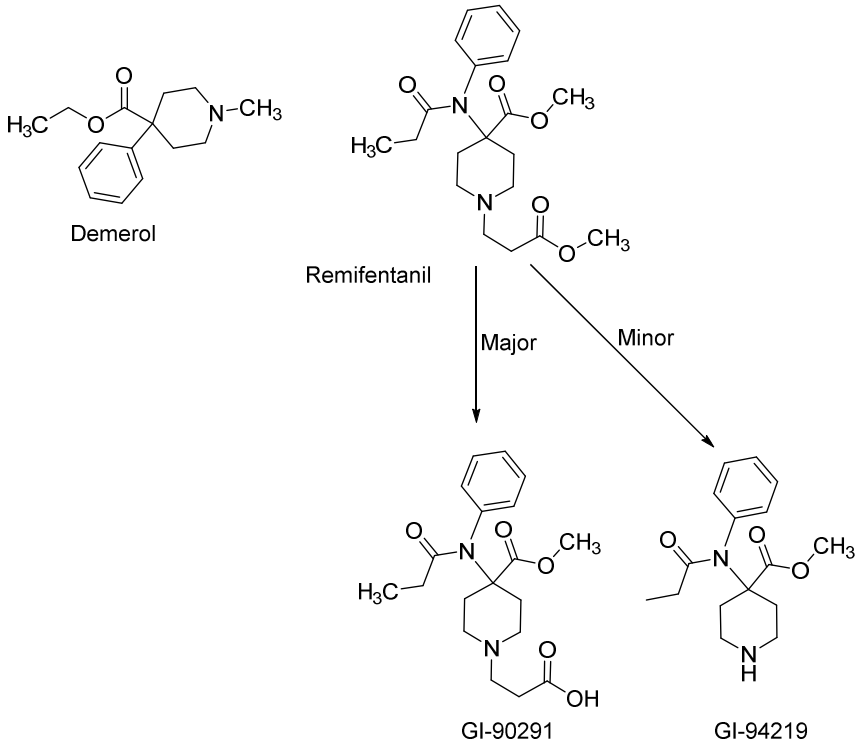

Figure 4.1. Chemical structure of remifentanil and major de-esterified metabolite (GI-90291) and minor P450 metabolite (GI-94219) and meperidine (IS).

was to further pharmacokinetic data available on inhaled remifentanil, as earlier research has shown inhaled remifentanil to have rapid onset and metabolism with marked efficacy in a rodent model.[3] To increase translatability to humans, this study utilized a porcine model, as pigs have breathing volumes and rates similar to humans due to a cardiopulmonary system that is similar to humans. Also, previous pharmacokinetic (PK) data on inhaled remifentanil in rodents were limited by blood volume preventing serial blood draws.[3] The larger blood volume of pigs allows for multiple serial blood draws from the same animal to assay circulating remifentanil concentrations.

Our hypothesis was that remifentanil would be rapidly absorbed and rapidly cleared following pulmonary administration to intubated pigs. We expect that inhaled remifentanil will have similar pharmacokinetic properties to intravenous remifentanil, although it is expected that an increased intrapulmonary dose will be required to produce comparable blood levels due to dead space in the anesthesia circuit, and loss of drug to rainout and exhalation compared to the amount of lung reaching distal airways. A nebulizer producing a small particle size of 2.5-4 microns was used to help facilitate deep lung deposition to maximize absorption. This research will develop a sensitive and selective method for the detection of remifentanil in blood using LC-MS-MS. This method was used for the pharmacokinetic analysis of inhaled remifentanil in intubated pigs.

Methods/Materials

Animals

The Institutional Animal Use and Care Committee (IACUC) at the University of Utah approved this study. Duroc pigs were purchased from Innovative Livestock

Solutions (Great Bend, KS, USA). Pigs weighing 26-32kg were housed in pairs in a fully staffed and AAALAC-approved vivarium. The vivarium was maintained at 20-26°C with a relative humidity of 40-50% under 12/12-h light/dark cycles. Pigs were provided water and standard pig chow; food was deprived for 8 hours preprocedure.

Drug and Reagents

Remifentanil was purchased from Mylan Inc (Canonsburg, PA, USA). Meperidine (IS) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Formic acid, acetonitrile (ACN) and methanol were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Ethyl Acetate was purchased from VWR International (Radnor, PA, USA). Propofol and fentanyl were purchased from Pfizer (NY, NY, USA). Bovine blood was purchased from Sierra Medical (Whittier, CA, USA).

Instrumentation

LC-MS-MS measurements were performed using a Thermo Finnigan TSQ Quantum AM (Thermo Scientific, Waltham, MA, USA). Separation of remifentanil and IS was achieved chromatographically using an Xbridge C8 (50 mm x 2.1 mm) HPLC column (Waters, Milford, MA, USA). The column was equilibrated with a flow rate of 0.25 mL/min with a mobile phase consisting of 10% ACN, 90% aqueous formic acid. Upon sample injection, the concentration of ACN was increased to 25% over 5 minutes. This held at 25% ACN, 75% formic acid for 0.5 minutes then ACN decreased to 10% for the final 5.5 minutes. The autosampler was maintained at room temperature and the injection volume was 20 μL.

Calibrator and Quality Control Solution Preparation

Stock solutions of remifentanil were prepared in saline at a concentration of 1000 ng/µL. Stock solutions of meperidine (IS) were also prepared at a concentration of 1000 ng/µL. Intermediate solutions of remifentanil were prepared at concentrations of 100, 10, 1, 0.1, and 0.01 ng/µL by performing serial dilutions. Intermediate solutions of IS were prepared by serial dilution to concentrations of 100, 10, and 2 ng/µL. All stocks and intermediate solutions were stored in glass tubes at -20°C for the duration of the study. Working solutions were prepared immediately prior to use.

Calibrator and quality control (QC) solutions were prepared from intermediate solutions by aliquoting 1 mL of bovine blood into 13 x 100 mm screw top glass culture tubes (Fisher Scientific, Fair Lawn, NJ, USA) fortified with 50 ng/mL IS (25 µL of 2 ng/µL IS). A total of 12 bovine blood samples were used for calibration curves with a final concentration of 0, 0.1, 0.25, 0.5, 1, 2.5, 5, 10, 25, 50, 75, and 100 ng/mL with a total of 50 ng/mL IS added. Liquid-liquid extraction was performed by adding 4 mL of ethyl acetate to each sample, vigorously shaking, then vortexing for 10 seconds. Samples were then centrifuged in a Beckman GPR centrifuge with a 20.4 cm rotor (GH 3.7) at 3,500 rpm for 10 minutes. The bottom organic supernatant was transferred into a new glass tube, and dried under a constant flow of air. The dried residue was reconstituted in 50 µL of 20%ACN/80% H_2O. Reconstituted samples were transferred to the autosampler for analysis. QC samples were prepared in the same manner in the following concentrations: 75, 50, 10, and 1 ng/mL.

Accuracy and Precision

Both intra- and interassay accuracy and precision were determined. The percentage of the expected and calculated analyte concentration using the mean (n=5) was used to determine the accuracy in a single batch of samples at concentrations of 75, 50, 10, and 1 ng/mL. Intra-assay precision was expressed as percent relative standard deviation (%RSD) and was determined by using the standard deviation of the actual measured concentration at each concentration divided by the mean assayed concentration. Interassay precision was determined by dividing the standard deviation of the assay concentration (75, 50, 10, and 1 ng/mL) by the concentration of the mean concentration for three separate batches (n=20 for each).

Recovery

Recovery of remifentanil from blood was determined using quality control samples at 2.5 and 75 ng/mL (n=5) and compared to unextracted samples. Recovery for remifentanil was calculated to be between 76 and 89%

Stability

Using quality control samples, the effect of various storage conditions on stability were tested. Autosampler stability of samples at 75, 50, 10, and 1 ng/mL (n=5) and stored at room temperature in the auto sampler overnight were re-run against freshly prepared calibrators and controls. There was no degradation of internal standard under these conditions. Concentrations of 75 and 2.5 ng/mL were stored for 1 hour in blood at room temperature on the bench top prior to extraction, and were compared to samples immediately extracted. Samples were analyzed as described. Stability was computed by

comparing the mean assayed concentration with the mean concentration of untreated QC samples.

Animal Exposure and PK Sample Collection

Each pig was sedated by intramuscular injection of a mixture of 4.4 mg/mL Telazol® (tiletamine), 2.2 mg/kg xylazine, and 2.2 mg/kg ketamine. Once adequate sedation was achieved, two separate 18g intravenous catheters were placed in the ears. IV sedation was maintained with a propofol infusion (4-5 mg/kg/hr) and fentanyl infusion (100 mcg/kg/hr). A solution of normal saline was infused at 50 ml/hr. Ventilation was maintained with an 8.0 endotracheal tube and volume-control ventilation with 2L flow of 100% oxygen, tidal volume of 15 ml/kg, and respiratory rate of 12/min. A 20-g arterial line was accessed through the femoral artery for purposes of blood sampling and continuous blood pressure monitoring. Pigs were also continuously monitored by electrocardiograph, blood pressure, and pulse oximetry to assess adequacy of anesthetic depth and ensure adequate ventilation.

Remifentanil was administered through a 2.5-4.0 μm volume median diameter Aerogen nebulizer unit (Aerogen Ltd. Galway, Ireland) fitted into the inspiratory arm of the breathing circuit (Figure 4.2). 100 μg/kg remifentanil reconstituted in normal saline to a final volume to 2.5 mL was administered over approximately 10 minutes. Blood samples of 1 mL each were collected at 0, 1, 2.5, 5, 7.5, and 9 minutes during drug delivery and at 10, 11, 12.5, 15, 20, 25, 40, 55, 70, and 130 minutes after drug delivery and processed as below.

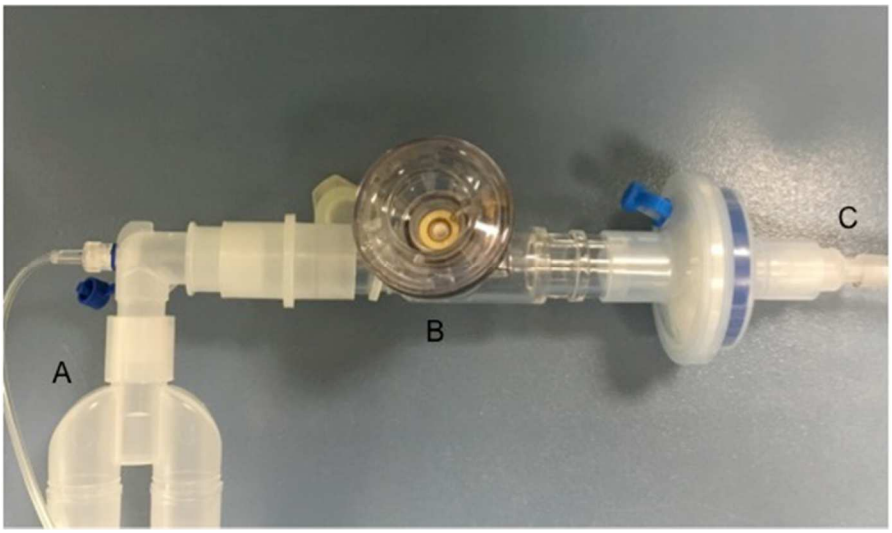

Figure 4.2. Vaporizer set-up in the anesthesia circuit:
A. Anesthesia circuit B. Aergoen vaporizer
C. Endotracheal tube

PK Sample Preparation

Fresh pig aliquots of 1mL were mixed in Fisherbrand 13 x 100 mm screw top glass culture tubes prefilled with 4 mL ethyl acetate fortified with 50 ng/mL IS (meperidine), shaken vigorously, and kept on ice. Following collection, samples were vortex mixed for 10 seconds, and centrifuged (3,500 rpm for 10 minutes); the organic solvent was transferred to a clean tube, and evaporated to dryness. Samples were stored at -70°C until analysis. To test ex-vivo stability, 1 mL bovine blood was spiked with 75 and 2.5 ng/mL remifentanil and left at room temperature for 1 hour.

Results

Liquid Chromatography-Tandem Mass Spectrometry

The analysis of remifentanil in blood exhibited a lower limit of quantification (LLOQ) of 0.25 ng/mL. The upper limit of quantification (ULOQ) was 100 ng/mL, which was arbitrarily determined based on expected concentrations in animals.

Remifentanil was readily detectable with a run time of 11 minutes. The predominant product ion had a mass to charge ratio of 285 (m/z 377.1→285.0) (Figure 4.3). The plot of the ratio of analyte and internal standard peak area versus calibrator concentration was nonlinear over the range of 0.25-100 ng/mL. Calibration curves were fit to a quadratic equation, weighted $1/X^2$, where X was the analyte concentration. Calibration curves generated in this manner exhibited a correlation coefficient (r^2) that was ≥0.98. The accuracy of the assay was ≥90% (n=5). The intra-assay precision was <8% RSD for all analytes at the four quality control concentrations.

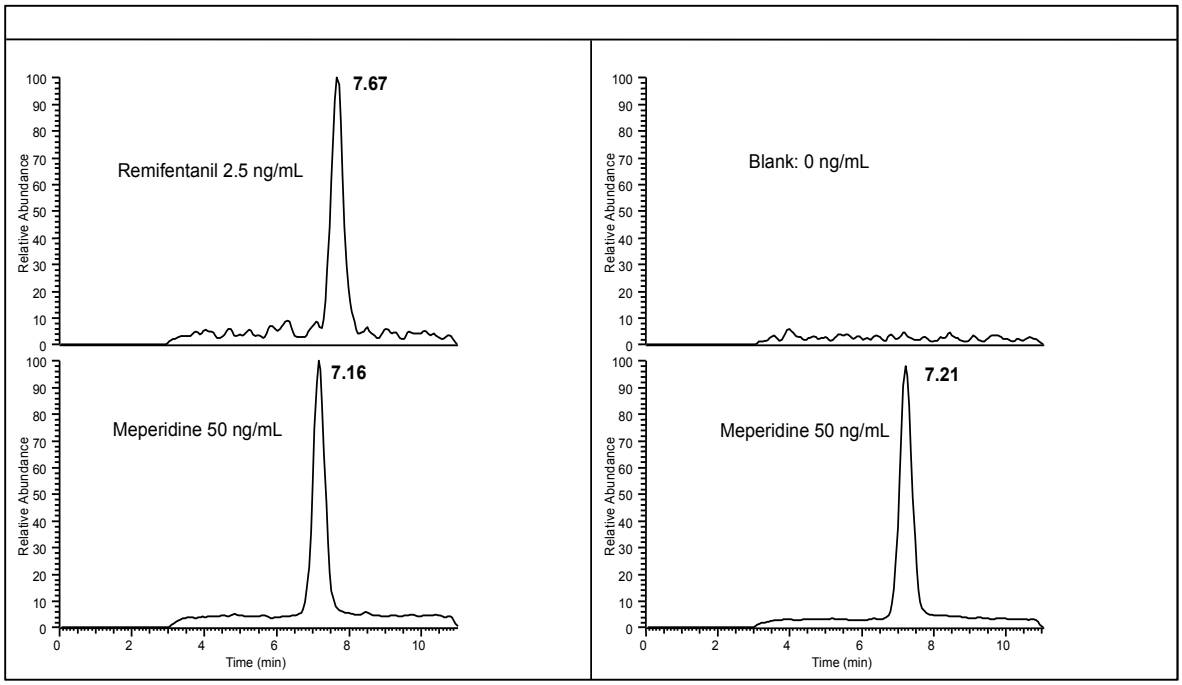

Figure 4.3. Chromatogram of remifentanil (top left) compared to a blank chromatogram (top right) and meperidine (bottom) peaks. Remifentanil peak is at a concentration of 2.5 ng/mL (top left) and 0 ng/mL (top right) and meperidine peak is at 50 ng/mL.

The interassay precision was <15% RSD and the accuracy of the assay was ≤88% for all quality control concentrations (Table 4.1). Recovery of remifentanil from blood was between 76-90% (Table 4.2). Samples were stable (71-100% initial target concentration) after 24 hours in the autosampler at room temperature (20% ACN/80% H_20). Samples left at room temperature on the bench top for 1 hour were also stable at 76-89% of the nonaged samples (Table 4.3).

Table 4.1. Accuracy and Intra- and Interassay Precision (%RSD) for Quality Control Standards Containing Remifentanil

Target Concentration (ng/mL)	Accuracy (% Target)	RSD (%)
Intraassay (n=5)		
1	86	5
10	91	3
50	93	8
75	80	5
Interassay (n=20)		
1	95	14
10	99	12
50	88	10
75	94	15

Table 4.2. Recovery of Remifentanil from Blood

Target Concentration (ng/mL)	Remifentanil % Target
2.5	90
75	76

Table 4.3. Stability of Remifentanil in Standards Stored under Various Conditions

Treatment and Target Concentration (ng/mL)	Remifentanil % Control
24 hr Autosampler at Room Temp	
1	90 ± 14
10	86 ± 14
50	71 ± 16
75	100 ± 12
1 hr on Bench top in Blood	
2.5	88 ± 7
75	84 ± 12

Analysis of Biological Samples

Analysis of blood collected from pigs following exposure to inhaled remifentanil demonstrates rapid uptake and rapid metabolism following cessation of administration (Figure 4.4). Peak concentrations of remifentanil in pig blood ranged from 40-100 ng/mL. Population pharmacokinetic parameters estimate the volume of distribution (V) to be 0.03 ± 0.02 liters (L) with a clearance of 0.41 ± 0.18 L/hr (Table 4.4). Individual pharmacokinetic parameters estimate volume of distribution (V) between 0.01 and 0.07 L and a clearance between 0.23 and 0.66 L/hr, with an estimated half-life between 0.03 and 0.07 hours, or between 1.8 and 4.2 minutes, with an average of 3 minutes (Table 4.5).

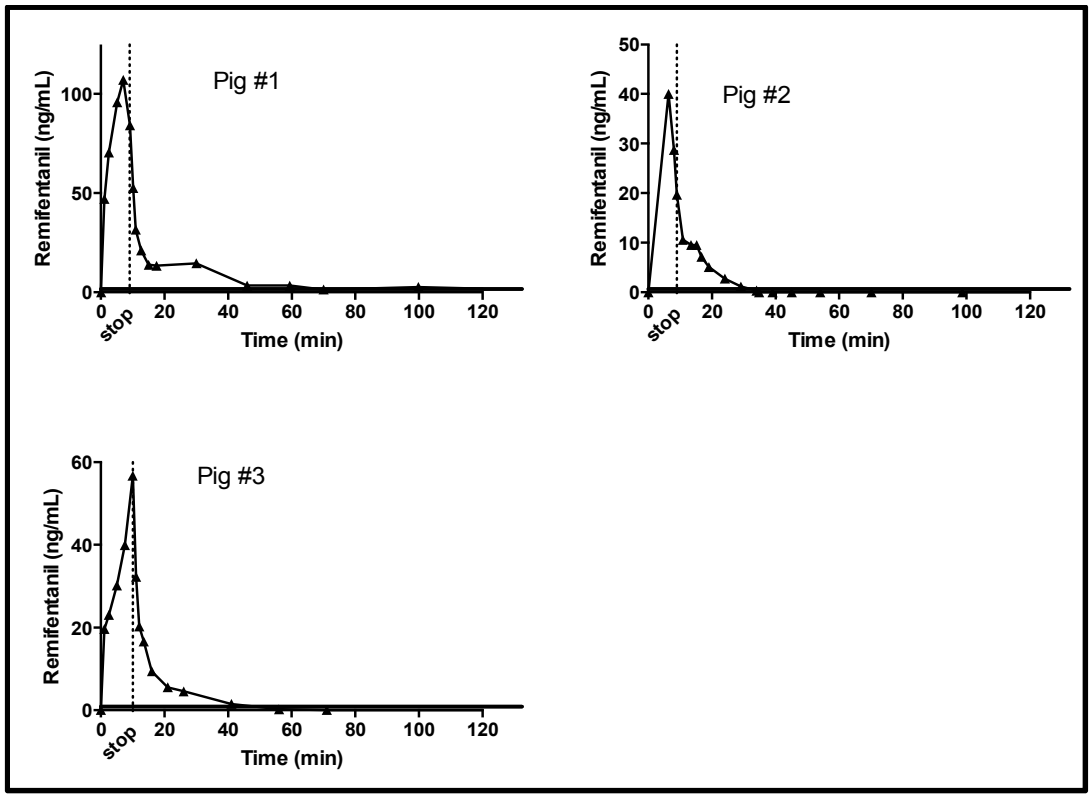

Figure 4.4. LC-MS-MS analysis of concentration of remifentanil in blood following timed blood draws during and after inhalation of remifentanil in pigs

Table 4.4. Population Pharmacokinetic Parameter Estimates of Inhaled Remifentanil in Pigs					
	Mean	SD	CV	Variance	Median
V	0.03	0.02	70.79	0.00	0.03
CL	0.41	0.18	44.61	0.03	0.34

V=plasma volume (L), CL=clearance (L/hr

Table 4.5. Individual Pharmacokinetic Parameters of Inhaled Remifentanil in Pigs				
	V	CL	Ke	Half-life
1	0.01	0.23	22.49	0.03
2	0.07	0.66	9.77	0.07
3	0.02	0.34	13.37	0.05

V=plasma volume (L), CL=clearance (L/hr),
Ke=elimination rate constant, Half-life (hrs)

Discussion

A sensitive and selective method was devfeloped for the detection of remifentanil in blood using LC-MS-MS. Using this method, the assayed concentrations for the fortified quality control samples were 90-114% of the target concentrations for analytes. This method produced very similar values for the quality control samples on separate days, exhibiting an interassay precision of <15% RSD. In general, remifentanil was not significantly affected by different storage and handling conditions. Level of detection is within therapeutic dose range.

Samples from pigs after exposure to inhaled remifentanil show that remifentanil is rapidly absorbed and metabolized. Pharmacokinetic analysis of intravenous remifentanil showed a peak effect within 1-3 minutes.[2] Inhaled remifentanil shows similar onset of action. Observed concentrations were slightly higher overall than predicted concentrations for inhaled remifentanil as the model is shifted somewhat to the left (Figure 4.5). Eighty-eight percent of the variability is explained by this model (R-squared

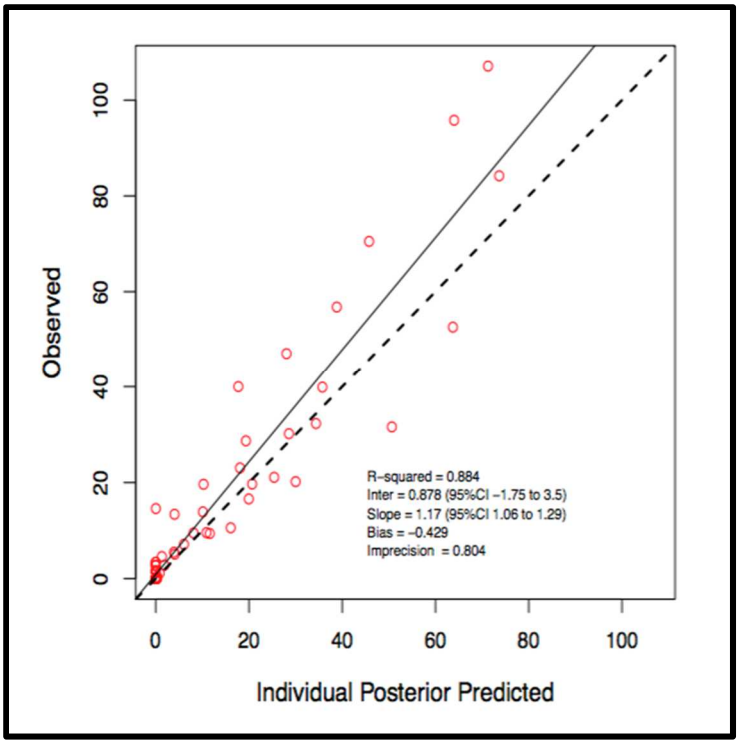

Figure 4.5. Observed (x-axis) versus predicted (y-axis) population pharmacokinetic model for inhaled remifentanil in pigs

= 0.884), despite the small sample size (n=3). Future samples will add power to this model. After a total of 10 pigs, the model will be re-evaluated and assessed for accuracy, at which point more pigs may be added.

The pharmacokinetics of intravenous remifentanil shows a half-life of 3 minutes, with a 10-minute terminal half-life.[1] Our data thus far also show a population half-life of 3 minutes. Additionally, aerosol exposure in one pig over 27 minutes shows the ability to deliver inhaled remifentanil over a longer period of time (Figure 4.6). This relative plateau in remifentanil blood concentration with a longer exposure period is consistent with clinical needs for longer anesthetics, while maintaining remifentanil's rapid elimination profile.

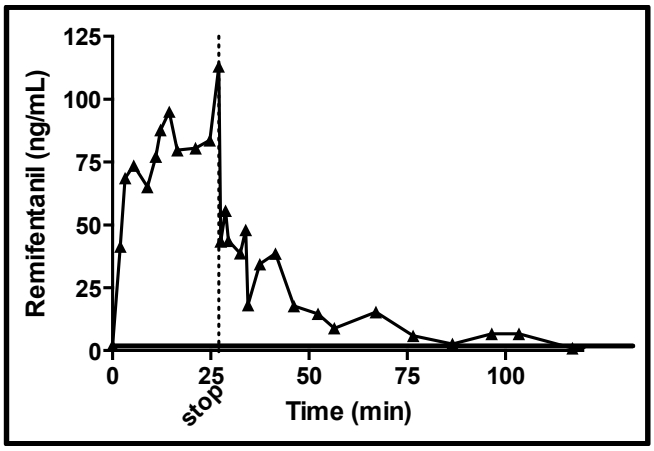

Figure 4.6: Pharmacokinetics of inhaled remifentanil
with a 27-minute exposure period

The administered dose of 100 μg/kg is a high dose, in part due to waste associated with intrapulmonary delivery through an endotracheal tube. This translates to greater drug waste and increased cost. Although Aerogen nebulizers average an impressive 35% lung deposition,[4] total amount of drug required to reach effective dose may be reduced by development of an intermittent nebulizer or reservoir so drug is nebulized only during inhalation.

There is currently a moderate amount of variability between peak drug concentrations detected between subjects. However, this variability is within normal limits, and it is expected that increasing the number of subjects will decrease variability in peak drug concentrations. A large variability in peak concentration would suggest unpredictable drug absorption, making dose estimation difficult.

This study shows that remifentanil delivered by inhalation to intubated pigs is rapidly absorbed and rapidly cleared, consistent with earlier research on inhaled

remifentanil in pigs. A larger sample size will allow for estimation of variability among subjects and further validate our pharmacokinetic model.

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CHAPTER 5

DETERMINATION OF REMIFENTANIL AND REMIMAZOLAM IN BLOOD BY LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY

Abstract

Remifentanil is an ester-based μ-receptor opioid agonist used for analgesia. Remimazolam is an ester-based benzodiazepine, which is an allosteric modulator on the $GABA_A$ receptor and is currently in stage III clinical trials as a new sedative agent. A sensitive and selective liquid-chromatography-tandem mass spectrometry (LC-MS-MS) method for the analysis of remifentanil and remimazolam in blood has been developed. This method uses a one step liquid-liquid extraction. Calibration curves of 0.25-2500 ng/mL of both remifentanil and remimazolam were constructed by plotting concentration versus peak area ratio (analyte/internal standard) and fitting the data with a weighted quadratic equation. The accuracy of the assay ranged from 83-139% for all analytes. The intra-assay precision (%RSD) for remifentanil ranged from 2.3-12.5% and from 1.7-18.8% for remimazolam. The interassay precision (%RSD) for remifentanil ranged from 5.2-9.9% and 8.4-25.1% for remimazolam.

Introduction

Remifentanil is an FDA-approved potent opioid μ agonist frequently used for analgesic purposes in the operative setting. Remimazolam is a new allosteric modulator benzodiazepine on $GABA_A$ receptors that is currently in phase III clinical trials. Both ester-based drugs are rapidly cleared by plasma esterases to metabolites with less than 1% potency of the parent compound, and are thereby considered inactive metabolites. This rapid metabolism is relatively independent of hepatic and renal function and infusion duration,[1-4] allowing for rapid recovery from the analgesic and sedative effects of these drugs. The purpose of this research was to develop a sensitive and selective method for the co-analysis of remifentanil and remimazolam in bovine blood for future evaluation of co-administration of these drugs by inhalation in animals.

Opioids and benzodiazepines are commonly given together for anesthetic purposes during procedures requiring sedation. Prior research has shown that inhaled remimazolam can significantly potentiate the analgesic effect of inhaled remifentanil when concurrently delivered (unpublished). A method for co-analysis of inhaled remifentanil and inhaled remimazolam has not been previously described.

Methods/Materials

Drugs and Reagents

Remifentanil was purchased from Mylan Inc (Canonsburg, PA, USA). Remimazolam was provided by PAION pharmaceuticals (Aachen, Germany). Meperidine was purchased from Sigma-Aldrich (St. Louis, MO, USA). Formic Acid, acetonitrile (ACN), and methanol were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Ethyl Acetate was purchased from VWR International (Radnor, PA, USA).

Bovine blood containing sodium citrate was purchased from Sierra Medical (Whittier, CA, USA).

Instrumentation

LC-MS-MS measurements were performed using a Thermo Finnigan TSQ Quantum AM (Thermo Scientific, Waltham, MA, USA). Separation of remifentanil, remimazolam, and IS was achieved chromatographically using an Xbridge C8 (50 mm x 2.1 mm) HPLC column (Waters, Milford, MA, USA). The column was equilibrated at a flow rate of 0.25 mL/min with a mobile phase consisting of 11% ACN, 89% aqueous formic acid (0.1% v/v). Upon sample injection, the concentration of ACN was increased at a constant rate to 30% over 4 minutes and held there for 0.5 minutes before further increasing to 95% ACN over 0.1 minutes and holding for 0.5 minutes, then reduced back to 11% ACN over 0.1 minutes and holding for 5 minutes. Flow rate remained constant at 0.25 mL/min. The autosampler was maintained at room temperature and the injection volume was 20 µL.

Calibrator and Quality Control Solution Preparation

Stock solutions of remifentanil were prepared in saline at a concentration of 1000 ng/µL. Stock solutions of remimazolam were prepared in a methanol solution at a concentration of 1000 ng/µL. Stock solutions of meperidine (IS) were also prepared at a concentration of 1000 ng/µL. Intermediate solutions of remifentanil and remimazolam were prepared at concentrations of 100, 10, 1, 0.1, and 0.01 ng/µL by performing serial dilutions. Intermediate solutions of IS were prepared by serial dilution to concentrations of 100, 10, and 2 ng/µL. All stocks and intermediate solutions were stored in glass tubes

at -20°C for the duration of the study. Working solutions were prepared immediately prior to use.

Calibrator and quality control (QC) solutions were prepared from intermediate solutions by aliquoting 0.5 mL of bovine blood into 13 x 100 mm screw top glass culture tubes (Fisher Scientific, Fair Lawn, NJ, USA) fortified with 50 ng/mL IS (25 µL of 2 ng/µL IS). A total of 14 bovine samples were used for calibration curves with a final concentration of both remifentanil and remimazolam of 0, 0.25, 0.5, 1, 2.5, 5, 10, 25, 50, 100, 250, 500, 1000, and 2500 ng/mL with a total of 50 ng/mL IS added. Liquid-liquid extraction was performed by adding 3 mL of ethyl acetate to each sample, vigorously shaking, then vortexing for 10 seconds. Samples were then centrifuged in a Beckman GPR centrifuge with a 20.4 cm rotor (GH 3.7) at 3,500 rpm for 10 minutes. The bottom organic supernatant was transferred into a new glass tube, and dried under a constant flow of air. The dried residue was reconstituted in 50 µL of 20%ACN/80% H_2O. Reconstituted samples were transferred to the autosampler for analysis. QC samples of remifentanil and remimazolam were prepared in the same manner in the following concentrations: 500, 50, 10, and 1 ng/mL.

Accuracy and Precision

The accuracy of this assay was determined as the percentage of the target analyte concentration using the mean (n=3) assayed concentration in a single batch of samples. Intra-assay precision was expressed as a percent relative standard deviation (%RSD) and was calculated for each batch using the standard deviation of the assayed concentrations of each analyte at concentrations of 500, 50, 10, and 1 ng/mL divided by the mean assayed concentration (n=3). Interassay precision (%RSD) was determined by dividing

the standard deviation of the assayed concentration (500, 50, 10, and 1 ng/mL, n=12) for three separate replicated batches by the mean concentration (n=12).

Recovery

The recovery of remifentanil and remimazolam from bovine blood was determined using quality control samples (n=3) at 500 and 10 ng/mL. Recovery was assessed by comparing the concentrations obtained for quality control samples processed as described to samples that were extracted and the internal standard added immediately prior to evaporation of solvent. The ratio of the two concentrations represented the percentage of analyte recovered by the extraction.

Stability

The effect of various storage conditions on sample stability was determined for each analyte using quality control samples. Quality control samples (n=3) at 500, 50, 10, and 1 ng/mL were stored at room temperature in the auto sampler overnight, and were re-run against freshly prepared calibrators and controls. There was no degradation of internal standard under these conditions. Concentrations of 500 and 10 ng/mL were stored for 1 hour in blood at room temperature on the bench top prior to extraction, and were compared to samples immediately extracted. Samples were analyzed as described. Stability was assessed by comparing the mean assayed concentration (n=3) for the stability controls to the mean concentration of untreated quality control samples.

Results

LC-MS-MS

The analysis of remifentanil and remimazolam in blood by LC-MS-MS exhibited a lower limit of detection (LLOD) of 0.25 ng/mL and a lower limit of quantification (LLOQ) of 1 ng/mL. The upper limit of quantification (ULOQ) was 2500 ng/mL for both drugs. Remifentanil was readily detectable at 3.26 minutes with a run time of 10.3 minutes. The predominant product ion had a mass to charge ratio of 285 (m/z 377.1→285.0). Remimazolam was readily detectable at 4.81 minutes with the predominant product ion having a mass to charge ratio of 406 (m/z 439.0→406.8). Meperidine (IS) was readily detectable at 3.3 minutes with a predominant product having a mass to charge ratio of 220 (m/z 248.0→220.1) (Figure 5.1 and Figure 5.2).

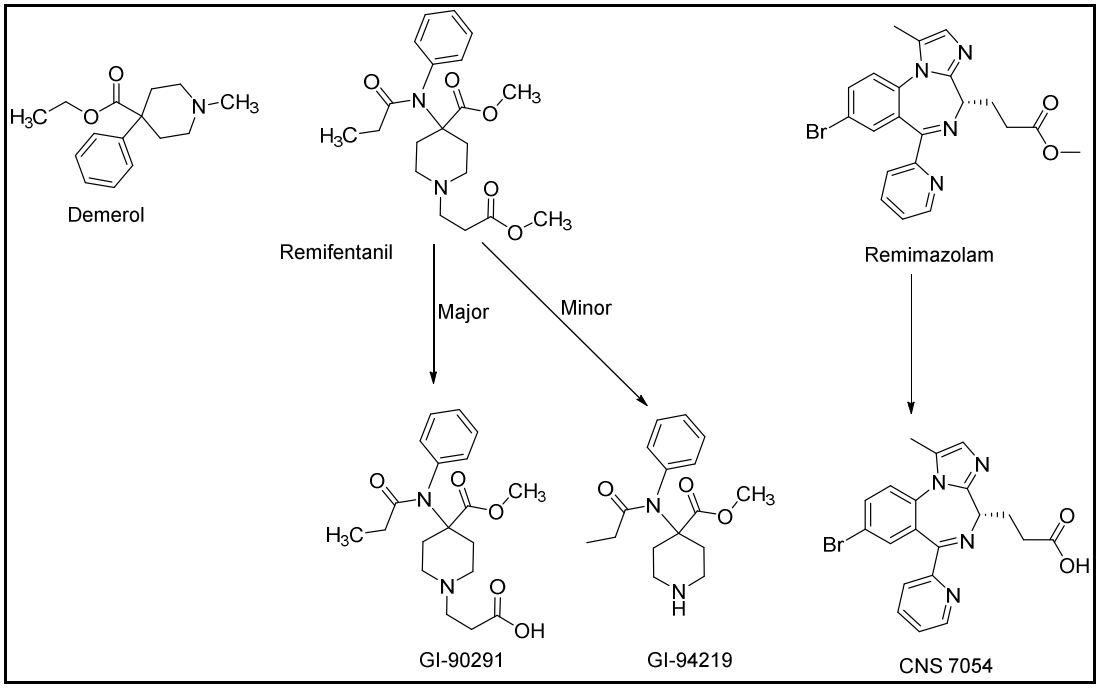

Figure 5.1. Structures of meperidine (IS), remifentanil, and metabolites GI-90291 and GI-94219 and remimazolam and metabolite CNS 7054.

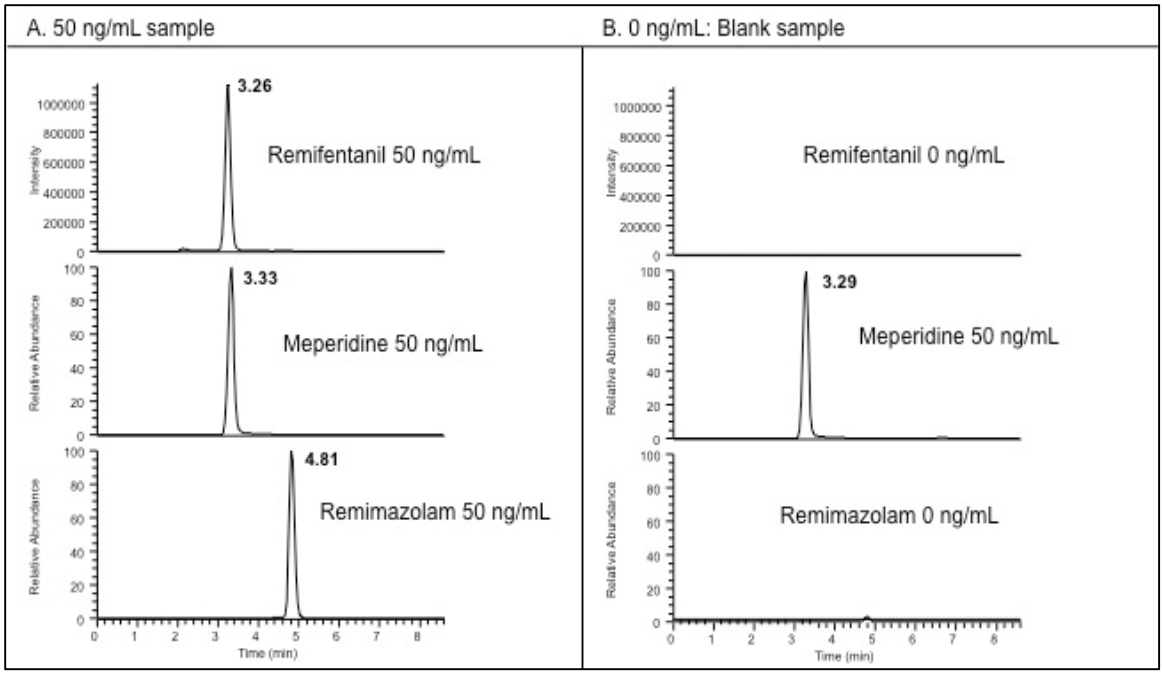

Figure 5.2. A. Chromatogram of remifentanil, meperidine, and remimazolam at a concentration of 50 ng/mL (left) B. Chromatogram of a blank sample of remifentanil and remimazolam at 0 ng/mL, meperidine concentration of 50 ng/mL (right).

The plot of the ratio of analyte and internal standard peak areas versus calibrator concentration was nonlinear over the range of 0.5-2500 mg/mL.

Calibration curves were fit to a quadratic equation, weighted $1/Y^2$, where Y was the analyte/IS. Calibration curves generated in this manner exhibited a correlation coefficient (r^2) that was typically ≥0.98. The accuracy of the LC-MS-MS assay was >79% (n=3).

Intra-assay precision was ≤12% for remifentanil and ≤15% for remimazolam for all quality control concentrations.

The interassay precision was ≤10% for remifentanil and ≤25% for remimazolam

for all quality control concentrations (Table 5.1). Recovery of remifentanil from blood was between 89-103%, recovery of remimazolam from blood was 79-104% (Table 5.2). Samples were recovered at 67-74% for remifentanil after 36 hours in the auto-sampler at room temperature, while remimazolam was recovered at 102-118%. Samples left at room temperature on the bench top for 1 hour were recovered at 66% for remifentanil and 58-68% for remimazolam (Table 5.3).

Table 5.1. Accuracy and Intra- and Interassay Precision (%RSD) for Quality Control Standards Containing Remifentanil and Remimazolam

	Remifentanil		Remimazolam	
Target Concentration (ng/mL)	Accuracy (% Target)	RSD (%)	Accuracy (% Target)	RSD (%)
Intra-assay (n=5)				
1	116	3	139	6
10	110	12	107	11
50	120	8	109	15
500	108	4	119	15
Interassay (n=20)				
1	112	5	103	25
10	101	10	101	9
50	110	9	107	8
500	100	10	111	11

Table 5.2. Recovery of Remifentanil and Remimazolam from Blood

Target Concentration (ng/mL)	Remifentanil % Target	Remimazolam % Target
10	103	79
500	89	104

Table 5.3. Stability of Remifentanil and Remimazolam in Standards Stored Under Various Conditions

Treatment and Target Concentrations (ng/mL)	Remifentanil % Control	Remimazolam % Control
36 hr Autosampler at Room Temp		
1	74 ± 11	118 ± 56
10	71 ± 4	108 ± 2
50	77 ± 2	102 ± 12
500	67 ± 11	108 ± 2
1 hr Bench top in Blood		
10	66 ± 7	58 ± 11
500	66 ± 9	68 ± 13

Discussion

We developed a method for the detection of remifentanil and remimazolam in blood using LC-MS-MS. Using this method, the assayed concentrations for the fortified quality control samples were 88-119% for remifentanil and 83-139% of the target concentration for remimazolam.

For quality controls, remimazolam showed more variability than remifentanil, especially at the very low concentrations (1 ng/mL). This may be due to some carry-over on the LC-MS-MS for remimazolam. The lowest concentration for remimazolam may need to be increased to 2.5 ng/mL. Also, larger sample sizes may be necessary. Level of detection is lower than therapeutic doses, allowing for detection of recovery from each drug.

When stored at room temperature in the autosampler, remifentanil showed expected degradation, but there was an increase in concentration for all remimazolam samples. This may also due to carry-over in the LC-MS-MS. The expected degradation was seen when blood was left for 1 hour on the bench top in blood.

Further refinement of this method for detection of remifentanil and remimazolam in combination will be necessary for pharmacokinetic sampling of remimazolam and remifentanil in animals.

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CHAPTER 6

CONCLUSION

Throughout this dissertation, the feasibility, efficacy, and safety of delivering the ultra-short acting analgesic agent remifentanil and the sedative agent remimazolam by inhalation has been evaluated. It was our hypothesis that inhaled remifentanil and remimazolam would be rapidly absorbed, pharmacologically active, rapidly cleared, and noninjurious to airways in rodent models. Following positive rodent findings, we continued this research in a porcine model.

Our conclusions were that inhaled remifentanil was rapidly absorbed, with onset of action within 2 minutes; it was also pharmacologically active, with detectable blood level and evidence of profound analgesic effect when measured with a tail flick meter. Inhaled remifentanil was also rapidly cleared, with recovery within 3 minutes. Additionally, there was no evidence of inflammation or irritation to rodent airways, and tissues were histologically normal.

Our research on inhaled remimazolam in rodents found that there was no apparent sedative effect in rats, most likely attributable to dosing limitations. There was, however, marked sedative effect in mice as evidenced by time to movement outside a perimeter. Follow-up research showed that low dose inhaled remimazolam potentiated the analgesic effect of inhaled remifentanil. There were detectable levels of both remifentanil and

remimazolam in rat blood following pulmonary exposure, and there was no evidence of lung irritation in mice when the drugs were combined.

Preliminary research on inhaled remifentanil in pigs shows rapid uptake and rapid metabolism, with a population half-life of 3 minutes, which is similar to the half-life of intravenous remifentanil. We have established that these drugs are rapidly absorbed and highly efficacious, while being nonirritating to the lungs. While moving forward with our large animal model in pigs, we are also looking forward to testing these medications in a humans.

There is debate regarding the future of inhalational anesthesia.[1,2] Currently, volatile anesthetics are used for most general anesthetics, with inhaled nitrous oxide being used for both general and sometimes sedation anesthesia. The first general anesthetics ever delivered were inhaled anesthetics, starting with diethyl ether in 1846.[3] Modern inhaled anesthetics are halogenated with fluorine, thereby limiting metabolism and thus toxicity. However, rate of inhaled anesthetic onset and offset still depend upon their solubility and vapor pressure. Volatile anesthetics are minimally soluble in the blood, allowing for rapid induction and emergence, and are minimally metabolized (0.02-5%), limiting toxicity to the liver and kidneys.[3,4] Additionally, surrogate measures of effect site concentration can be inferred from the pulmonary percentage or partial pressure of these gases.[4,5] However, the use of inhaled anesthetics is falling out of favor with some anesthesia providers.

Volatile anesthetics and nitrous oxide have a tendency to be emetogenic. They are also very resistant to biologic degradation and thus deactivation, which can result in residual sedation if spontaneous ventilation and thus clearance is impeded at the end of an

anesthetic. This can present a safety risk in patients such as those with obstructive sleep apnea. This is especially true if the patient has been significantly dosed with supplemental opioids during the case. Additionally, another drawback of nitrous oxide and volatile anesthetics, which are chlorofluorocarbons, is that they are all greenhouse gases.[6]

Total intravenous anesthetics (TIVA) has become increasingly popular due to the development of an ultra-short acting class of intravenous anesthetics, or "soft drugs", such as remifentanil, as well as improved hypnotics with attenuated side effects, such as propofol. These drugs afford the anesthesia provider a new level of control, with rapid intravenous inductions and rapid emergence, increasing safety, facilitating shorter turn around times, and increasing the efficiency of the operating room.[7,8]

However, TIVA has its own limitations. Direct access to the circulation is required for IV anesthetics. This type of anesthesia is vulnerable to drug dilution and dosing errors as well as contamination. It lacks the surrogate effect site concentration measurement that the volatile anesthetics and nitrous oxide allow. It is also more complex to setup.

This research shows that all the pharmacokinetic benefits of these "soft drugs", as shown during intravenous administration, are maintained during the easier administration technique of inhalation. To reiterate, these pharmacokinetic benefits include liver and kidney independent metabolism and a short context-sensitive half-life[9,10] These benefits admonish criteria that relegate some of these medications strictly to intravenous administration.

Intravenous medication dosing for sedation or general anesthesia is inconvenient for patients and providers. Although necessary for safety, establishing intravenous access is painful for the patient and at times, time consuming for the practitioner. This is especially true for very minor procedures such as skin tag removal and/or cataract surgery. In order for drugs to be infused, some require dilution and a majority, if not all, are delivered based on weight. Additionally, multiple IV pumps with separate programming are required when multiple medications are infused. Sometimes, certain combinations of medications cannot be administered in one carrier line due to precipitate formation or other incompatibility issues. Over-dosage of intravenous medications for sedation, whether by infusion or boluses, is frequent and results in respiratory or hemodynamic depression. This generally will require life-sustaining measures by the practitioner. Of course, this is balanced against under-sedation in which the patient is anxious or in pain.

We envision a respiratory-dosed, multimodal and thus balanced anesthetic for maintenance of general anesthesia. A short acting, inhaled benzodiazepine dynamically mixed with a short acting inhaled opioid would be the amnestic/analgesic combination to accommodate extremes of patient age, weight, and comorbidities. Kinetically, taking into account the potentiating effects of benzodiazepines and opioids, combinations to produce general anesthetic maintenance could be dynamically calculated and altered based on needs due to age, cardiac comorbidities, and tolerance. Opioid tolerant patients may require more longer-acting opioids (fentanyl, sufentanil, morphine, or hydromorphone) to be titrated instead of the shorter acting opioid. These opioids could also be administered via inhalation. Again, to reiterate, this type of anesthetic for maintenance of general

anesthesia is clinically superior to the current inhaled anesthetics because of rapid esterase metabolism of both drugs and the ability to selectively reverse either drug when a patient fails to adequately emerge from general anesthesia. Currently, when patients are slow to emerge from general anesthesia, there is not a competitive reversal agent for volatile anesthetics. Thus, sadly, the clinician may try to reverse the synergist aspect of the opioid. This can leave a patient in significant postoperative pain.

Currently, intravenous dosing of amnestics and analgesics for sedation is even more complex because of the complex balance between adequate sedation and maintaining spontaneous respiration during rapidly changing surgical stimuli. Sedation level is targeted by arbitrary intravenous infusions and/or bolus dosing. Also, unlike most general anesthetics, sedation does not have a secure or semisecure airway. Handling apnea requires in-depth knowledge and an advanced airway skill sets. Respiratory dosing of these same medications for sedation has the advantage of patient-controlled dosing during the procedure. When the surgical stimulus becomes intense, the patients' minute ventilation will increase, thereby self-dosing more medication. When the medications cause more sedation and/or the surgical stimulus abates, the patients' minute ventilation will decrease, thereby decreasing self-dosing. The inherent safety mechanism is that these specific medications are esters and are broken down by the non-specific esterases in the patients' blood. This will help prevent dangerous, apnea-producing levels in the patients' blood. Further studies could help elucidate if, when a patient is breathing oxygen with these medications, if they will be adequately sedated but can self-rescue themselves before oxygenation desaturation or hypoxia occurs. Additional safety mechanisms for sedation would include the ability to intravenously

reverse either the opioid or benzodiazepine. Further, since these medications are not volatiles, scavenging these medications may be less complex in the office-based environment.

The practice of anesthesiology has become more challenging. Adequate care of patients is threatened by drug shortages and complicated by rules as put forth by USP 797. So even though drug shortages are prevalent, USP 797 states that unless drugs are drawn in aseptic conditions in the pharmacy, syringe drawn drugs must be administered within an hour. This can have an impact on the efficiency and cost of anesthetic administration. New controlled and regulated systems in which these drugs could be administered via inhalation could help.

Development of these drugs and delivery devices will require collaboration between many disciplines, including pharmacology, bioengineering, and anesthesiology. With the development of any new drug or device, there are obstacles to overcome. Continued pig testing and human testing must evaluate for intersubject variability. Large variability between subjects would make it difficult to estimate required dose. Currently, during general anesthesia, gas analyzers test the percentage of drug in a patient's exhaled breath to estimate depth of anesthesia when volatile anesthetics are used. However, these drugs are not metabolized and are essentially ideal gases that are governed by the ideal gas law and rules of partial pressure and solubility. An alternative system would need to be developed to assess depth of anesthesia for inhaled opioids and benzodiazepines.

Another hurdle to overcome will be potential for abuse. A noninvasively delivered opioid and benzodiazepine would have significant abuse potential. Ways would have to be developed to mitigate this type of behavior. While delivery through an

anesthesia machine could eliminate the need to reconstitute, and could prevent drug waste by preventing contamination caused by intravenous use, there would also be the added expense of altering the configuration of the anesthesia machine to accommodate, deliver, and monitor drug cartridges.

Testing would still have to be ongoing with respect to tolerability of the drugs. Human testing would still need to occur. Investigation would have to occur with regard to allergies. Patients can have more allergies to esters. Also, a question of how the drug tastes when administered to awake humans will also have to be investigated.

Drug deposition variability would have to be studied for both general anesthetic and sedated patients. There would be obvious differences in deposition during positive pressure ventilation and spontaneous ventilation. Differences in deposition differences between males and females and extremes of age would have to be studied. Many comorbid conditions would also have to be studied. A few would include COPD, restrictive lung disease, and pulmonary hypertension.

Use of inhaled ester-based drugs presents a paradigm shift for anesthesia. These drugs are metabolized at a constant rate by plasma esterases independent of kidney and liver metabolism, are fully reversible by intravenous injection, and therefore present a safer option for extremes in age, the morbidly obese, and patients with multiple co-morbid conditions whether they are delivered by IV or by inhalation. The added benefit of inhalation delivery is a noninvasive delivery method and dynamic respiratory dosing which has an increased margin of safety. Throughout this dissertation, we have shown that inhalation of these drugs is feasible. It is our hope that future research in this area will allow these drugs delivered by inhalation to be yet one more tool in our arsenal.

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APPENDIX

AEROSOL PARTICLE SIZE AND DEPOSITION

AEROSOL PARTICLE SIZE AND DEPOSITION

Useful information on factors affecting the choices of nebulizers and aerosol deposition in the lungs:

1. Particle size

2. Mode of inhalation: speed, volume, frequency, breath holding

3. Anatomy and morphology: variation and disease states

- Peak alveolar deposition occur with diameter of 2-4 microns
- Slow lung inhalation (30L/min) enhances both lung deposition and clinical effect of inhaled drugs

Jet Ventilators:

Work on Bernoulli principle. As kinetic energy of the air increases, its potential energy and pressure falls, allowing liquid from the nebulizer reservoid to be pulled up and released as droplets. These droplets range from 1-100 microns. Larger droplets are not released, and run back into the reservoir, and are recycled. It takes several minutes to nebulize a dose of drug contained in 2-5 mL, a minimum of 2 mL is required, most of the drug is nebulized in the first 5 minutes.

Ultrasonic Nebulizers:

Contain synthetic ceramic piezoelectric crystal, usually vibrating at 1-3 MHz in response to an applied alternating electric field. The vibration is then transmitted to the

nebulized fluid, forming a fountain above the crystal. Small droplets leave the nebulizer by being entrained in patient's airflow. Ultrasonic nebulizers are smaller and quieter than jet ventilators, and have higher output rates, leading to shorter nebulization times. However, they do tend to have slightly larger droplet size. Ultrasonic nebulizers have difficulty aerosolizing viscous solutions.

Vibrating Mesh Nebulizers:

Use ultrasonics to generate the aerosol, but have a different principle of operation. The mesh is stainless steel and has holes drilled by a laser drilling process. The mesh vibrates around 100 kHz, which causes liquid to be ejected from the holds forming droplets of relatively uniform size. There is no need to eliminate large droplets. The residual volume is much smaller, and volumes as small as 1 mL can be nebulized. Aeroneb (a vibrating mesh nebulizer) has up to 1,000 holes. This nebulizer reduces waste, decreases nebulization times, and improves convenience.

Deep Lung Deposition:

Highest in compliant, ambulating patients who can take slow deep breaths through a mouthpiece (to minimize nasal deposition). However, mechanically ventilated patients can benefit from choice of nebulizer, placement of nebulizer in the circuit, and adjustment of ventilator settings to increase inspiratory times so that deposition can be almost the same as ambulatory patients.

Source: Newman S. *Respiratory Drug Delivery: Essential Theory and Practice.* Richmond, VA: Respiratory Drug Delivery Online; 2009.