ACCELERATED INDUCTION OF ETORPHINE IMMOBILIZATION IN BLUE WILDEBEEST *(CONNOCHAETES TAURINUS)* BY THE ADDITION OF HYALURONIDASE

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by

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i

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ABSTRACT

Wild animal capture has progressed over the years from trapping or physical capture, which was dangerous to both animal and man, to chemical immobilization. Opioids and butyrophenones are the most common classes of drugs used for ungulate immobilization; however newer drugs and drug combinations are commonly used in an attempt to reduce time to immobilization in wildlife. The enzyme hyaluronidase is often added to drug combinations in the belief that it reduces time to immobilization by improving drug absorption.

The primary objective of this study was to ascertain if the addition of hyaluronidase to an etorphine and azaperone drug combination would be of value in reducing time to immobilization in blue wildebeest. The study also tried to ascertain if the added hyaluronidase enabled one to reduce the etorphine and azaperone doses required to immobilize blue wildebeest, without affecting time to immobilization.

The study made use of a four-way cross-over study design, with four treatment groups, four sequences and four periods. The four treatment groups were etorphine and azaperone; etorphine, azaperone and 5000 international units (IU) hyaluronidase; etorphine, azaperone and 7500 IU hyaluronidase; and 75 % of the original etorphine dose, 75% of the original azaperone dose and 7500 IU hyaluronidase. Each animal was immobilized with each of the above four drug combinations randomly over an eight week

period with a two week interval between each period. The times to first effect, first down and immobilization were recorded.

The etorphine and azaperone treatment group was used as the control group. The difference in time to first effect between the control group and the etorphine, azaperone and 7500 IU hyaluronidase treatment group was statistically significant (95 seconds versus 67 seconds; p = 0.007). When compared to the time to immobilization in the control group (323 seconds) the time to immobilization in the etrophine, azaperone and 5000 IU hyaluronidase (228 seconds); etorphine, azaperone and 7500 IU hyaluronidase (210 seconds) and the low dose etorphine, low dose azaperone and 7500 IU hyaluronidase (268 seconds) groups were statistically significantly reduced (p=0.002, p=0.001 and p=0.045 respectively).

It is therefore concluded that the addition of 5000 or 7500 IU hyaluronidase to an etorphine and azaperone combination significantly reduced the time to immobilization in blue wildebeest. The unexpected decrease in time to immobilization in the low dose etorphine, low dose azaperone and 7500 IU hyaluronidase treatment group requires further investigation.

TABLE OF CONTENTS

FIGURES

TABLES

Chapter
1

INTRODUCTION

$\mathcal{B}$efore effective immobilizing agents were available the capture of wild animals was facilitated through trapping or physical capture. Whilst these methods were effective, they were ethically questionable as they routinely caused injuries to the animals and were probably equally as dangerous to the handlers.[31] As capture techniques improved and immobilization drugs were discovered, immobilizations became easier, safer and quicker.[28]

During the chemical immobilization of animals it is important to ensure that the time to immobilization is kept as short as possible. To facilitate this effect, most immobilizing agents are combined with either a sedative or tranquilizer due to their synergistic/anaesthetic sparing effects i.e. this enables a decrease in the immobilization time while at the same time using lower doses of the immobilizing agent(s) with the net effect being the reduction of potential side effects.[26]

Another method commonly used to facilitate faster immobilization is the addition of hyaluronidase to drug combinations. The added hyaluronidase is believed to reduce the viscosity of the interstitial barrier between cells thereby aiding with the diffusion of substances within the tissues.[50,58] As a result hyaluronidase is believed to enhance the rate of absorption, thereby facilitating faster immobilization at lower concentrations of immobilization drugs.

While the anaesthetic sparing effect of sedative drugs, in combination with etorphine, has been well elucidated, qualitative information on the benefits of the hyaluronidase combinations is sparse. The study therefore intends to determine if the addition of hyaluronidase to an etorphine/azaperone mixture could reduce the "time to first effect" and the "time to immobilization" in blue wildebeest (*Connochaetes taurinus*).

1.1. HYPOTHESIS

The addition of hyaluronidase to an etorphine/azaperone dart combination in blue wildebeest does not reduce the time to immobilization.

1.2. BENEFITS OF THE STUDY

The results of this study will contribute towards the future use of hyaluronidase:

- By ascertaining the benefit of hyaluronidase in dart combinations to reduce the time to immobilization in wildlife
- By determining if hyaluronidase contributes to a dose sparing effect on the total dose of etorphine required for wildlife immobilization which will reduce the risk of potential life threatening side effects to the animal and reduce the cost of wildlife immobilizations

The following study will also contribute to the completion of the degree: Masters in Veterinary Medicine with specialization in Wildlife Medicine.

1.3. OBJECTIVES OF THE STUDY

To determine the "time to first effect", "time to first down" and "time to immobilization" in blue wildebeest immobilized with:

1. a standard dose of etorphine and azaperone; in comparison to the same dose of etorphine and azaperone combined with one of two doses of hyaluronidase (5000 and 7500 IU).

2. a standard dose of etorphine and azaperone; in comparison to a low dose of etorphine and azaperone combined with 7500 IU of hyaluronidase.

| Chapter 2 | # LITERATURE REVIEW |

2.1. WILDLIFE CAPTURE

Wild animals are usually immobilized chemically with the aid of drugs for translocation purposes, treatment, testing/sampling and for the fitting of tracking devices.[26] Before the current effective immobilizing agents were available, the capture of wild animals was accomplished by trapping or physical capture. Whilst these methods were effective, they were dangerous not only to the animals being captured, but also the handlers facilitating the capture.[31] As a result improved methods for capturing wildlife were sought. From this the field of chemical immobilization was borne whereby wildlife is immobilized through the use of pharmacologically active chemicals.

2.1.1. Chemical immobilization: A brief history

The use of progenitor immobilization drugs lessened, but did not eliminate, the risks involved with handling wildlife. Some of the first immobilizing agents to be used were paraldehyde, curare, flaxedil, succinylcholine chloride and the nicotine alkaloids.[8,16,28]

The effects of paraldehyde, curare and flaxedil were first studied on white-tailed deer (*Odocoileus virginianus*) by the intramuscular route. Paraldehyde proved to be ineffective in inducing complete paralysis and in addition was characterized by a narrow range between the stupefying and lethal doses. Curare produced complete paralysis but, as with

paraldehyde, the difference between the non-fatal relaxation and lethal doses was too close. However, flaxedil was easily available, inexpensive, had a wide safety margin, low incidence of adverse effects and had an indirect antagonist in endophonium (3-hydroxyphenyl-dimethylethyl-ammonium chloride) (Tensilon®, E. L. Sevringhaus, Hoffman-LaRoche, Inc., Nutley 10, N. J.) being available. Not surprisingly flaxedil became the agent of choice in producing muscular relaxation in white-tailed deer.[28] Post[47] also goes on to describe flaxedil as the drug of choice for the immobilization of elk (*Cervus canadensis*), although with moderate precaution due to its ability to induce suffocation from intercostal muscle and diaphragm skeletal muscle paralysis.

Nicotine salicylate was also used with varying success in numerous species. The drug however never gained in popularity due to interspecies differences in response and toxicity e.g. while the drug was found to be safe in the immobilization of 17 white-tailed deer,[16] the response was largely unsatisfactory when used on fatigued or excited moose (*Alces alces*).[48] In most cases the moose were incompletely immobilized with the concurrent mortality being excessively high at 26%.[48] In Uganda Kobs (*Adenota kob thomasi*), the use of nicotine alkaloids was associated with convulsions and catalepsy.[8]

Diazepam bait was another method used for the immobilization of wild animals. In one trial animals were fed ear corn coated with diazepam. Once the animals showed signs of sedation, they were physically caught. While the method was in theory sound, an unexpected problem encountered was the ability of excitation to override the effects of sedation. As a result 13 out of 29 animals had to be subsequently darted with nicotine

alkaloids.[43] In a second study greater success was achieved when the method was adapted by baiting a mixture of horse and mule pellets with diazepam. The experimental animals had their food cut by half two days before dosing and all food was withheld the day prior to dosing. In total 105 deer from six different species were caught in large pens using this method. The most effective and economical dosage range was 15.4 to 33.0 mg/kg of bodyweight however clinical effects lasted anywhere between twelve hours and five days.[55]

2.1.2. Chemical immobilization: Current

2.1.2.1. The ideal immobilization agent

Following numerous years of wildlife chemical immobilization, the ideal immobilizing agent is described as one that has the following properties:

- Volume and potency – the more concentrated a drug the greater its potency. This is necessary to ensure that the effective dose does not exceed the volume of the dart.[40]

- Safe – a high safety factor allows for a margin of error in doses. This is necessary as body weights are estimated from a distance; and in addition there will be intraspecies variation in physiological response to the immobilizing agents.[40]

- Reversible – the immobilizing agent should be reversible by means of a pharmacological antagonist. This is necessary as immediate reversal may be required to prevent severe adverse effects or death in the immobilized animal.[40]

- Non irritant – the immobilizing agent should not cause tissue irritation. This is necessary as agents usually get administered intramuscularly. If these drugs are irritant they could induce permanent muscle damage which will interfere with the ability of the animal to survive in the wild after immobilization.[40]

- Stability – chemical stability of the drug. This is necessary as immobilization occurs under a variety of conditions during field work.[40]

- Side effects – fewer side effects. This is necessary as severe side effects like convulsions or respiratory depression can lead to fatalities.[40]

- Rapid induction – rapid time from drug administration to effect. This is necessary as the risk of hyperthermia, acidosis and capture myopathy increases from the time of dart impaction to immobilization through excessive muscle exertion.[40]

At present, no one immobilizing agent is able to achieve all the above mentioned properties.[40] As a result the primary immobilization agent (the primary agent is the one that produces the most marked central nervous system (CNS) depression, such as the opioid compounds or the cyclohexamines) needs to be combined with a secondary agent such as a sedative or tranquilizer (the secondary agent usually only induces mild CNS depression at the doses at which they are used, such as the alpha-2 agonists and the benzodiazepines).[26] In combination, one usually benefits from a reduction in the induction time, a lower dose of the immobilizing agent being required, and the adverse effects of the primary immobilizing agent being reduced.[26] As a result of the need for combination of agents, a new property of compatibility can be added to the above list i.e. it is important that these drugs are compatible both physically and chemically.[40]

2.1.2.2. Current immobilization agents

A variety of drugs are currently used as immobilization agents and include the opioids, butyrophenones, phenothiazines, alpha-2 agonists, benzodiazepines and the cyclohexamines.

a) The Opioids

The opioids are the primary agents used in ungulate immobilizations. These drugs work through the stimulation of the opioid endorphin receptors, of which the three main receptor types are mu (μ), kappa (κ) and delta (δ).[36] The opioid receptors are found in high densities in the dorsal horn of the spinal cord as well as in the brain, kidney, heart, adrenal glands and the myenteric plexus of the gastrointestinal tract.[6,9,24] The μ receptors are divided into μ_1 and μ_2 receptor subtypes and are associated with analgesia (spinal and supraspinal), respiratory depression, gut motility inhibition, sedation, euphoria and increased appetite. The κ receptors are divided into κ_1, κ_2 and κ_3 subtypes and their effects include analgesia (spinal and supraspinal), gut motility inhibition, sedation and increased appetite. The δ receptors are responsible for analgesia (spinal and supraspinal), immunomodulation and increased appetite.[36,49]

The affinity of a drug is the drug's ability to bind to receptors within the body, and the ability of the drug to cause an action in or on the cell where its receptor occurs is termed intrinsic activity. Based on their affinity and activity, opioids can be classified into the following:

- Full agonists – have affinity and intrinsic activity at all clinically relevant receptors. They produce profound analgesia but have significant side effects such as respiratory depression (e.g. morphine and etorphine).[7]

- Full antagonists – have affinity for, but no intrinsic activity at opioid receptors and are used as reversal agents for the agonists. Antagonists need to bind to receptors and displace the agonist in order to effectively reverse the agonists. One can either use an antagonist with a higher receptor affinity than the opioid, or use a larger dose of antagonist (e.g. naltrexone which is given at a larger volume than the opioid).[7]

- Partial agonists – have affinity for some of the opioid receptors, in addition to possessing lower intrinsic activity. The maximal analgesic effect induced by partial agonists is less than that for full agonists (e.g. buprenorphine).[7]

- Agonist-antagonists – have affinity for all opioid receptors but only have agonistic effects at some of the receptors. As a result they can be used to partially reverse the effects of full agonists or more importantly themselves at higher doses (e.g. butorphanol which is a mu receptor antagonist at lower doses and a kappa receptor agonist at higher doses).[7]

While the opioids are well absorbed following oral, subcutaneous or intramuscular administration, the plasma concentrations achieved may be erratic following oral administration due to a substantial first-pass effect. The opioids are well distributed throughout the body, with plasma protein binding being variable and dependent on the individual drug under investigation. They are also lipophilic weak bases that tend to

accumulate intracellular with a resultant large volume of distribution. The highly lipophilic opioids (e.g. fentanyl) also undergo rapid redistribution from the well perfused tissues, such as the central nervous system, to tissues with poorer perfusion, such as fat. Metabolism occurs predominantly via glucuronidation into polar compounds which are excreted in the urine, with a small amount of the unchanged drug also being eliminated unchanged in the urine.[36] While metabolism generally results in inactivation of the active compound, some of the opioid metabolites may retain a degree of the parent compound's pharmacological activity.[7]

The adverse effects associated with this group of drugs are numerous and includes potentially life-threatening clinical signs such as respiratory depression, hypertension and CNS excitement/muscular hypertonicity associated hyperthermia.[6,40] Renarcotization, or 'recycling', is always a possibility when using potent opioids. Some of the possible ways in which renarcotization can occur are enterohepatic recirculation, antagonist underdosing, release of the agonist from depots in the body or rapid metabolism of short-acting antagonists.[26] This may result in hyperthermia, myopathy and death.[1] In order to prevent renarcotization a suitable antidote must be administered at the correct dose and at times for the correct duration. The two antagonists that are used to reverse the effects of the opioids are diprenorphine hydrochloride and naltrexone.[36]

- Diprenorphine is a mixed agonist-antagonist.[40] Diprenorphine must be administered, either intramuscularly or intravenously, at double the etorphine dose

in order to fully reverse the effects of the etorphine.[7] Due to the mixed agonist-antagonist effects, a degree of depression may persist after administration.[40]

- Naltrexone (Fig 2-1) is a pure antagonist at opioid receptors and possesses a long half-life.[21] Naltrexone given to animals that had received carfentanil prevented renarcotization in all cases; however 5.3% of animals that had received diprenorphine experienced renarcotization.[1]
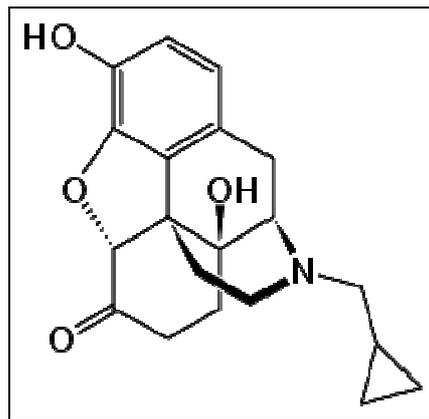
Figure 2-1: Chemical structure of naltrexone (Trexonil[®])
Source: Wikipedia

b) The Butyrophenones

The butyrophenone derivatives are a group of tranquilizers which include azaperone and the longer acting haloperidol.[19] Although the butyrophenones have been referred to as selective dopamine antagonists, they do also possess some affinity for the 5-hydroxytryptamine (5-HT) and alpha-1 adrenoreceptors (α_1). Dopamine receptor

blockade is primarily responsible for the neuroleptic effects, although other receptor type interactions may play a role.[23]

Azaperone is a shorter acting tranquilizer which can be used as a single agent for tranquilization of up to four hours, or it can be used in immobilization combinations where it acts synergistically with the primary agent.[40] Haloperidol on the other hand is used predominantly for tranquilization during transportation or boma adaptation as its effects may last for up to eighteen hours.[19] When used at the correct dose this class of drugs is safe with minimal adverse effects (possible aggression in some species e.g. gemsbok, hypotension and extrapyrimidal effects e.g. allotriophagia, torticollis,).[2,15,23,40]

c) Alpha-2 Agonists

The alpha-2 agonists, xylazine, detomidine and medetomidine, are sedatives that are used widely in both domestic and wild carnivores/herbivores to provide sedation, muscle relaxation and analgesia.[40] While these drugs are used alone in domestic animals to facilitate sedation for simple procedures, they are often used in combination with opioids to minimize their significant adverse effects of marked salivation in ruminants, ruminal atony and bloat, possible abortion in the last trimester, hypotension and bradycardia. Atipamezole hydrochloride and yohimbine are specific antagonists recommended for the reversal of the alpha-2 agonists.[23] Reversal of the sedative effects is achieved within 2-4 minutes following administration.[40]

d) Phenothiazines

The phenothiazine derivatives are commonly used for tranquilization, neuroleptanalgesia (a profound degree of CNS depression attained through the combination of a potent opioid and a tranquilizer) and premedication prior to general anaesthesia.[23,54] Acepromazine is the shortest acting tranquilizer in this group while perphenazine enanthate and pipothiazine palmitate are more long-acting tranquilizers.[54] The long-acting tranquilizers are used to calm animals during long journeys, control aggressive and dominant animals, and to reduce anxiety in recently captured animals adapting to confinement.[40] The most important adverse effects of the phenothiazines are hypotension, disruption of the thermoregulatory system and extra pyramidal effects (e.g. torticollis and head pressing). There are no antidotes available for this group of drugs.[54]

e) Benzodiazepines

The benzodiazepines are a group of sedatives which include diazepam, midazolam and zolazepam and are often used for the relief of anxiety.[23,54] When working with wildlife these drugs are most commonly used together with immobilizing drugs, particularly the cyclohexamine anaesthetics. The benzodiazepines are generally well tolerated although hyperexcitement and respiratory depression has been recorded at higher doses.[54] Flumazenil is an effective antagonist for the benzodiazepines, however its expense often precludes its use in wildlife.[23,54] Following the intramuscular administration of flumazenil in cheetahs (*Acinonyx jubatus*) immobilized with tiletamine-zolazepam, recovery was smooth and completed in 66.8 ± 15.2 minutes.[57]

f) Cyclohexamines

The cyclohexamines (ketamine and tiletamine) produce a dissociative anaesthesia characterized by open eyes, and muscular hypertonicity which may result in hyperthermia. Low doses may cause excitement whereas high doses have been known to cause convulsions. No antagonist exists for this group of drugs and thus recovery is dependant on the metabolism and excretion of the drug.[40]

2.2. A MAJOR SHORTCOMING OF CURRENT IMMOBILIZING AGENTS

If one looks at the general requirements for the perfect immobilization agent, the majority of the criteria listed above (2.1.2.1 The ideal immobilization agent) are met with opioids.[40] The use of the newer generation opioids allows for immobilization that is potent, reversible, non-irritant, stable and rapid acting: while the addition of a sedative and/or tranquilizer to this dart combination allows for safer immobilization through a decreased incidence of side effects.[40] However, in reality there is always room for improvement. One such area that is considered important is the time to immobilization i.e. it is strongly believed that time to immobilization can be further shortened. This can perhaps be achieved through the manipulation of the pharmacokinetics of the molecule(s) used.[12]

2.2.1. Pharmacokinetics

The pharmacokinetics of any drug is important. The pharmacokinetics of a drug includes absorption, distribution, metabolism and elimination which are all ultimately involved in

the movement, and thus concentration, a drug achieves within a body. The ability of drugs to traverse membranes depends on its molecular size and shape; degree of ionization; relative lipid solubility of the ionized and non-ionized forms; and the extent of serum or tissue protein binding. This movement across cell membranes may be by either passive processes (e.g. diffusion) or carrier-mediated transport which may or may not require energy.[12]

Absorption can be described as the movement of a drug from the site of administration into the central compartment (circulation) and the degree to which this occurs. Of primary concern is the fractional extent to which a dose of drug reaches its site of action, and is termed bioavailability. The most common routes for drug administration are oral, intravenous, subcutaneous and intramuscular. Drug absorption, and ultimately bioavailability, varies between these sites and a thorough understanding of the drug, and the advantages and disadvantages of each route of administration is important when a drug formulation is designed. Intravenous drug administration circumvents the absorption process as the drug is administered directly into the central compartment.[12]

Distribution involves the movement of a drug from the central compartment into the interstitial and intercellular spaces. This process is influenced by factors that include cardiac output, regional blood flow and capillary permeability. Well-perfused organs like the brain, kidney and liver receive the majority of the drug whereas delivery to the muscle, skin and fat occurs at a slower rate. Some drugs are bound to either plasma, or tissue, proteins and this may affect their distribution within the body.[12]

The primary aim of metabolism, which occurs predominantly in the liver, is to convert lipophilic drugs into more hydrophilic metabolites and involves two phases. The first phase generally results in the loss of pharmacological activity and the second phase results in highly polar conjugates that are rapidly excreted. Nonetheless some lipophilic drugs may be excreted unchanged by the kidneys, albeit as a minor role in the overall elimination of the therapeutic agents, with the exception of the gas anaesthetics which undergo excretion in large quantities via the lungs. The most important organ for the excretion of drugs and their water-soluble metabolites is the kidney. Drug metabolites may also be excreted in the bile or directly into the intestinal tract for elimination via the faeces.[12]

Of all the pharmacokinetic principles discussed above, the one that is considered the most important is drug absorption as this is the driving force behind the formulation desired response as it controls both the actual concentrations achieved and speed at which this concentration is achieved within the body. This is important as all drugs follow a dose-response relationship within their effective tissues (biophase) in that an increase in dose will result in an increased response to a certain maximum effect (study known as pharmacodynamics).[12] Likewise most drugs also have a lower effective concentration, in that any concentrations lower than this threshold will not result in any desired response. While the effective concentration is determined directly at the biophase, a general pharmacokinetic plasma concentration versus time profile will provide an indirect representation of this concentration once equilibrium has been achieved i.e. the plasma

concentration at equilibrium may be used as a marker that the correct concentration has been reached at the biophase.

2.2.2. Manipulation of the pharmacokinetic profile

As a result of the close relationship between plasma pharmacokinetics and the effective concentration at the biophase, it is believed that an increase in absorption may result in faster entry of the drug into the central compartment and thereby faster induction times (Fig 2-2). To enhance the absorption of the currently used dart combinations, it has been hypothesized that the addition of hyaluronidase to the dart may provide the more rapid absorption desired.
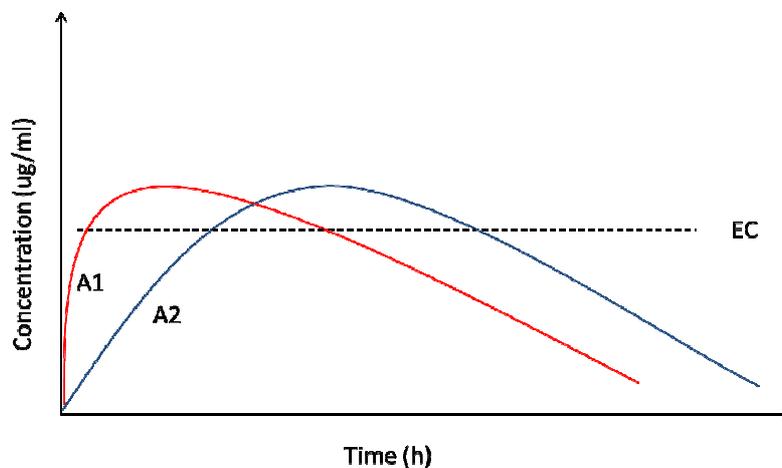
Source: Adapted from Wikipedia

Figure 2-2: Illustration of the relationship between plasma concentration versus time for two different rates of absorption. A1 (red): rapid absorption results in the effective concentration being achieved faster. A2 (blue): slower absorption results in the effective concentration being achieved at a later stage.[12]

2.3. HYALURONIDASE

2.3.1. The enzyme
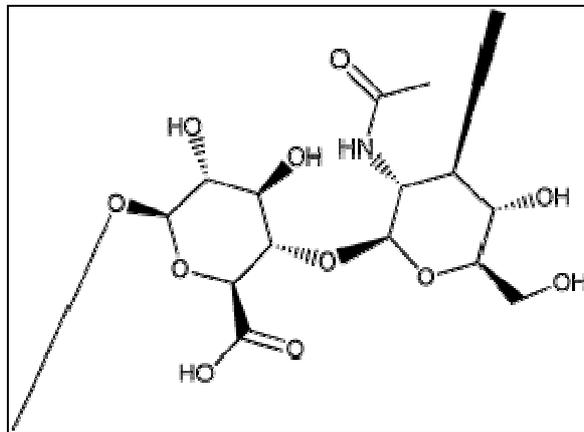
Figure 2-3: Chemical structure of hyaluronidase
Source: Wikipedia

Enzymes that degrade hyaluronic acid are termed hyaluronidases.[41] Hyaluronidase is a protein that was initially demonstrated in mammalian testes.[14] Hyaluronidase plays an important role in fertilization, as sperm possess the protein PH-20 which has hyaluronidase activity that enables the acrosome of the intact sperm to penetrate the barrier of cumulus cells surrounding the typical mammalian egg.[38] It has since also been demonstrated that hyaluronidase is present in other human organs (testis, spleen, skin, eye, liver, kidney, synovial tissue, uterus and placenta) and body fluids (tears, plasma and urine).[5,41] Hyaluronidase has also been isolated from rat (*Rattus rattus*) and guinea pig (*Cavia porcellus*) lung, liver and kidney.[5]

Hyaluronidase is not unique to mammals and has also been described to occur in other organisms. Both bee and wasp venoms are known to contain hyaluronidase,[25] which is significantly homologous to the hyaluronidase found the in mammalian testes.[20] While

the hyaluronidase has no local or systemic toxicity, it does enable the spread of the other venomous constituents in the venom.[25] Hyaluronidase has also been associated with various bacterial strains. It is believed that the hyaluronidases produced by the gram-positive bacterium *Clostridium perfringens* aids its spreading in tissues,[13] whereas the hyaluronidase associated with *Streptococcus pyogenes* may possibly break down hyaluronic acid for nutritional purposes.[51]

Injected hyaluronidase is responsible for the breakdown of the interstitial barrier between cells, which is accomplished by cleaving the β-1,4 glycosidic bond between 2-acetamido-2-deoxy-D-glucose and D-glucuronic acid. Tetrasaccharides are formed and this process is reversible. As a result of this action the intercellular matrix, which is responsible for tissue integrity, is broken down which enables the drugs to reach the central compartment in a shorter time.[50,58]

Therefore, as a result of the beneficial effects of the hyaluronidase under normal physiological conditions, it has been hypothesized that this effect may be useful clinically i.e. if hyaluronidase can be used to break down intercellular bonds in a localized area, it may reduce the time taken for an injected drug to reach the capillaries, thereby enabling more rapid drug absorption and a faster time to clinical response.

2.3.2. Current uses

The major use of hyaluronidase in human medicine is in the field of ophthalmology.[58] During a trial in people hyaluronidase added to a lignocaine anaesthetic protocol for

peribulbar blocks, at 300 international units (IU)/ml increased the speed of onset of the block without adversely affecting the quality of the block.[17] The addition of hyaluronidase at 15 IU/ml to lignocaine was shown to a significantly reduce the volume (up to 2.4 fold) of local anaesthesia required for cataract surgery.[44,50] Interestingly, although hyaluronidase was shown to improve the quality of regional anaesthesia of the eye, increasing the dose of hyaluronidase from 3.75 IU/ml to 7.5 IU/ml did not offer any additional advantage.[32]

As a result of the dose sparing effect and faster onset of anaesthetic effects during ophthalmic surgery, hyaluronidase has become widely used in the wildlife industry as the perceived benefit of the enzyme in immobilization agents during capture is quicker onset of immobilization meaning less distance traveled by the animal from darting to first effect and the less likely the chance of losing the animal in mountainous or thick areas.[40]

Although commonly used in commercial capture, there are only a few published studies which have studied the influence of hyaluronidase on drug absorption in wildlife. Lloyd, Reichard and Odum[39] found that immobilized Cuban crocodiles *(Crocodylus rhombifer)* recovered significantly sooner when hyaluronidase was included in the antidote and that no repeat dosing of reversal agent was required. During black rhinoceros *(Diceros bicornis)* immobilization, Kock[33] found that xylazine (100mg), hyaluronidase (4500 IU) and an increase in the etorphine dose (3.9 ± 0.18mg) significantly lowered induction times and reduced stress when compared to a previous capture where xylazine (100mg) and a lower dose of etorphine (3mg) was used. During a moose capture it was found that

the addition of hyaluronidase to a fentanyl/xylazine mixture appeared to decrease immobilization times by between 36 and 45 % (although not confirmed statistically).[27]

To facilitate the immobilization of African wildlife the dose of hyaluronidase used in combination with immobilization drugs is highly variable. Between 1000 and 5000 IU hyaluronidase has been used for white rhinoceros (*Ceratotherium simum*) capture.[35,59] Impala (*Aepyceros melampus*) have been immobilized with the addition of 7500 IU hyaluronidase and 6000 IU hyaluronidase was used for the capture of giraffe (*Giraffa camelopardalis*).[11,56] For the immobilization of elephants (*Loxodonta africana*) between 2000 IU and 4500 IU hyaluronidase has been included in immobilization cocktails.[34,47] It is evident that the recommended dose for hyaluronidase in wildlife immobilizations has not been finalized and that the quantity used is currently based on personal preference.

Following extensive work for the South African National Parks and in the private wildlife sector in South Africa, J.P. Raath believes that the addition of at least 7000 IU hyaluronidase would significantly reduce immobilization times when added to an immobilizing drug cocktail. As a result we decided to include 5000 IU and 7500 IU hyaluronidase in our darts to determine at what level, if any, the hyaluronidase provided a significant difference in time to first effect, time to first down and time to immobilization in the wildebeest (*Connochaetes taurinus*).

2.4. WILDEBEEST

2.4.1. Description

Figure 2-4: Blue wildebeest (*Connochaetes taurinus*)

C. taurinus consists of five subspecies distributed across the African continent with only one, the blue wildebeest (*Connochaetes taurinus taurinus*), also known as the brindle gnu, being indigenous to southern Africa. Blue wildebeest are usually found in savannah woodland that has adequate shade and drinking water. They are naturally grazers and prefer grass shorter than 100-150 mm high. Within South Africa they are commonly distributed across the country.[42]
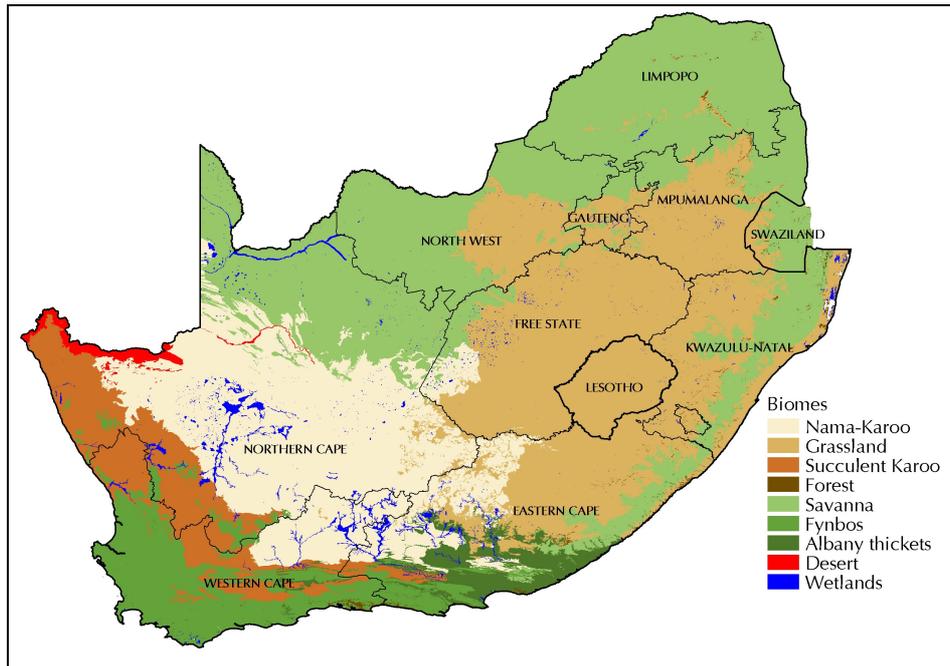
Figure 2-5: South Africa's biomes
Source: National Spatial Biodiversity Assessment 2004

The blue wildebeest's colour varies from a silvery-grey to a bluish-grey and may contain a tinge of brown. The adult males have an average shoulder height of 1472cm and an average weight of 251.7 kg, while the females have an average shoulder height of 1353cm and an average weight of 214.8 kg. The blue wildebeest is a herd animal with between 20 – 30 animals in the average herd which consists of females and their young, or bachelor groups. Herds of several thousand animals are, however, found in Kenya and Tanzania. This phenomenon is primarily due to the lack of fences and the ability of smaller herds to join together while they follow the rains in search of better grazing. The species has a gestation period of approximately 250 days, with a single calf of birth mass of about 22 kg being born between mid-November and the end of December.[42]

2.4.2. Immobilization

Blue wildebeest may require immobilization for translocation purposes, treatment, testing/sampling or for the fitting of tracking devices. Various drug combinations have been used for the capture of blue wildebeest, from fentanyl and azaperone; xylazine, etorphine and acepromazine; to xylazine, fentanyl and azaperone with varying degrees of success.[22] Nowadays etorphine and azaperone are commonly used as this is an effective combination for ungulate immobilizations. Under free-ranging conditions the recommended dose for blue wildebeest bulls is 6-8 mg etorphine and either 100 mg azaperone or 10 mg detomidine. For blue wildebeest cows the dose is 4-5 mg etorphine to which either 80-100 mg azaperone, 10 mg detomidine or 10-15 mg xylazine is added.[10]

2.4.2.1. Etorphine hydrochloride

Etorphine hydrochloride (6,14-ethenomorphinan-7-methanol,5,5-epoxy-3-hydroxy-6-methoxy-ά-propyl-,(5ά,7ά-®)-hydrochloride) is an active derivative of 6,14-endoethenotetrahydro-oripavine and was first synthesized by Bentley and Hardy.[3] It is a semi-synthetic opiate closely related to morphine and is a pure opiate agonist of all three major classes of opioid receptors: mu, kappa and delta.[36] Etorphine was one of the first potent opiate agonists to be used on animals and was shown to have a rapid incapacitating effect in goats (*Capra aegagrus hircus*) and donkeys (*Equus africanus asinus*), and its potential for use in wild ungulates was recognized.[29] Etorphine, as an analgesic, was found to be 2000 times more potent than morphine when administered intravenously to rats.[4] Etorphine is registered in South Africa under the trade name Captivon®.
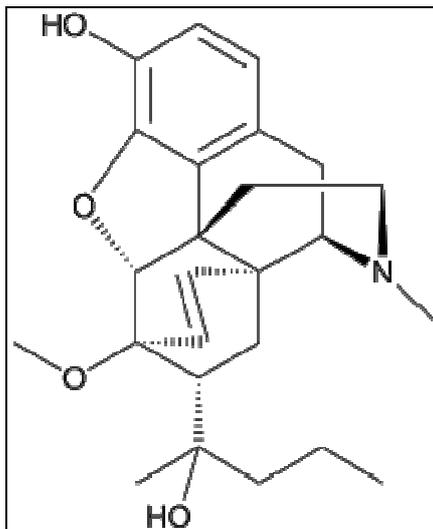
Figure 2-6: Chemical structure of etorphine hydrochloride (Captivon®)
Source: Wikipedia

The dose requirements and time to effect for opioids including etorphine, follow the general pattern for administration routes, intravenous < intramuscular< subcutaneous.[26] Following intramuscular injection in rats, etorphine reached peak levels in the brain and skeletal muscle approximately 20 minutes after administration. Etorphine concentrations in the liver and kidney, relative to the blood, were also high which was attributed to the role these organs play in excretion of the drug. Following intramuscular administration in the rat the levels of etorphine in the liver peaked after 30 minutes and that the major route for etorphine elimination was via the bile and that urine concentrations of etorphine, when administered alone, peaked 12 hours after administration, whereas the levels of etorphine in the faeces peaked 60 hours after administration.[18]

When compared to morphine, etorphine depresses the lower parts of the respiratory centre to a greater extent resulting in severe respiratory depression. Etorphine also blocks

conditional reflexes in rats more readily than morphine and causes a greater degree of catatonia.[4]

2.4.2.2. Azaperone

The opioids use as immobilization agents is associated with excitement; muscular hypertonicity and the subsequent hyperthermia; hypertension and respiratory depression which pose certain risks to the animal. In order to reduce these adverse effects and reduce time to immobilization, the tranquilizer or sedative drugs are routinely used together with opioids to reduce the dose required of the opioid being used. The butyrophenone azaperone is regularly used in conjunction with etorphine for wild ungulate immobilizations.[40]
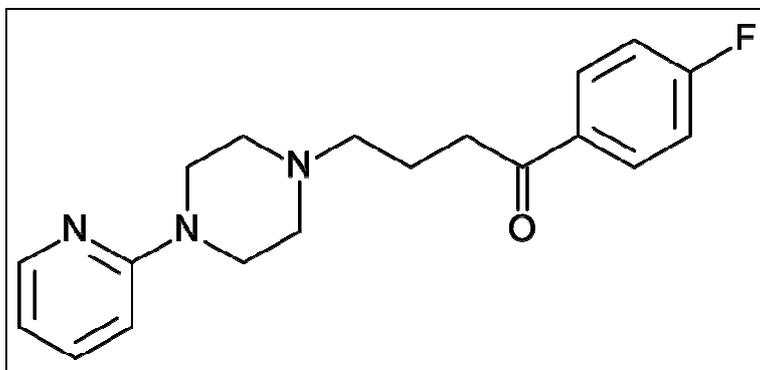
Figure 2-7: Chemical structure of azaperone (Stresnil[®])
Source: Wikipedia

Azaperone (4′-fluoro-4-[4-(2-pyridyl)-1-piperazinyl]butyrophenone) can be classified as a short-acting tranquilizer, neuroleptic or antipsychotic, that is primarily a dopamine receptor antagonist, with some minor α_1-receptor antagonistic activity.[23,46] The dopaminergic effects cause sedation and potentiation of anaesthesia, while alpha-1

adrenergic blockade results in peripheral vasodilation and bradycardia.[23,40,54] While azaperone generally has a wide therapeutic index, possible side effects include extrapyrimidal effects (allotriophagia, torticollis), hypotension and possible aggression in some species e.g. gemsbok.[2,23,40] Azaperone has no specific antidote as the drug is in itself an antagonist.[30]

Azaperone may be administered intravenously, intramuscularly or subcutaneously. Following administration the onset of action is 20-30 minutes with the drug being effective for approximately 2-4 hours.[40] The effects of azaperone when administered as treatment may be unpredictable with no observable changes being evident, to the animal becoming indifferent to auditory and visual stimuli.[30] Azaperone has been used extensively in pigs to reduce aggression and fighting in recently mixed groups.[46] Azaperone may be used alone or in drug combinations as it is chemically compatible , and acts synergistically, with a number of immobilizing agents e.g. xylazine, ketamine, fentanyl and etorphine.[22,37,40,52,53,60]

2.5. CONCLUSION

Drug combinations have become the "norm" in the immobilization of wildlife, all in the hope of making the immobilization procedure safer. One area, in which it is believed that vast improvements can still be made, is quicker times to the onset of immobilization. From literature, it would appear that hyaluronidase added to the drug combination may be able to produce the required increase in knock-down time required due to preferential absorption. The aim of this study is to, therefore, ascertain if the addition of

hyaluronidase to an etorphine/azaperone dart combination will be of value in reducing immobilization times in blue wildebeest and to ascertain if the added hyaluronidase enables one to reduce the etorphine and azaperone dose required to immobilize blue wildebeest, without there being any appreciable effect on the immobilization times.

Chapter
3

MATERIALS AND METHODS

3.1. MODEL SYSTEM

Eight blue wildebeest, four males and four females, between the ages of 1.5 and 5.5 years were used for the project. The animals were housed in two bomas on Ngongoni Game Ranch, Nelspruit for 12 weeks and upon completion of the project were released onto the game ranch.

The eight blue wildebeest were mass captured in Hoedspruit, Mpumalanga, by J.P. Raath and transported by road to bomas on Ngongoni Game Ranch. Once in the bomas the animals were fed high quality teff, lucerne and game pellets with borehole water being provided. The animals had four weeks to adapt to their new boma situation prior to the project commencing. Two weeks prior to the start of the project each animal was immobilized with etorphine and azaperone, for weighing and the placement of ear tags. The drug doses the animals received for this initial immobilization prior to the project commencing were as follows:

Table 3-1: Drug doses used for weighing and ear tag placement

Animal Number	Captivon		Azaperone	
	Mg	ml	mg	Ml
1	3	0.3	30	0.75
2	3	0.3	30	0.75
3	2	0.2	20	0.5
4	3	0.3	30	0.75
5	2.5	0.25	25	0.63
6	2.5	0.25	25	0.63
8	2	0.2	20	0.5
9	2	0.2	20	0.5

3.2. STUDY DESIGN

The study made use of a four-way cross-over study design, with four treatment groups, four sequences and four periods. A wash-out period of two weeks was included prior to each period. The sample size for the study was determined by conducting a power analysis using mock test data. This data was analyzed using the G*Power 3.1.0 (Franz Faul, Uni Keil, Germany) and a sample size of six animals generated. An extra two animals were included in the study to account for unpredictability, and the possibility of mechanical dart failure.

The treatments were divided into four sequences according to table 3.2. Animals were subsequently randomized into four sequences using a table of random numbers (Table

3.3). The final treatments were standard dose etorphine and azaperone (Treatment 1); standard dose etorphine, azaperone and 5000IU hyaluronidase (Treatment 2); standard dose etorphine, azaperone and 7500IU hyaluronidase (Treatment 3) or 75% of the original etorphine dose, 75% of the original azaperone dose and 7500IU hyaluronidase (Treatment 4).

Table 3-2: Treatments allocated to sequences

Sequence	Period 1	Period 2	Period 3	Period 4
Seq 1	A1	A2	A3	A4
Seq 2	A4	A1	A2	A3
Seq 3	A3	A4	A1	A2
Seq 4	A2	A3	A4	A1

A1 - etorphine and azaperone; A2 - etorphine, azaperone and 5000IU hyaluronidase; A3 - etorphine, azaperone and 7500IU hyaluronidase; A4 - 75 % of the original etorphine dose, 75% of the original azaperone dose and 7500IU hyaluronidase

Table 3-3: Treatments, by period and sequence, allocated to each animal

Animal	Sequence	Period 1 21/08/09	Period 2 04/09/09	Period 3 18/09/09	Period 4 02/10/09
1	Seq4	A2	A3	A4	A1
2	Seq3	A3	A4	A1	A2
3	Seq2	A4	A1	A2	A3
4	Seq1	A1	A2	A3	A4
5	Seq3	A3	A4	A1	A2
6	Seq4	A2	A3	A4	A1
8	Seq1	A1	A2	A3	A4
9	Seq2	A4	A1	A2	A3

A1 - etorphine and azaperone; A2 - etorphine, azaperone and 5000IU hyaluronidase; A3 - etorphine, azaperone and 7500IU hyaluronidase; A4 - 75 % of the original etorphine dose, 75% of the original azaperone dose and 7500IU hyaluronidase

3.3. WILDEBEEST DARTING

The animals were darted by J.P. Raath a qualified, experienced veterinarian. The animals were darted using a Dan-Inject® (Fritz Rohr, Private Bag X402, Skukuza, 1350) dart gun and 3ml Dan-Inject® darts using collared 2 mm x 38 mm needles. All animals were darted in either the left or right hindquarters. All dart sites were recorded as well as any abnormalities e.g. excessive bleeding from the dart sites.

The anaesthetic agents used were etorphine 0.98% M/V (Captivon®, Wildlife Pharmaceuticals South Africa, South Africa), azaperone 4% M/V (Stresnil®, Janssen Pharmaceuticals (Pty.) Ltd., South Africa) and hyaluronidase (Hyalase, 801 IU dry

powder per milligram, Merck, South Africa). The dart combinations were made up for each animal using their weights from the previous immobilization and a standard dose of 0.01 mg/kg etorphine and 0.1 mg/kg azaperone was used. The hyaluronidase was weighed using an ESA 300 milligram scale (Salter Brecknell, USA) ensuring that exactly 5000 and 7500 IU was included in the respective darts. The darts were then filled with sterile water to ensure that each animal received the same dart volume. J.P. Raath was solely responsible for the making up of darts so that the monitoring of the response could be blinded.

Once immobilized, dart penetration wounds were treated with 5 ml procaine penicillin 15% M/V (Lentrax®, Merial, South Africa) injected directly into the dart wound and all the animals were monitored daily by the staff on the premises for any signs of lameness or swelling or discharge from the dart wounds.

Once all the readings were taken the animals were weighed and the immobilization reversed through the administration of the antidote, naltrexone 5% M/V (Trexonil®, Wildlife Pharmaceuticals South Africa, South Africa), intravenously in the ear at twenty times the etorphine dose. The animals were observed until fully conscious and standing.

3.4 MONITORING OF ANIMALS

Each animal was immobilized for a minimum of twenty minutes prior to the administration of the antidote. Each immobilized animal was placed in sternal

recumbency and a total of four readings at five minute intervals after immobilization were taken. The following readings were recorded:

- Systolic and diastolic blood pressures (mmHg) using a digital wristwatch with pressure cuff (Geratherm® wristwatch, Geratherm Medical AG, Germany) placed around the base of the tail in the larger animals and around the medial aspect of the forelimb slightly proximal to the carpus in smaller animals.

Figure 3-1: Digital blood pressure wristwatch proximal to the carpus

- Blood oxygen saturation (SpO_2) (mmHg) and heart rate (beats per minute) using a pulse oximeter (Nellcor®, Austvet (Pty.) Ltd., Australia) with the probe placed on the vulva in female animals and on a fold of skin below the anus in male animals
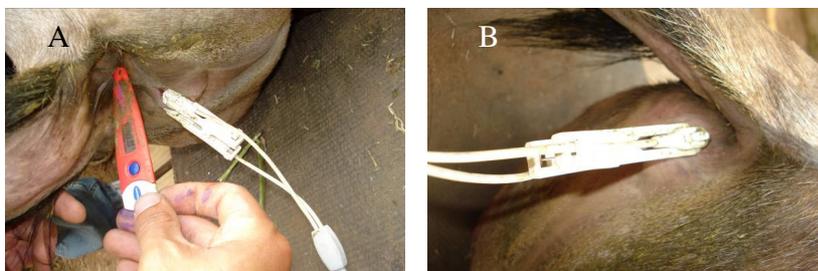
Figure 3-2: Pulse oximeter placed on the vulva (A) and on a skin fold below the anus (B)

- Respiratory rate by counting the chest movements over a 20 second period and multiplying the result by three to get breaths per minute.

- Body temperature ($^{\circ}$C) using a digital thermometer (Thermoval®, Hartmann-Vitamed, Germany) placed inside the rectum.

- Ambient temperature ($^{\circ}$C) using an outdoor thermometer.

- Weight using a Salter 300 kg hanging scale (Salter Brecknell, USA).

3.5. MONITORING OF ANAESTHETIC EFFECT

3.5.1. Time to first effect

The time to first effect was the time taken from the injection (darting) of the animal to the time the animal started showing signs that the drugs were taking effect. Common signs included slight imbalance, a stumble or mild ataxia.

3.5.2. Time to first down

The time to first down was the time taken from injection (darting) of the animal to the time the animal became recumbent for the first time, but rose when approached.

3.5.3. Time to immobilization

The time to immobilization was the time taken from the injection (darting) of the animal to when the animal could be safely approached and blindfolded. When animals were not fully recumbent following darting, the animal was deemed immobilized if the animal

allowed a person to walk up to it for the placement of a blindfold over its eyes without resistance.

3.5.4. Response to monitoring

The response of the animal was recorded every five minutes over a twenty minute period. The following grading system was used: 1 – no response; 2 – slight muscular tensing; 3 – head movement; 4 – increasing general body tone; 5 – struggling to rise; 6 – standing.

3.6. STATISTICAL ANALYSIS

Results are presented as means ± standard error of the mean (SE). Differences between the treatment groups for the parameters stage of immobilization, temperature, pulse, respiration, systolic blood pressure, diastolic blood pressure and oxygenation (PaO_2) were determined using a univariate analysis of variance (ANOVA) with sequence, period, treatment, subject, sex and dart site being factors. Post-hoc analysis made use of a Dunnett's t-test for effects (time to first effect, time to first down, time to immobilization, response to monitoring) being significantly lower than the control group. Changes in the following parameters: pulse, respiration, systolic and diastolic blood pressures, temperature and stage of anaesthesia over the twenty minute observation period were evaluated with a paired t-test while a Wilcoxon signed ranked test was used for stage. In all the cases the first available time point (five minutes) after immobilization was used as the point of comparison. All analysis was undertaken in SPSS, version 17.0 (SPSS Inc., Chicago, Illinois 60606, USA).

| Chapter |
| 4 |

RESULTS

4.1. GENERAL PROGRESS WITH STUDY

All animals appeared relaxed, ate and drank well in the bomas and maintained body condition and were thus deemed healthy prior to induction into the study. The time from darting to first effect and immobilization was recorded for each animal in seconds. Stage of immobilization, temperature, pulse, respiration, systolic blood pressure, diastolic blood pressure and oxygenation was recorded for each animal at 5 minute intervals following immobilization.

The study in general progressed as per the study design. A problem was encountered after the first session of darting when 2 wildebeest bulls were accidentally placed together in the same boma resulting in one of the bulls (animal 6) suffering a compound fracture of the left forelimb. The bull was re-immobilized that day using a ketamine/medetomidine combination and a cast was fitted. Due to the fact that the bull was immobilized using a different drug combination to the combination specified for this study, it was decided that the additional immobilization would have no bearing on future results, and the bull remained in the study. However at the third darting session the cast was removed and it was evident that the limb was showing no signs of healing. After obtaining the required data over the 20 minute period the bull was euthanazed on humane grounds. As a result only 3 sets of data were recorded for animal 6.

Figure 4-1: Wildebeest bull with fractured left forelimb

During the fourth session of darting, the dart used for animal 1 malfunctioned resulting in only half of the intended dose being administered. As a result the times obtained for first effect and immobilization (incomplete) were protracted and deemed invalid and the animal was excluded from evaluation. The animal was physically caught and the antidote administered intramuscularly.

4.2. TIME TO FIRST EFFECT

The time to first effect was the time taken from drug administration to the first sign of the drug having an effect on the animal. Although signs such as slight imbalance or a stumble are indications that the drugs are taking effect, the parameter is largely subjective and tends to vary between observers. For this study, the parameter was standardized by having the same person undertaking all observations. Nonetheless, no significant differences in the time to first effect were evident between the control group (95 ± 10 s)

and either the etorphine, azaperone and 5000 IU hyaluronidase group (98 ± 9 s; p=0.859), or the low dose etorphine, azaperone and 7500 IU hyaluronidase group (96 ± 5 s; p=0.768). However, a significant difference was evident between the control group (95 ± 10 seconds) and the etorphine, azaperone and 7500 IU hyaluronidase group (67 ± 5 s; p=0.007).

4.3. TIME TO FIRST DOWN

The time to first down is the time taken from drug administration until the animal goes down for the first time, but rises when approached. This parameter was not evaluated in this study, as none of the animals attempted to rise when approached.

4.4. TIME TO IMMOBILIZATION

The time taken to immobilization was measured as the time from drug administration until the animal either became recumbent and unable to rise, or when the animal was standing but did not attempt to avoid human contact or resist handling. For this study, the parameter was standardized by having the same person undertaking all observations. All three test groups were significantly different to the control group (323 ± 37 s). The results obtained for the etorphine, azaperone and 5000 IU hyaluronidase group; etorphine, azaperone and 7500 IU hyaluronidase group; and the low dose etorphine, azaperone and 7500 IU hyaluronidase groups were 228 ± 24, 210 ± 23 and 268 ± 14 seconds respectively (p=0.002, p=0.001 and p=0.045 respectively).
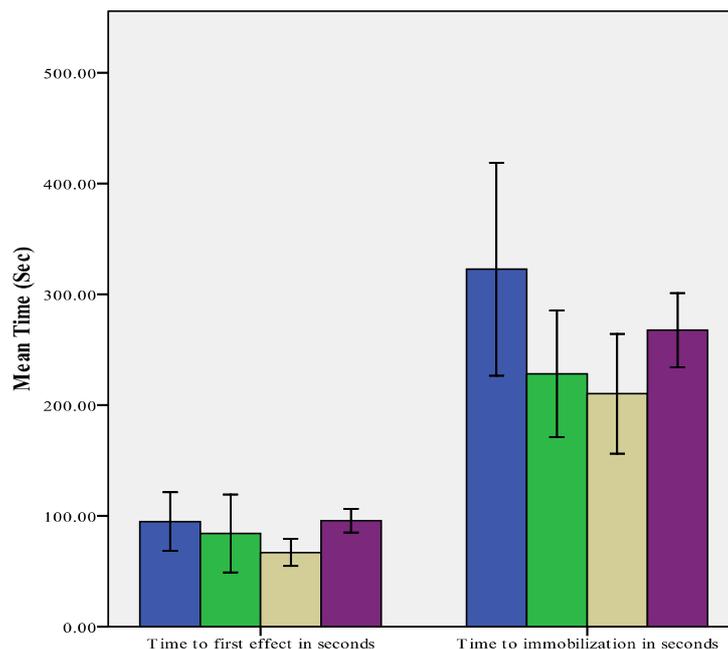
Figure 4-2: Graph depicting times to first effect and immobilization in seconds. First effect is the time taken from the injection (darting) of the animal to the time the animal started showing signs that the drugs were taking effect. The time to immobilization was the time taken from the injection (darting) of the animal to when the animal could be safely approached and blindfolded. The blue columns represent the captivon and azaperone (control) group. The green columns represent the captivon, azaperone and 5000 IU hyaluronidase group. The tan columns represent the captivon, azaperone and 7500 IU hyaluronidase group. The purple columns represent the low dose captivon and azaperone group with 7500 IU hyaluronidase group.

4.5. BIOLOGICAL PARAMETERS

Means and standard error of the means (SE) for systolic blood pressure (systolic BP), diastolic blood pressure (diastolic BP), blood oxygen saturation (SpO_2), pulse rate,

respiration rate and body temperature (temperature) in each treatment group during the 5 minute intervals are presented in table 4-1. Stage of anaesthesia is presented as the mode as the value is non-parametric.

Table 4-1: Means and standard error of the means for parameters, monitored at 5 minute intervals after the animals were immobilized, per treatment group

Parameter	Time Period	Treatment 1		Treatment 2		Treatment 3		Treatment 4	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Systolic BP	5 Minutes	132	14	124	6	132	14	134	13
Diastolic BP		71	16	69	8	79	13	70	7
SpO$_2$		91	2	88	1	86	3	87	2
Pulse		93	10	103	7	88	4	93	6
Respiration		26	3	26	2	26	3	25	3
Temperature		39.1	0.1	38.9	0.2	38.8	0.1	38.9	0.2
Stage		3	0	3	0	2	0	3	0
Systolic BP	10 Minutes	116	14	140	12	125	14	126	12
Diastolic BP		57	10	70	10	75	13	65	8
SpO$_2$		94	2	88	4	89	3	88	2
Pulse		81	6	89	9	87	5	92	10
Respiration		24	2	23	1	22	2	21	2
Temperature		39	0	39	0	39	0	39	0
Stage		2	0	2	0	2	0	2	0
Systolic BP	15 Minutes	130	17	131	13	122	7	135	18
Diastolic BP		51	8	57	8	58	5	69	8
SpO$_2$		92	2	88	3	89	3	90	2
Pulse		75	6	86	11	74	4	83	9
Respiration		22	1	21	2	20	2	20	1
Temperature		38.9	0.1	38.7	0.2	38.6	0.2	38.8	0.2
Stage		2	0	2	0	2	0	2	0
Systolic BP	20 Minutes	128	25	121	14	131	10	133	18
Diastolic BP		52	9	56	8	58	4	68*	8
SpO$_2$		94	1	88	3	93	1	92	1
Pulse		71	6	79	9	71	3	82	9
Respiration		21	2	20	2	20	2	19	1
Temperature		38.7	0.2	38.8	0.2	38.5	0.1	38.7	0.2
Stage		3	0	2	0	2	0	2	0

Treatment 1: Captivon + Azaperone; Treatment 2: Captivon + Azaperone + 5000IU Hyaluronidase; Treatment 3: Captivon + Azaperone + 7500 IU Hyaluronidase; Treatment 4: Low dose Captivon + Azaperone + 7500 IU Hyaluronidase; BP-Blood pressure; SpO$_2$- Blood oxygen saturation. SpO$_2$, BP presented at mmHg. Pulse presented as beats per minute. Respiration presented as breaths per minute. Stage is presented as the mode as the value is non-parametric. *Denotes a statistically significant difference ($p < 0.05$) when compared to the control using a univariate analysis of variance (ANOVA).

Based on the above results the only parameter found to be statistically significantly different to the control group over 20 minutes was the diastolic blood pressure in the low dose captivon, azaperone and 7500 IU hyaluronidase treatment group during the fourth 5 minute interval (p=0.017). None of the remaining biological parameters evaluated were significantly different from the control group which led us to conclude that the use of the hyaluronidase did not interfere with the immobilization procedure.

When the parameters were compared for changes over time (Table 4.2), a significant difference was present for the following parameters:

- Treatment group 1: temperature was marginally reduced at 20 minutes (0.045).

- Treatment group 2: systolic BP was significantly increased at 10 minutes (p=0.043 value), while pulse and respiration was deceased at 20 minutes (p=0.024 and 0.07 respectively).

- Treatment group 3: SpO_2 was significantly increased at 15 and 20 minutes (p = 0.014 and 0.023 respectively), while both pulse (p= 0.08 and 0,10 respectively) and respiration (p = 0.028 and 0.021 respectively) was significantly reduced for the same time period at 15 and 20 minutes. Temperature was marginally decreased at 20 minutes (p = 0.021).

- Treatment group 4: SpO_2 was significantly different at 20 minutes (p = 0.024).

Table 4.2: Parameter means and standard error of means for the four treatment groups presented over time

Parameter	Rx	5 Minutes		10 Minutes		15 Minutes		20 Minutes	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Stage[!]	1	2.67	0.21	2	0	2.33	0.21	2.5	0.34
Systolic BP		132.2	13.6	116.33	14	130	17.5	127.6	25.3
Diastolic BP		71	16	56.67	10.5	51.33	8.19	52	9.17
SpO_2		91.17	1.58	94	1.65	92.17	1.51	94.17	0.95
Pulse		92.5	10.3	81	5.82	75	6.48	70.5	6.38
Respiration		26.33	3.03	23.67	2.22	22.33	1.5	20.67	2.17
Temp.		39.07	0.06	39.02	0.09	38.92	0.1	38.68*	0.16
Stage[!]	2	2.5	0.27	2.25	0.16	2.13	0.13	2.13	0.13
Systolic BP		123.5	6.15	140.00*	11.8	131.1	12.6	120.8	14.2
Diastolic BP		69	7.69	70.38	10.2	57.25	8.28	55.63	8
SpO_2		88.38	0.92	87.63	3.77	88.38	2.62	88.13	3.36
Pulse		102.9	6.52	89.13	9.11	85.88	11.4	78.63*	9.37
Respiration		26.25	2.19	23	1.46	21.25	1.96	19.75*	1.79
Temp.		38.86	0.19	38.91	0.2	38.71	0.16	38.79	0.23
Stage[!]	3	2.38	0.18	2.25	0.16	2	0	2	0
Systolic BP		132.4	13.9	125	13.9	121.5	7.05	130.5	9.89
Diastolic BP		79.25	13.3	75	13.5	57.75	4.8	58.38	3.91
SpO_2		85.88	2.92	88.88	2.83	89.38*	2.69	92.75*	1.29
Pulse		87.62	4.05	87.13	4.56	74.00*	3.57	71.38*	3
Respiration		25.63	3.37	21.88	1.91	20.13*	1.61	20*	2.14
Temp.		38.79	0.13	38.74	0.13	38.61	0.16	38.53*	0.14
Stage[!]	4	2.63	0.18	2.25	0.16	2.13	0.13	2.25	0.16
Systolic BP		133.6	13.2	125.5	12.3	134.9	18.2	132.5	18.5
Diastolic BP		70.25	6.65	64.75	7.93	69.38	8.45	68	7.76
SpO_2		87.12	2.45	87.62	2.27	90.38	1.74	91.63*	1.03
Pulse		92.5	6.22	92.38	10.1	82.63	8.89	82.25	8.99
Respiration		25.25	2.7	20.5	1.59	20.25	1.33	19.25	1.31
Temp.		38.85	0.23	39.08	0.22	38.8	0.15	38.73	0.15

BP-Blood pressure; SpO_2- Blood oxygen saturation; Rx - treatment

Rx 1: Captivon + Azaperone; Rx2: Captivon + Azaperone + 5000IU Hyaluronidase;Rx 3: Captivon + Azaperone + 7500 IU Hyaluronidase; Rx 4: Low dose Captivon + Azaperone + 7500 IU Hyaluronidase;. BP presented at mmHg, Pulse presented as beats per minute, Respiration presented as breaths per minute, Stage is presentedas the mode as the value is non-parametric.*Denotes a statistically significant difference ($p < 0.05$) when compared to the 5 minute parameter reading using a paired t-test.

When the data is evaluated for trends, a trend of change over time was evident for all parameters except systolic blood pressure. For diastolic blood pressure (mmHg), pulse (beats per minute), respiration (breaths per minute) and temperature ($^{\circ}$C) a decrease was observed while a trend for increase in SpO_2 (mmHg) was observed. The trend was only statistically significant for the treatment 3 for SpO_2, pulse and respiration.
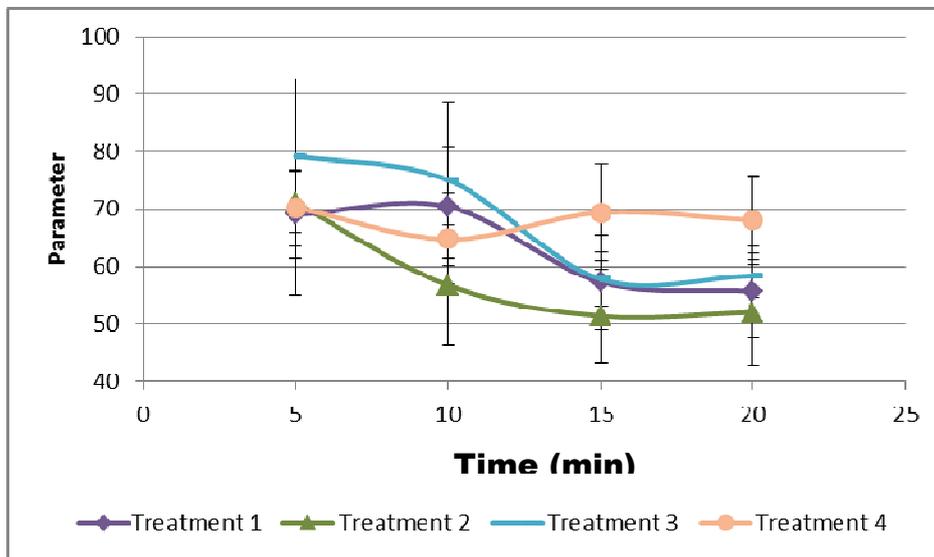

Figure 4-3: Illustration of the change in diastolic blood pressure (mmHg) values over time for the four treatment groups
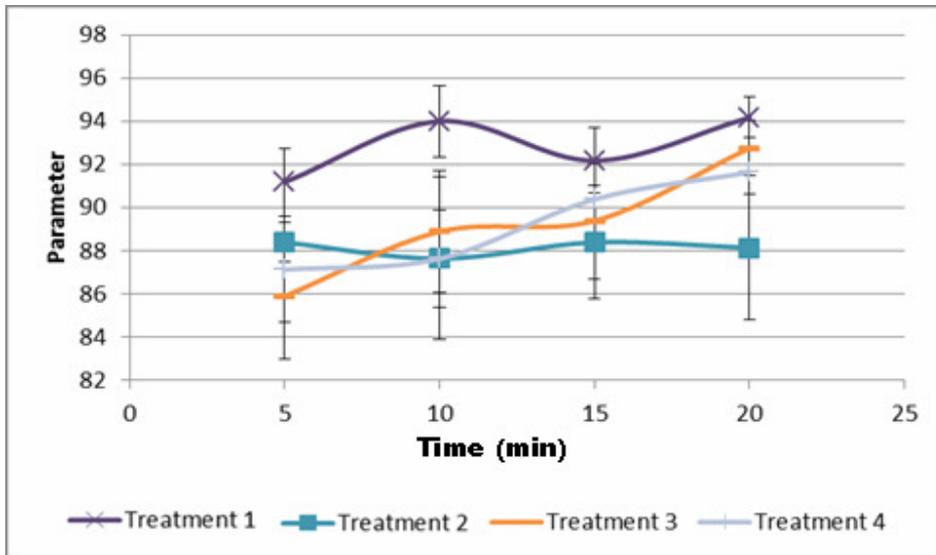
Figure 4-4: Illustration of the change in SpO_2 (mmHg) values over time for the four treatment groups
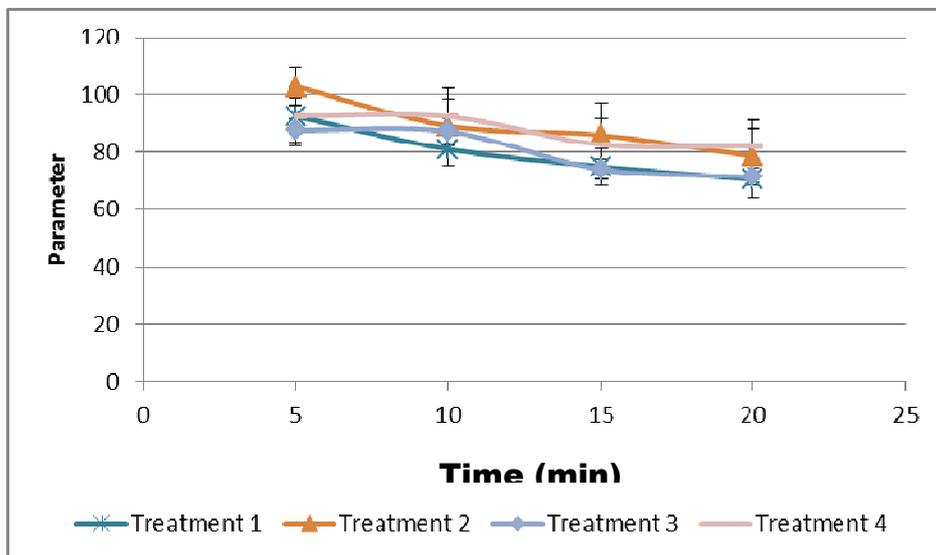

Figure 4-5: Illustration of the change in pulse (beats per minutes) over time for the four treatment groups
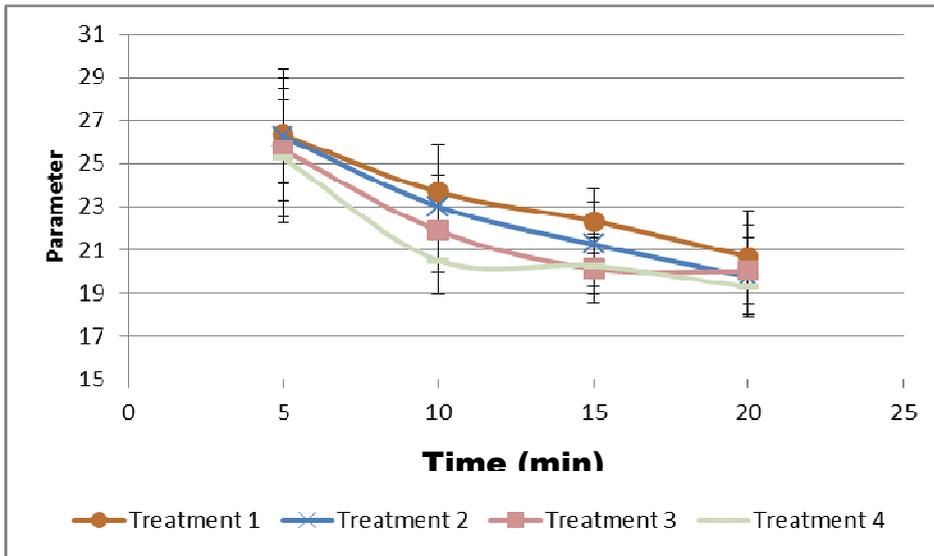
Figure 4-6: Illustration of the change in respiration (breaths per minutes) over time for the four treatment groups
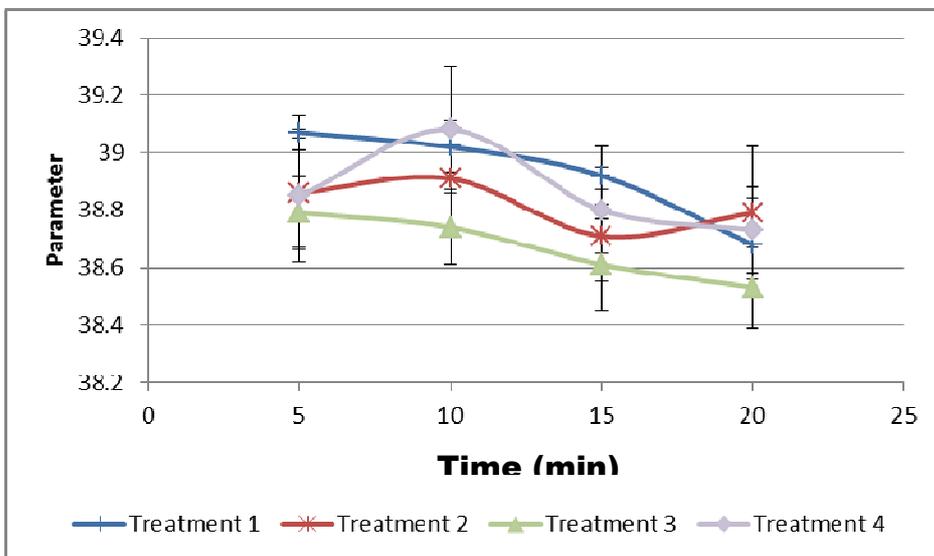

Figure 4-7: Illustration of the change in temperature ($^{\circ}$C) over time for the four treatment groups

<table>
<tr><td>Chapter
5</td><td># DISSCUSSION</td></tr>
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5.1. HYALURONIDASE IN IMMOBILIZATION COMBINATIONS

For this study, we speculated that the addition of hyaluronidase to the dart combination offered no significant benefit in reducing time to immobilization in blue wildebeest. When the 5000 and 7500 IU hyaluronidase treatment groups are compared to the control, to which no hyaluronidase was added, both treatment groups showed significantly reduced times to immobilization. The control group's time to immobilization was 323 ± 37 seconds whereas the time to immobilization in the 5000 IU hyaluronidase treatment group was 228 ± 24 seconds (p=0.002) and the and the time to immobilization in the 7500 IU hyaluronidase treatment group was 210 ± 23 seconds (p=0.001). Therefore the animals in the 7500 IU hyaluronidase treatment group showed a significantly reduced time to immobilization in comparison to the control group, which in real terms amounts to 100 seconds.

The benefit of a reduced time to immobilization is that less distance is covered by the animal prior to immobilization. For the 100 seconds of time saved we estimated that the addition of hyaluronidase could result in the animal being immobilized with a saving of approximately 550 meters, if the animal runs at an estimated 20 kilometers per hour. This reduced distance run prior to immobilization firstly reduces the risk hypoxaemia, hypercapnia, hyperthermia, acidosis and the resultant capture myopathy which may lead

to death of the animal, and secondly reduces the risk of losing an animal in thick bush or mountainous areas.[16,40]

Kock[33] found that by adding 4500 IU hyaluronidase and increasing the etorphine dose from 3 mg to 3.9 ± 0.18mg that the time to immobilization and capture-related stress could be reduced in black rhinoceroses. From our study we found that by simply adding 5000 or 7500 IU hyaluronidase to immobilization combinations the time to immobilization could be significantly reduced without the need to increase the etorphine dose. We believe that the addition of hyaluronidase to the immobilization combination increased the rate of etorphine absorption resulting in faster entry of the drug into the central compartment which led to the effective concentration being reached more rapidly than for the etorphine and azaperone treatment group. This ultimately led to a reduced time to immobilization bypassing the need to increase the etorphine dose.

We found that increasing the hyaluronidase dose from 5000 to 7500 IU (50%), further reduced the time to immobilization which is similar to the effect seen when the hyaluronidase dose was doubled during regional anaesthesia of the human eye.[32] In this particular study the addition of 3.75 IU/ml and 7.5 IU/ml hyaluronidase to a bupivicaine-lidocaine mixture improved the success of the initial retrobulbar/palpebral block (p<0.001 and p=0.006 respectively) when compared to the control group. No significant difference was however present between the two hyaluronidase groups (p-value not provided).[32] This study was able to demonstrate a dose/response relationship as time to immobilization within the 7500 IU hyaluronidase treatment group (210 ± 23 seconds)

was lower than for the 5000 IU treatment group (228 ± 24 seconds) although for both treatment groups the times to immobilization were significantly reduced when compared to the control (p=0.001 and p=0.002 respectively). The reason for the differences may therefore reside in the response of the tissue at the biophase as opposed to increased absorption seen i.e. the local anaesthetic may not be able to produce a faster effect on the peripheral nerves.

5.2. HYALURONIDASE AND LOW DOSE ETORPHINE

We expected the low dose etorphine and azaperone treatment group to which 7500 IU hyaluronidase was added to demonstrate times to immobilization similar to the control group (323 ± 37 seconds) but surprisingly the times to immobilization were 268 ± 14 seconds. Although this represents a statistically significant reduction in time to immobilization when compared to the control group (p=0.045), the reduced times to immobilization were not as significantly reduced, when compared to the control group, as the etorphine, azaperone and 5000 IU hyaluronidase group (228 ± 24 seconds, p=0.002) and the etorphine, azaperone and 7500 IU hyaluronidase group (210 ± 23 seconds, p=0.001).

A possible explanation could be that the control treatment group had a slower rate of absorption which resulted in a longer time to reach effective concentration. The results would suggest that when hyaluronidase (5000 or 7500 IU) was added to the standard etorphine and azaperone dose, the rate of absorption was increased which reduced the time to effective concentration. When 7500IU hyaluronidase is added to the low dose

etorphine and azaperone treatment group we suspect that the rate of absorption is increased resulting in the effective concentration being reached at a faster time than the control treatment group although the total extent of absorption is less for the low dose etorphine and azaperone treatment group.

This concept is best expressed by the following pharmacokinetic equation, where C_p represents the concentration at any time (t); K_a is the absorption rate constant, F is the fraction of drug absorbed, D the dose administered, Vd the volume of distribution, and K_{el} the elimination rate constant:

$$Cp = \frac{K_a FD}{V_d (K_a - k_{el})} (e^{-k_{el}t} - e^{-k_a t})$$

As evident in the equation, changes to K_a, dose or both with result in a change in the plasma concentration achieved.[12] This concept is best theoretically illustrated in the figure below (Fig 5-1). In this figure, the theoretical effective concentration is set at is 3 ug/ml, with F and V_d being fixed. Treatment one is administered at a dose of 350 mg with a ka of 0.4h[-1]. Treatment 2 is administered at a lower dose of 200 mg and the same k_a (0.4h[-1]) of treatment 1. Treatment 3 is administered at the same dose at treatment 1 (350 mg) but absorption is assumed to result at a lower K_a of 0.10h[-1]. As evident can be seen, the three curve all reach the EC at different time, which supports the conclusion that an increase in the rate of absorption will result in a faster effect, even with a change in dose.
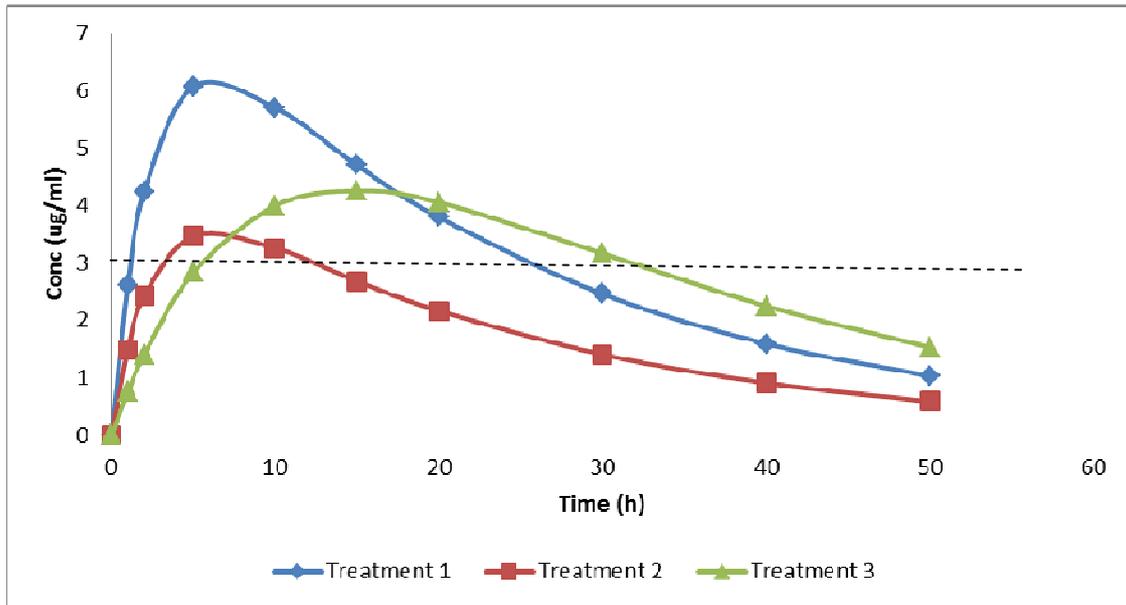
Treatment 1 is administered at a dose of 350 mg with a ka of 0.4h^{-1}. Treatment 2 is administered at a lower dose of 200 mg and the same k_a (0.4h^{-1}) of treatment 1. Treatment 3 is administered at the same dose at treatment 1 (350 mg) but absorption is assumed to result at a lower K_a of 0.10h^{-1}. Vd was fixed at 43L and F at 0%.

Figure 5-1: Theoretical representation of how dose and rate of absorption impact on the time to reach effective concentration

5.3. BIOLOGICAL PARAMETERS

Besides the statistically significant differences in times to immobilization between the treatment and the control groups, no statistically significant differences in any of the biological parameters were evident between the treatment groups.

Trends were evident in diastolic blood pressure, SpO$_2$, pulse, respiration and temperature. The changes were only significant for the normal etorphine dose with 7500 IU hyaluronidase. The reason for systolic blood pressure to remain unchanged over time and for the diastolic blood pressure to decrease is unknown and will require further investigation. SpO$_2$ changes are possibly explained by the respiratory depression induced

by etorphine as a side effect, in combination with the animals initially being in lateral recumbency following immobilization which leads to a ventilation-perfusion mismatch.[59] This slow correction in SpO_2 hereafter most likely reflects the improved oxygenation that resulted from the animals being placed in sternal recumbency for the 20 minute monitoring period.

The trends seen for the pulse and respiratory rate are not known at this stage. A similar trend was, however, seen in rhinoceros immobilizations where the heart and respiratory rates decreased over time and only stabilized at a certain level 20 to 30 minutes post immobilization.[59]

The decrease in body temperature most likely reflects a slow return of the animal's basal temperature to a more normal point, as opposed to a true drop in basal temperature. From literature it is known that etorphine induces central nervous system-excitement and muscular hypertonicity with a resultant hyperthermia prior to immobilization.[6,40] Once immobilized the animals in this study were placed in a shady, cool area that enabled their body temperatures to gradually decrease.

5.4. HYALURONIDASE AND DEAD SPACE

Although it is believed that the action of hyaluronidase on the interstitial barrier is short-lived and reversible,[58] a potential concern when using hyaluronidase is the onset of infection in the dead space created when the interstitial barrier between cells is broken down. During our study each dart wound was treated with an antibiotic and no animal

experienced any pain, discomfort or swelling at the dart sites. This supported the findings of Moore[44] who found no sloughing or inflammation at the sites of injection (no antibiotic administered) in 519 cases of local and nerve block analgesia using pontocaine hydrochloride together with hyaluronidase.

The wound, and subsequent dead space, created by the entry of the dart's needle into the animal's muscle is quite possibly larger than the dead space created by the hyaluronidase and for this reason it remains important to treat dart wounds with an appropriate antibiotic prior to the recovery of the immobilized animal.

Chapter 6 CONCLUSIONS

*B*ased on the times to immobilization, the results of this study show that when either 5000 or 7500 IU hyaluronidase is added to a standard etorphine and azaperone drug combination, the time to immobilization in blue wildebeest is reduced.

The lower etorphine and lower azaperone combination together with 7500 IU hyaluronidase was expected to produce times equivalent to the control, but produced times to immobilization that were statistically significantly lower than the control. Based on these results, the hypothesis is rejected as the addition of either 5000 or 7500 IU hyaluronidase to an etorphine and azaperone drug combination reduced the time to immobilization in blue wildebeest.

The addition of hyaluronidase into immobilization combinations should definately be considered during all wildlife immobilizations and especially in those involving endangered animals and animals prone to capture related complications. It would be advisable to incorporate hyaluronidase, at a dose of either 5000 or 7500 IU per dart, with the higher dose being the most beneficial.

6.1. FUTURE TRENDS

As a result of significantly reducing times to immobilization in blue wildebeest with the addition of hyaluronidase to a drug combination, we agree with Lloyd et al.[39] that the addition of hyaluronidase to an antidote could reduce recovery times when administered intramuscularly. This therefore warrants investigation for those cases in which the administration of the antidote intravenously is not possible.

Further studies to determine the pharmacokinetics that result in the reduced times to immobilization when using the low dose of etorphine and azaperone together with 7500 IU hyaluronidase would be of benefit. If this finding can be consistently reproduced, it will enable one to reduce the etorphine dose in future immobilizations which will not only reduce the risk to the animal but will also reduce capture costs.

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