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A NOVEL PROTOCOL FOR ANAESTHESIA OF CHACMA BABOONS

UUDNE PROTOKOLL KARUPAAVIANIDE ANESTEESIAKS

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The object of this study was to evaluate the efficacy and safety of the combination of butorphanol, azaperone and medetomidine with a low dose of ketamine (BAM-Ket) for immobilisation of chacma baboons (Papio ursinus). Six male chacma baboons were used in a dose titration study and another fifteen baboons were immobilized and monitored for physiological response to BAM-Ket. The dose titration study showed that ketamine is needed in the protocol since BAM on its own cannot produce complete immobilisation in baboons. In the second part of the study, following doses were used: BAM 0.01 ± 0.005 ml/kg (butorphanol 0.31 ± 0.15 mg/kg, azaperone 0.12 ± 0.06 mg/kg, medetomidine 0.12 ± 0.06 mg/kg) and ketamine 2.04 ± 0.22 mg/kg. BAM-Ket protocol produced a minimum of 40 minutes of immobilisation, with induction times of 3.46 ± 1.36 minutes and full recovery in 4.8 ± 2.8 minutes after receiving reversals (atipamezole, naltrexone). The main side-effects seen were hypoxia (SpO2: 62 ± 13%; PaO2: 37 ± 10 mmHg) as well as slightly elevated EtCO2 (63 ± 9 mmHg) and PaCO2 (63 ± 9 mmHg). In conclusion, BAM with a low dose of ketamine produces short-term general anaesthesia in baboons that allows for minor veterinary procedures such as blood collection and microchipping.

Keywords: baboon, immobilisation, butorphanol, azaperone, medetomidine
Uudne protokoll karupaavianide anesteesiaks

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Selle uuringu eesmärk oli hinnata butorfanooli, asaperooni ja medetomidiini kombinatsiooni koos ketamiini madala annusega (BAM-Ket) efektiivsust ja ohutust karupaavianide (Papio ursinus) immobiliseerimiseks. Sobiva BAM annuse määramiseks kasutati kuut isast karupaaviani. Veel viisteist paaviani immobiliseeriti ja jälgiti selgitamaks karupaavianide füsioloogilisi muutusi BAM-Ket anesteesia ajal. Sobiva annuse uuring näitas, et ketamiini lisamine on vajalik, kuna ainuüksi BAM ei taga paavianidel vajalikku immobilisatsiooni taset. Uuringu teises osas kasutati järgmisi annuseid: BAM 0,01 ± 0,005 ml/kg (butorfanool 0,31 ± 0,15 mg/kg, asaperoon 0,12 ± 0,06 mg/kg, medetomidiin 0,12 ± 0,06 mg/kg) ja ketamiin 2,04 ± 0,22 mg/kg. Kasutatud BAM-Ket anesteesia tagas minimaalselt 40-minutilise immobilisatsiooni, induktsioon oli 3,46 ± 1,36 minutit ja täielik taastumine toimus 4,8 ± 2,8 minutit peale antidoodi (atipamezool, naltreksoon) manustamist. Peamised täheldatud körvaltoimed olid hüposia (SpO2: 62 ± 13%; PaO2: 37 ± 10 mmHg), samuti pisut kõrgenenud EtCO2 (63 ± 9 mmHg) ja PaCO2 (63 ± 9 mmHg). Kokkuvõtteks võib öelda, et väikese ketamiiniannusega BAM tekitab paavianides lühiajalise üldanesteesia, mis võimaldab väiksemaid veterinaarprotseduure, nagu vere kogumine ja mikrokiibistamine.
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LIST OF ABBREVIATIONS

AUC – Area under the curve

BAM – Fixed-dose combination of butorphanol, azaperone and medetomidine

BAM-Ket – Fixed-dose combination of butorphanol, azaperone and medetomidine with a low dose of ketamine

BP – Blood pressure

CRT – Capillary refill time

DBP – Diastolic blood pressure

EtCO₂ – End-tidal carbon dioxide

RR – Respiratory rate

HR – Heart rate

IM – Intramuscular

MAP – Mean arterial blood pressure

PaCO₂ – Partial pressure of arterial carbon dioxide

PaO₂ – Partial pressure of arterial oxygen

SBP – Systolic blood pressure

SpO₂ – Haemoglobin oxygen saturation
INTRODUCTION

There are various methods of handling and restraining primates, and occasionally chemical immobilisation is needed, especially when working with semi-wild or wild individuals. Chemically immobilising animals makes it possible to handle, transport or treat animals that would otherwise be too dangerous to approach and handle. It also reduces stress related injuries such as capture myopathy, associated with physical restraint (Paterson, 2014). Different agents have been tested in primates, most commonly ketamine in combination with either benzodiazepines or α2-agonists. In this thesis, a fixed-dose combination of butorphanol, azaperone and medetomidine with a low dose of ketamine (BAM-Ket) is considered as an alternative protocol to previously used immobilisation protocols in baboons (Papio). The fixed-dose combination of butorphanol, azaperone and medetomidine (BAM) has shown to produce safe and reliable immobilisation in both different ungulate and carnivore species (e.g. Wolfe et al., 2008; Miller et al., 2009; Semjonov et al., 2017). It has recently also been shown that BAM can be used to produce immobilisation in macaques (Macaca mulatta) (Malinowski et al., 2019). The compound is both reversible and is believed to have good analgesic properties, making it useful for mildly painful procedures during immobilisation. The low dose of ketamine is used to enable complete immobilisation, without having significant impact on the reversibility of the combination. For evaluating the effects of BAM-Ket in baboons, this study was performed in two parts. In the first part a titration study of BAM was performed. The second part of the study consisted of a prospective clinical trial in which physiological parameters of immobilised baboons were monitored. The main objective of the study was to determine the efficacy of the protocol as an anaesthetic for baboons. For this, the combination’s ability to produce immobilisation was evaluated. This was done by analysing induction times, depth and duration of anaesthesia, as well as the recovery processes. A second aim was to evaluate the safety and risks of using BAM-Ket in baboons, which was done by detailed monitoring of physiological parameters during the immobilisation. Any adverse effects were documented, with an extra focus on the parameters describing the cardiorespiratory system.
1. LITERATURE REVIEW

1.1. Natural history of baboons

Baboons are primates belonging to the family *Cercopithecidae* of the suborder *Haplorhini*. The chacma baboon is one of five baboon species within the family. Baboons are omnivores and their diet consists mostly of different plants, fruits, nuts, and they might occasionally feed on insects and small rodents. Chacma baboons live in strict social structures called troops. A troop is led by an alpha male who forms strong social bonds with the females and their offspring. The role of the alpha male is vital for maintaining the structure and harmony within the troop. Females usually inherit their rank from their mother, whereas the ranks between adult males are in a constant turnover. The competition among males for female partners is intense and may result in violent fights (Kalbitzer *et al.*., 2015). An alpha male showing signs of weakness may be challenged by other males that wish to take his place, therefore younger males of lower rank will be suppressed or chased away from the troop. Young males usually migrate from their native troop when they reach puberty and may then join other troops (Barton *et al.*., 1996). Being an opportunistic species, baboons may migrate into urban areas in search for food and new territories, where they may end up feeding on agricultural fields or waste left by humans. Baboons are disliked in many human societies, as they may be dangerous, aggressive, and are known to cause damage to property e.g. by destroying crops (Hill & Wallace, 2012) This is why conflicts with humans are considered one of the biggest threats to the survival of the wild-life populations (Hoffman *et al.*, 2012). Another matter of concern is that, since baboons are closely related to humans, we share many diseases with them, e.g. *Mycobacterium tuberculosis* is a zoonotic pathogen that raises concern (Keet *et al.*, 2000). This pathogen is found in different species of primates and can cause serious illness in humans. Other pathogens that were recently detected in a screening of wild chacma baboons in South Africa included hepatitis A virus, cytomegalovirus, Epstein-Barr viruses and herpes B virus (Drewe *et al.*, 2012; Dickens *et al.*, 2012; ...
To prevent conflicts between humans and wild animal populations, there is a need for developing different strategies for controlling the movements of the troops. This requires cooperation of many different instances as well as new, safe and efficient techniques in handling these animals, both as entire troops as well as on an individual level. Wild baboons are not only captured for population control purposes but also for conservational reasons. There are different organizations that aim to preserve the wild troops by moving problematic animals away from populated areas, nursing orphans and working with disease control (e.g. Centre for Animal Rehabilitation and Education (C.A.R.E.) and Riverside Wildlife Rehabilitation Centre).

1.2. Handling of baboons

1.2.1. Physical restraint of baboons

Working with primates presents many challenges, and there are several different approaches for handling these animals. Some approaches are more invasive than others, however, safety of both the animal as well as of the handler should be the primary concern. Restraint is always stressful for the animal and simply approaching a wild animal for physical restraint might be both dangerous and impractical (Ølberg & Sinclair, 2014). Some captive individuals can be trained to cooperate, e.g. reaching out an arm for blood sampling or injections. This technique minimizes the stress for the animal; however, it is time consuming to train animals and the technique is unsuitable for wild individuals. Physical restraint for simple procedures or measurements can be achieved by using specific squeeze cages or restraint chairs (figure 1).
A downside in using such methods is that they may cause significant stress to the animal. Stressed animals are not only more dangerous to handle, but may develop severe complications, including metabolic acidosis due to increase in blood lactate levels and induced capture myopathy (Bush et al., 1977; Paterson, 2014). This applies especially when working with semi-wild or wild individuals. Furthermore, it is unethical to cause unnecessary stress and suffering to animals (European commission, 2007). Therefore, especially any procedures causing pain or discomfort should be performed under anaesthesia.
1.2.2. Restraint of baboons using chemical immobilisation

For the above-mentioned reasons, the chemical immobilisation may be the only reasonable option, especially if any painful manipulations have to be performed. In some cases, the animals may be caught in cages prior to injection. This allows for closer approach, which in turn may allow the use of pole-syringes for administering the drug. Alternatively, the delivery can be done by using remote administration techniques. Remote drug delivery is challenging since it requires good darting skills and the person handling the gun needs to get close enough to ensure a safe shot (Isaza et al., 2014). Safe intramuscular injection sites in baboons are in the shoulder muscles or the hindquarters (gluteal muscles). The skin and muscles of the baboons are thin, and soft tissue or even bone damage is common. Therefore it is essential to choose the correct dart and needle size as well as carefully adjust the darting speed according to the distance, to prevent unnecessary trauma (Shury, 2014). Monkeys tend to pull out the dart as soon as it hits them, making quick release darts useful, to ensure that a full dose of the drug is injected. By ensuring a safe environment prior to darting, the risk of injuries associated with induction can significantly be reduced (Arnemo et al., 2014). Darted animals may try to climb trees in search of safety, which is problematic since they can fall and injure themselves once the effect of the anaesthetic sets in. A real challenge when working with monkeys is that their intelligence enables them to quickly learn to discover the source of danger. It may be easy to dart the first animals in a troop, but as the rest of the animals start to recognize the source of the darts, they learn quickly to hide whenever they see a dart gun. It is also vital to make sure that the baboons are fully recovered after immobilisation before re-joining them with the troop. Due to the strict hierarchy in the troop, poorly recovered animals showing signs of weakness may be attacked by rival troop-members (Ølberg & Sinclair, 2014).
1.2.3. Handling and monitoring of anaesthetised baboons

Baboons should be kept in lateral recumbency during the immobilisation, and to decrease external stimulation, the use of a blindfold is advised. To ensure safety, it is vital to monitor the animal during the procedure – starting from the induction, throughout the immobilisation until the animal has reached complete recovery. A minimum requirement is to monitor the respiration, however, once the animal is approachable, most common reflexes, heart rate (HR) and body temperature will give valuable information about the depth of anaesthesia and individual response to the anaesthetics. Anaesthetic depth is mainly evaluated based on palpebral reflex, reaction to stimuli (micro-chipping, injections) and jaw tonus. In case of painful stimulation, changes in HR, blood pressure (BP) and respiratory rate (RR) may be expected if the anaesthetic depth is light. The availability of antagonists to reverse the immobilisation is of great value, both to reverse any observed adverse effects and to ensure a quick and smooth recovery (Wenkler, 1998). Primates have a large surface-area-to-body-mass ratio and therefore they are prone to heat loss during immobilisation (Ølberg & Sinclair, 2014). This effect could further be accentuated by the choice of anaesthetics, e.g. both opioids and α2-agonists affect thermoregulation (Gutstein & Akil, 2001; Sinclair, 2003). Additionally, one concern regarding immobilisation of primates is that they are known to commonly exhibit signs of hypoxia when these types of anaesthetics are used (Butelman et al., 1995; Liguori et al., 1996; Sinclair, 2003). It is also common especially for wild primates to regurgitate under anaesthesia. Due to this it may be advisable to intubate the animal and pay close attention to the oxygenation e.g. by using a pulse oximeter. For more exact measurements arterial blood gas analysis can be performed as advanced monitoring. If the baboon exhibits severe hypoxia, supplemental oxygen should be provided.
1.3. Anaesthetics

1.3.1. Ideal chemical immobilisation protocol

None of the drugs used today, fulfil all the criteria for an ideal anaesthetic agent. An anaesthetic agent should support a safe and easy drug delivery, meaning that the drug does not pose any significant danger to the person handling it, and that the therapeutic dose of the drug is of a convenient volume and consistency (solubility) for administration. Substances that are used for immobilisation of wild animals should always be suitable for intramuscular (IM) administration, as other ways of administration are highly impractical or even impossible. The therapeutic interval for the drug should be broad, and the toxic dose should be significantly higher than the effective dose. In other words, the drugs should have no substantial side-effects with minimal effect on either the cardiovascular or the respiratory system and they should be easily metabolized by the organism without producing toxic metabolites (Digger & Viira, 2008). By using a combination of different substances, the synergism of the combined drugs allows for decreasing of the doses of each substance and thereby theoretically also minimizing the dose dependent adverse effects of these compounds (Kreeger, 2002). In addition, the compound should be effective and safe to use on a broad variety of species. Ideally the drug should reach full action quickly, with a short or no excitement phase, and the length of effect should be suitable for simple procedures. Such procedures may include wound treatments, injections, micro-chipping, or short distance transportation. As some of these procedures are considered mildly painful, anaesthetics with analgesic properties are beneficial. It should also be safe to top-off the drug in case longer anaesthesia is needed. The drug should provide a fast and smooth induction, followed by a stable and uneventful recumbency. The recovery should be smooth and complete, without re-sedation. Ideally, an agonistic agent should also be available for quick reversal of the anaesthesia.
1.3.2. Anaesthetics generally used in primates

Several different pharmaceuticals have been used in primates to date, however the challenge lies in finding a compound that is safe and reliable for every individual, as well as in several different species of primates. The most used anaesthetics in primates are dissociative anaesthetics. These are either administered alone or more commonly in combination with α2-agonists or benzodiazepines (Jalanka, 1989; Horne, 2001; Sun et al., 2003). For example, ketamine used alone produces around 30 minutes of immobilisation at a dose of 10 mg/kg in macaques (Sun et al., 2003). But since ketamine, when used alone, tends to produce rough inductions, poor muscle relaxation and eventful recoveries, it is preferably used in combination with other compounds (Mion & Villevieille, 2013). Langoi et al. (2009) conducted a study on olive baboons, in which a ketamine-xylazine (10 mg/kg; 0.5 mg/kg) protocol produced an immobilisation lasting for approximately one hour before spontaneous recovery. During recent years also other protocols have been suggested for primates, for example, ketamine used in combination with medetomidine (3.0 mg/kg; 0.15 mg/kg) produces immobilisation times up to 70 minutes in macaques, and in golden-headed lion tamarins a duration of 45 minutes when a dose of 10 mg/kg of ketamine and 0.02 mg/kg of medetomidine is used (Sun et al., 2003; Selmi et al., 2004). Lee et al. (2010) conducted a study where ketamine in co-administration with various sedatives was evaluated in both rhesus and crab-eating macaques. The study suggested that by combining with medetomidine, ketamine does not only produce deep and reliable anaesthesia, but also provides better muscle relaxation, in comparison to when ketamine is used alone, or when a ketamine-midazolam combination is used. Another study conducted by Woolfson et al. (1980) showed that good muscle relaxation as well as reduced anaesthesia associated epileptoid movements can be achieved in olive baboons by combining ketamine with benzodiazepines. In addition to ketamine, other dissociatives such as tiletamine/zolazepam combinations have been used in primates (Naples et al., 2010). The tiletamine/zolazepam protocol is fast acting, with the immobilisation lasting up to several hours. However, since there are no readily available reversal agents to this protocol, the recovery is prolonged (Ølberg & Sinclair, 2014). The availability of a complete reversal of the anaesthetics is of high value, and for that purpose BAM can be considered a viable alternative. The reversible BAM protocol was also recently tested in rhesus monkeys with promising results (Malinowski et al., 2019).
1.3.3. BAM

BAM is a fixed-dose combination of three different active components: butorphanol (30 mg/ml), azaperone (12 mg/ml) and medetomidine (12 mg/ml). The effect of BAM can be fully reversed. In primates atipamezole is used to reverse medetomidine at 10 times the actual dose of medetomidine in mg (1:10) and naltrexone hydrochloride is used to reverse butorphanol at one time the actual dose of butorphanol in mg (1:1). Azaperone is a short-acting tranquillizer lacking a reversal agent, however, due to its low dose and therefore negligible impact on the recovery, no reversal is needed. The enzyme hyaluronidase is added to the drug combination to increase absorption and reduce induction time. Semjonov et al. (2018) conducted a study in which the influence of the enzyme hyaluronidase on drug absorption was evaluated in blesbok, and the results showed that hyaluronidase significantly reduces the induction time of BAM from 9.6 ± 3.2 minutes when only BAM was used to 5.1 ± 0.8 minutes when hyaluronidase was added to BAM.

Butorphanol is an opioid that can be classified as a narcotic agonist-antagonist and is used in veterinary medicine both for its species-specific analgesic properties as well as in co-administration with other drugs for chemical immobilisation. In many species it is known to have excellent sedative properties and it acts as an agonist on κ-receptors and as a competitive antagonist on µ-receptors (Pathan & Williams, 2012). The κ-receptor activity is thought to be mainly responsible for the strong sedative effect of butorphanol. In primates, however, studies show that butorphanol acts as a partial agonist on µ-receptors, with a weaker effect on κ-receptors. This results in a weaker sedative effect in primates; however, the analgesic properties may be enhanced as µ-agonism is mainly responsible for the analgesic effect of the drug (Butelman et al., 1995; Liguori et al., 1996). The analgesic effect of opioids is derived from blocking the transmission of nociception in the dorsal horn of the spinal cord, resulting in inhibition of afferent pathways, activation of descending inhibitory pathways and decrease in the release of neurotransmitters (Pathan & Williams, 2012). Butorphanol has a duration of action of about two to three hours, depending on species, type of pain, dosage, and administration route. In comparison to more potent opioids, butorphanol is considered to have minimal effect on the cardiovascular system and it can be administered intravenously because it does not cause
histamine release (Branson et al., 2001). One concern of opioid use is the suppressing effects on the respiratory system. Butorphanol has shown to produce significant decrease in oxygen saturation in primate species. In rhesus monkeys, respiratory depression was seen in doses ranging from 0.001–0.32 mg/kg without an evident ceiling effect (Butelman et al., 1995). In another study of the same species, the minute volume of respiration was decreased to one third of base values when butorphanol was given at a dose of 0.3 mg/kg (Liguori et al., 1996). Also, in humans, doses equivalent to 0.03–0.06 mg/kg reported to produce significant respiratory depression (Zucker et al., 1987). However, in case of severe respiratory suppression, supplemental oxygen can be given, and the action of the opioid can be completely reversed by opioid antagonists such as naltrexone.

Azaperone is a tranquilizer, most widely used in pigs for controlling aggression when mixing animals from different groups (Blackshaw, 1981). Lately it is also more frequently used in various wild animals and studies have been published about azaperones’ tranquilizing effects in species such as white-tailed deer and African buffalo (Miller et al., 2009; Szabó et al., 2015). There is, however, very little known about the effect of azaperone in primates. Apart from the study by Malinowski et al. (2019) on the use of BAM in macaques, there is a short study by Meltzer et al. (1988), describing azaperone’s suppressing effect on electro-ejaculation in chacma baboons. Azaperone is a butyrophenone that is classified as a neuroleptic drug with anti-psychotic properties. The action of the drug is mediated through blocking of the D2-dopamine receptors in the central nervous system, and to some extent, serotonergic and adrenergic transmission. Azaperone has an effect on the behaviour and autonomic functions and causes decreased response to environmental stimuli without sedative effect or interference with motor functions. The drug tends to decrease the mean arterial blood pressure (MAP) and, depending on the dose, the stroke volume and cardiac output. The duration of action is considered reasonably short with effects usually lasting less than 4 hours. In case of overdosing, possible side-effects, such as hypotension, and extrapyramidal signs, such as nervousness and abnormal motic movements (dystonia, tremor and bradykinesia), may be seen, and the antidopaminergic action of azaperone can impair thermoregulation (Barletta & Eichstadt-Forsythe, 2017).
Medetomidine is a selective and potent $\alpha_2$-receptor agonist. It has sedative, analgesic and anxiolytic effects, which have been evaluated in both domestic and wild species (e.g. Sinclair, 2003; Sun et al., 2003; Lee et al., 2010). The $\alpha_2$-agonist shows synergistic effects in combination with several other drugs. With butorphanol it produces good CNS sedation and reduces the amount of ketamine needed for anaesthesia (Kreeger, 2002). After administration of $\alpha_2$-agonists, a pronounced vasoconstriction-associated hypertension can be expected. This in turn is rapidly followed by hypotension due to the increase in vagal tone, which reduces both the HR and cardiac output (Scheinin et al., 1989; Sinclair, 2003). Medetomidine’s inhibitory effect on the respiratory system results in a decrease in RR, especially when combined with other sedatives (Sinclair, 2003). In some cases, it may be difficult to evaluate true hypoxia since peripheral vasoconstriction may falsely exaggerate a low oxygen saturation if a pulse oximeter is used. There are several studies in which medetomidine was used in various primate species without marked signs of hypoxia. Macaques anaesthetised with a combination of medetomidine and ketamine showed minimal negative cardiopulmonary effect (Lee et al., 2010). Tamarins immobilised with a ketamine-medetomidine combination exhibited peripheral oxygen saturation (SpO$_2$) above 94% throughout the immobilisation, although decreased RR was observed (Selmi et al., 2004). Other physiological responses to $\alpha_2$-agonists include emesis and impaired thermoregulation. Since medetomidine acts on the same receptor as adrenaline, the onset of medetomidine action may be prolonged if the animal exhibits high levels of stress, which is often the case when working with wild animals (Sinclair et al., 2003). A great benefit of medetomidine is its reversibility. Atipamezole is a competitive central antagonist to medetomidine and can completely reverse the action of medetomidine (Virtanen et al., 1989).

The use of different butorphanol-azaperone-medetomidine mixtures has been evaluated and has shown promising results in a variety of wild animals. The first studies were carried out in black bears (Wolfe et al., 2008) and white-tailed deer (Miller et al., 2009; Siegal-Willott et al., 2009). In these species, BAM provided good reversible immobilisation, while keeping physiological parameters within safe limits (Miller et al., 2009; Wolfe et al., 2008). Other ratios of the combination were evaluated in species such as Nubian Ibex (Lapid et al., 2015) and Bennet’s wallabies (Watson et al., 2016) with varying results. As the ratios of the compounds differ between the studies, the results are not entirely comparable. Furthermore, the results indicate that the effective doses are species specific. The commercially produced fixed-dose drug
combination BAM (Wildlife Pharmaceuticals (Pty) Ltd., South Africa) has been evaluated in several wild species such as African lion (Semjonov et al., 2017), cheetah (Semjonov et al., 2019) and blesbok (Semjonov et al., 2018) with very promising results. In a more recent study, BAM was evaluated in macaques (Malinowski et al., 2019). Results showed that the monkeys were fully immobilised using BAM in doses ranging from 0.016–0.024 ml/kg. However, some adverse effects were observed, as about two thirds of the macaques showed signs of either bradycardia or hypotension, and hypoxia was seen in all animals.

1.4. Conclusions of the literature review

BAM may be considered as an alternative to commonly used protocols for the immobilisation of baboons. Both butorphanol and medetomidine have previously been used in immobilisation protocols for primates, and BAM has shown to be an efficient immobilising agent in a variety of ungulate and predator species. By combining butorphanol, azaperone and medetomidine, the aim is to produce deep enough immobilisation, with an analgesic effect that would be suitable for mildly painful procedures (e.g. wound treatment). One of the greatest benefits of the drug combination is its reversibility, whereas limitations may include low efficacy and negative effects on the respiratory system. In case BAM is not able to produce complete immobilisation of baboons, ketamine can be added to the protocol. To determine the effects and safety of BAM, the drug combination is tested in a clinical trial in which both qualitative and quantitative effects can be analysed.
2. AIMS OF THE STUDY

This study aims to explore chemical immobilisation in primates, more specifically in chacma baboons (*Papio ursinus*). To do this, the benefits and limits of anaesthetic protocols readily used in baboons and related species are evaluated, and the prospect of using a combination of butorphanol, azaperone and medetomidine (BAM) as an alternative protocol for the immobilisation of baboons is explored.

The aims are listed as follows:

- Determining the ability of BAM to produce immobilisation in baboons.
- Evaluating the physiologic response to BAM in baboons.
- Determining the safety of BAM by documenting potential adverse effects.
3. MATERIALS AND METHODS

3.1. Study design

To obtain the necessary data, the study was conducted in two parts. In the first part, a dose titration of BAM was performed, with the aim of determining an optimal dose to be used in the second part of the study, constituting a prospective clinical trial, in which the aim was to further determine the safety and physiological response of the drug combination in chacma baboons.

3.2. Animals and ethical approval

All procedures and the use of animals were approved by the Wildlife Pharmaceuticals Animal Ethics Committee. The baboons were handled and maintained in accordance with the South African National Standard Zoo and aquarium practice (SANS, 2005), the Animals Protection Act 71 of 1962 (Republic of South Africa, 1962) as well as the Guidelines for the accommodation and care of animals used for experimental and other scientific purposes (European Commission, 2007). All clinical studies were completed in April 2018 in the province of Limpopo, South Africa.

The animals used in the first part of the study were housed at the Centre for Animal Rehabilitation and Education in Limpopo, South Africa (C.A.R.E.). The sample sizes were determined based on sample sizes used in similar previously published studies. Six animals were considered a sufficient cohort size to establish a suitable BAM dosage for this species. Thus, the dose titration study of BAM included six adult male chacma baboons, that were immobilised
for the purpose of inserting deslorelin implants (Suprelorin®, Peptech Animal Health/Virbac, Australia).

In the second part of the study, chacma baboons from the Riverside Wildlife Rehabilitation Centre in Limpopo, South Africa, were observed for detailed physiological monitoring and evaluation of the immobilisation. An entire troop of semi-wild chacma baboons living in a large enclosure were immobilised for micro-chipping, full physical examination and anatomical measurements before reintroduction and release into the wild. A sample size of 15 baboons was considered sufficient to obtain reliable data about the physiological response to the drug, while simultaneously taking into consideration individual variance. The individuals were chosen by systematic sampling out of a population of 97 baboons. The baboons were captured in small groups (clusters), and the last baboon darted in each group received BAM-Ket. The rest of the baboons were immobilised with a previously known immobilisation protocol of ketamine and medetomidine and were thereby not included in the study. After the clinical trial, all the animals were reintroduced back into their troop.

3.3. Drugs and administration techniques

The specific solution of BAM (Wildlife Pharmaceuticals (Pty) Ltd., South Africa) used in this study contained the following active pharmaceutical ingredients: 30 mg butorphanol, 12 mg azaperone and 12 mg medetomidine per each ml of solution. Additionally, ketamine (Ketanil, Wildlife Pharmaceuticals (Pty) Ltd., South Africa) at the dose of 200 mg/ml was used. For the reversal of the effects of medetomidine, atipamezole (20 mg/ml, Wildlife Pharmaceuticals (Pty) Ltd., South Africa) at 10 times the actual dose of medetomidine in mg (1:10) was used. Butorphanol was reversed by naltrexone hydrochloride (Trexonil, 50 mg/ml, Wildlife Pharmaceuticals (Pty) Ltd., South Africa) at one time the actual dose of butorphanol in mg (1:1). All the injections were given IM either in the muscles of the hindquarters or the muscles of the shoulder. It is known that medetomidine, if overdosed, can cause adverse effects especially on
the cardiovascular system. Therefore, the dose of medetomidine was chosen to be the limiting factor for defining the dose of BAM. Relying on earlier publications on the use of medetomidine in primates, a maximum dose of 0.025 ml/kg was set as the upper safety limit. The dose of BAM was thereby calculated based on total medetomidine dosage per kilogram of body weight. A starting dosage of 0.0155 ml/kg BAM (containing 0.186 mg/kg medetomidine) was used. The increase in dose between animals was not more than 0.005 ml/kg. In the case a dose proved to be too low, an additional injection of ketamine was given to fully immobilise the animal to complete the required procedure. Ketamine has shown to be safe in primate species as a sole agent or in combination with other immobilisation protocols, therefore it was considered to be suitable to use as a top-up to BAM.

3.4. Capture

Study part I:

All but one of the six baboons at C.A.R.E that were included in the dose titration study were pre-captured and kept in small cages at an indoor facility. The pre-captured baboons were secured in a squeeze cage and hand injected. Once the animals reached recumbency, they were blindfolded and moved from the cage to a working area for weighing, intubation and monitoring. The last baboon was darted using a Teledart gun (Teledart GmbH & CO. KG, Germany) as it was kept outdoors in a small enclosure. The monitoring of said baboon was preformed within its enclosure.

Study part II:

Due to the large group of semi-captive baboons being housed together in the enclosure at Riverside, and due to a change in behaviour of animals towards the end of the capture period in response to troop-members being darted and removed, it was impractical to standardize the capturing method. Before being darted, most of the baboons were baited with food, such as fruit
and nuts, into a smaller, secondary enclosure of approximately 20 m², adjacent to the large enclosure. A small group of baboons were captured at a time in the feeding enclosure. Only one baboon in each group was darted with BAM-Ket, and the rest of the baboons caught simultaneously were not included in the study. A Dan-inject dart gun with Dan-inject darts (volume 1.5 mL with 1.5 mm x 25mm needle, Dan-Inject ApS, Borkop Denmark) were used for remote drug delivery. The distance for the remote darting was estimated to between 3 and 6 meters. Towards the end of the capturing period, some animals had to be captured in smaller individual overnight traps within the larger enclosure, since they were refusing to enter the adjacent feeding enclosure. The drug was administered to these animals using a pole syringe. Once immobilised and approachable, the baboons were blindfolded and carried into the shade approximately 150 meters away from the enclosure. At the working area, they were immediately weighed, intubated and placed in lateral recumbency and kept so throughout the monitoring procedures.

3.5. Monitoring

3.5.1. Data collection

In order to determine efficacy and safety of BAM, the baboons were carefully monitored, paying attention to several different factors throughout the procedure. This included: subjective evaluation of the induction and recovery processes, recording of the duration of different phases of immobilisation and monitoring of physiological parameters during the recumbency phase. In Study part I the total time of manipulations for each baboon was approximately 15 minutes, with three measurements taken with a five-minute interval throughout the immobilisation. Monitoring of physiological parameters during the immobilisation was done in the same way in both parts of the study (see below). However, the focus in the dose titration study (Study I) lay on efficacy of the drug, therefore parameters such as time until recumbency, depth of anaesthesia
as well as recovery rate were prioritized. In the second part of the study more focus was laid on safety and detailed physiological effects. Therefore, in addition to other measurements, also arterial blood analysis was performed, which was not included in Study part I. Physiological values were recorded every 5 minutes, starting within 15 minutes after darting. Five to six measurement points were taken for each baboon. In addition, external factors such as ambient temperature and barometric air pressure were recorded at the time of darting.

3.5.2. Induction

After darting, each baboon was carefully observed in order to notice any effects of the drug. Signs of sedation were based on visual observations such as lowering of the head, open mouth breathing and ataxic gate. Induction time was defined as the time from injection until full recumbency. For the few animals that fell asleep in a sitting position or leaning against the fence, the time of induction was estimated based on their reaction to stimulation. Once an animal was fully recumbent, non-reactive to environmental stimuli and considered safe to approach, it was blindfolded and moved to the working area. The animals were weighed and intubated before physiological monitoring could begin. The diameter for the endo-tracheal tubes used varied from 6–9 mm, depending on the size of the baboon.

3.5.3. Anaesthetic depth

The depth of the anaesthesia was evaluated based on the presence of palpebral reflex, muscle tone and reaction to painful stimulation (intubation, micro-chipping, injections). As variations in physiological parameters (HR, BP, RR, or muscle activity) are expected in case of painful stimulation or changes in anaesthetic depth, all these kinds of changes were recorded.
muscle relaxation was evaluated throughout the immobilisation in each baboon and a muscle tonus score was given, using a 3-level muscle tone grading system of the muscle tonus in the lower jaw: 1 – no tone, 2 – slight tone, 3 – strong tone. The animals were given a palpebral reflex score in accordance with the following scoring system: 1 – no reflex, 2 – slight reflex, 3 – strong reflex (blinking). An immobilisation score ranging from 1–4 was given to all baboons in accordance with the scoring system seen in table 1.

Table 1. Description of the different levels of anaesthetic depth and how they are determined

<table>
<thead>
<tr>
<th>Immobilisation Scoring</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Limited effect of drug: Re-dosing required</td>
</tr>
<tr>
<td>2</td>
<td>Strong sedation without complete recumbency: Some motor activity, cannot be manipulated without physical restraint, reflexes present (anal, palpebral), reactive to painful stimulation</td>
</tr>
<tr>
<td>3</td>
<td>Light anaesthesia: can be handled safely but reactive to painful stimulation, no anal reflex, slight palpebral reflex, slight muscular tonicity</td>
</tr>
<tr>
<td>4</td>
<td>Moderate anaesthesia: can be handled safely, complete relaxation, loss of palpebral reflex, no reaction to mildly painful stimulation</td>
</tr>
</tbody>
</table>

3.5.4. Cardiovascular- and respiratory system

The following physiological parameters were monitored using a veterinary monitor (Mindray, iMEC8 vet, Shenzhen Mindray Bio-electronics Co. Ltd., China): HR, RR, SpO₂, end tidal carbon dioxide (EtCO₂), non-invasive BP and electrocardiography (ECG). Oscillometric BP measurements were taken with the cuff (Mindray CM 1500E 8 to 15 cm) placed on the animal’s upper arm. Peripheral pulse and capillary refill time (CRT) were recorded for evaluating circulation and perfusion. The probe for measuring peripheral oxygen saturation was attached to the tongue. Auscultation of the heart was done with a stethoscope (3MTM Littmann® Classic
II S.E. Stethoscope, 3M United States, USA) and the rectal temperature was measured using a digital thermometer.

3.5.5. Arterial blood analysis

Arterial blood was obtained either from the femoral artery or the caudal tail artery for blood gas analysis. In case of arterial the blood collection, the arterial pulse was palpated and the needle was introduced percutaneously into the artery, confirmed by pulsating flow and colour of the blood (figure 2).

Figure 2. Monitoring of baboon immobilised with BAM-Ket at Riverside Wildlife Rehabilitation Centre in Limpopo, South Africa. Arterial blood sample is taken from femoral artery by palpating femoral pulse. Photograph courtesy of Andrew Butt.
Samples were collected anaerobically using a 23G needle and a heparinised syringe. Firm pressure was applied to the sample site for 5 minutes to avoid hematoma formation. A total of three samples were collected at 15, 25 and 35 minutes of monitoring, shortly before the measurement points for the physiological values. After collecting the arterial blood samples, they were immediately analysed using a portable analyser (EPOC Reader Blood Analysis and EPOC BGEM smart cards; Epocal; Kyron Laboratories). The following parameters were obtained: blood pH, partial pressure of arterial oxygen (PaO$_2$), partial pressure of arterial carbon dioxide (PaCO$_2$), bicarbonate, sulphur oxide, glucose, lactate, creatinine, haematocrit, haemoglobin, sodium, potassium, chloride and ionized Ca levels.

3.5.6. Recovery

The recovery was observed in similar way as the induction, by visual observations. After delivering the antidotes, the duration of recovery was recorded in two stages. The first stage entailed the time elapsed until first signs of recovery could be seen. These signs included blinking or subtle movements of head and limbs. The second stage marked the time of full recovery. This was defined as the time when the animal was fully awake, reactive and either standing or sitting up in the cage.

3.6. Statistical analyses

The area under the curve (AUC) was calculated for all the measurements of each parameter during the monitoring period using a trapezoid method. For the analysis of the dose effect of BAM-Ket, the mean AUCs were used as response variables in linear regression models on following parameters: HR, RR, BP (including: systolic blood pressure (SBP), diastolic blood
pressure (DBP) and MAP, SpO₂, PaCO₂ and PaO₂. To achieve a normal distribution of the response variables, square root transformation of PaCO₂, logarithmic transformation of PaO₂, and inverse square root transformation of SpO₂ was used. The given dose of BAM-Ket was calculated based on weighing the animal after immobilisation. The animals were divided into three dose categories depending on dose (low: D1, medium: D2, high: D3) which were used as categorical explanatory variables. In addition, gender and age (two levels: sub-adult and adult) were used as categorical explanatory variables, and body weight and ambient temperature were used as continuous explanatory variables.

Induction (recumbency) and recovery time were analysed using similar linear regression models, in which induction time and recovery time were used as response variables and dose (low, medium and high), age (sub-adult and adult) and gender were used as categorical explanatory variables. Logarithmic transformation of recumbency and recovery time was used to achieve normal distribution. Body weight and ambient temperature were used as continuous explanatory variables.

For investigation of the overall time effect on lactate, arterial blood pH and rectal temperature as well as time trend differences between the dosage groups, linear regression mixed models were used. To achieve normal distribution of lactate, an inverse square root transformation was used. Isotropic spatial exponential covariance structure was used to account for serial correlations of repeated measurements in all models, with baboons included as a random variable. Polynomials of time, with interactions with the dosage group, were added as fixed effects in increasing order. Initially gender, age group and ambient temperature as two-level categorical variable (<25°C and ≥25°C) were included as fixed factors.

A backward elimination procedure was performed in all models. All fitted model assumptions were verified by scatter and normality plots of standardized residuals. A $p$-value of $\leq 0.05$ was considered as statistically significant. Data is reported as mean ± standard deviation (range). For all models, STATA 14.0 (Stata Corporation, Texas, USA) statistical software was used.
4. RESULTS

4.1. Study part I: Dose titration

By using the data obtained from six male baboons (28.3 ± 2.07 kg, range 26–32 kg), the optimal doses of BAM and ketamine to be used in the second part of the study could be determined. The first three baboons were given only BAM, with no added ketamine. The dose of BAM was increased between animals from 0.0155 ml/kg to 0.025 ml/kg. Though becoming strongly sedated, none of the baboons became recumbent even at the higher concentrations. To enable immobilisation, in order to handle these animals, a dose of ketamine had to be given as a supplemental injection. This resulted in the decision to add ketamine to the immobilisation protocol (BAM-Ket), as BAM on its own at reasonable concentrations could not produce complete immobilisation. As strong sedative effects were evident already at the lower concentrations of BAM, a set dose as low as 0.01 ml/kg was chosen. This dose of BAM was expected to produce a strong enough sedation while keeping the risk for adverse effects to a minimum. Once the dose of BAM was fixed, the next aim was to determine the smallest possible dose of ketamine needed to enable the induction of the immobilisation. Different dosages of ketamine were tested ranging from 1.03 mg/kg to 3.0 mg/kg. The lower doses of ketamine (<1.15 mg/kg) proved not to be sufficient for producing complete recumbency. And once again, these animals had to be given an additional dose before becoming recumbent. Finally, full immobilisation was reached with a dose of 3 mg/kg of ketamine. However, the dose was later decreased to 2 mg/kg of ketamine, which was combined with the already set dose of 0.01 ml/kg BAM. Using the aforementioned protocol, the first signs of sedation were seen after 2 minutes and full immobilisation was reached within 6 minutes of darting. A detailed description of the dose titration study, in which induction times in relation to doses of BAM and ketamine are presented, can be found in table 2.
Table 2. The influence of different doses of BAM (butorphanol-azaperone-medetomidine) and ketamine in chacma baboon is shown. When given only BAM, the animals would not reach full recumbency (animal 1 to 3). These animals were given an additional dose of ketamine to induce full immobilisation. Ketamine was added to the protocol and given from the start to animals 4 to 6. Full immobilisation without additional ketamine was reached only in baboon 5 and 6.

<table>
<thead>
<tr>
<th>Animal</th>
<th>BAM [ml/kg] + Ketamine [mg/kg]</th>
<th>Ketamine of top-up Time* [min]: dose [mg/kg]</th>
<th>Sign of first sedation** [min]</th>
<th>Time to recumbency [min]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0155</td>
<td>22 min: 1.03 mg/kg</td>
<td>1.5</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>0.0193</td>
<td>22 min: 1.07 mg/kg</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>0.025</td>
<td>34 min: 0.71 mg/kg</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>0.01 + 1</td>
<td>16 min: 1.11 mg/kg</td>
<td>1.5</td>
<td>18</td>
</tr>
<tr>
<td>5</td>
<td>0.01 + 3</td>
<td>22 min: 2.00 mg/kg</td>
<td>3</td>
<td>22</td>
</tr>
<tr>
<td>6</td>
<td>0.01 + 2.1</td>
<td>-</td>
<td>2</td>
<td>6</td>
</tr>
</tbody>
</table>

Notes:
* Time of top-up after initial injection.
** Time until first sign of sedation after the last top up.

In addition to induction times, an immobilisation score was given for each baboon, based on the quality of recumbency, reflexes and muscle relaxation. The immobilisation scores as well as the results of the physiological measurements (HR, RR, SpO₂, EtCO₂) taken can be seen in table 3. HR (59.6 ± 8.0 bpm) and RR (19.0 ± 2.1 bpm) remained stable in all the animals throughout the immobilisation. The respiration was, however, somewhat affected with SpO₂ (73.7 ± 16.5 %) and EtCO₂ (54.5 ± 5.3 mmHg) being outside of normal range (SpO₂ < 90% and EtCO₂ > 45 mmHg) in all animals.
The table shows the physiological parameters measured during a titration study of BAM (butorphanol-azaperone-medetomidine) with a low dose of ketamine in baboons. The depth of the immobilisation in response to different doses of BAM and ketamine is also presented. The physiological values are given as a mean of three measurements for each baboon.

<table>
<thead>
<tr>
<th>Dose of BAM [ml/kg]</th>
<th>Total dose of ketamine [mg/kg]</th>
<th>HR (bpm)</th>
<th>RR (bpm)</th>
<th>SpO2 (%)</th>
<th>EtCO2 (mmHg)</th>
<th>Palpebral reflex</th>
<th>Jaw tone score</th>
<th>I score</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0155</td>
<td>1.03</td>
<td>52</td>
<td>18</td>
<td>85.7</td>
<td>NA</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>0.0193</td>
<td>1.78</td>
<td>56</td>
<td>19</td>
<td>90</td>
<td>47</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>0.025</td>
<td>1.11</td>
<td>57</td>
<td>23</td>
<td>64</td>
<td>54</td>
<td>1</td>
<td>1.3</td>
<td>2</td>
</tr>
<tr>
<td>0.01</td>
<td>3</td>
<td>58</td>
<td>18</td>
<td>83</td>
<td>55</td>
<td>1.6</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>0.012</td>
<td>2.1</td>
<td>75</td>
<td>17</td>
<td>NA</td>
<td>62</td>
<td>1.3</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>


4.2. Study part II: Physiological effects of BAM-Ket

4.2.1. Animals and doses

Fifteen baboons were used in the study for monitoring of physiological parameters while being immobilised with BAM-Ket. The animals included six female baboons weighing 16.1 ± 2.7 kg (range 12.5–19.9 kg) and nine male baboons weighing 22 ± 8.9 kg (range 6.7–38.0 kg). The weight of each baboon was initially estimated by visual appearance, and the dose was calculated based on this estimation. The aim was to get three different dose categories of BAM: 0.007 ml/kg, 0.01 ml/kg and 0.02 ml/kg, while keeping the dose of ketamine fixed at 2.0 mg/kg in all categories. The actual mean doses for all animals were as follows: volume of BAM was 0.007–0.022 ml/kg (0.01 ± 0.005 ml/kg), with the actual doses of the different components:
butorphanol 0.31 ± 0.15 mg/kg, azaperone 0.12 ± 0.06 mg/kg, medetomidine 0.12 ± 0.06 mg/kg. The total mean dose of ketamine was 2.03 ± 0.22 mg/kg. The three dose categories obtained for BAM were: D1 (n = 5): 0.0067-0.0079 ml/kg, D2 (n = 6): 0.0080-0.0099 ml/kg, D3 (n = 4): ≥0.01 ml/kg.

4.2.2. Induction

All the baboons had smooth and calm inductions. First signs of sedation were observed after 1.86 ± 0.70 minutes and the animals reached recumbency after 3.46 ± 1.36 minutes. No significant differences were seen in the time to full recumbency between the dosage groups or between the different genders. Regurgitation or vomiting was not seen in any of the animals despite having access to feed when darted.

4.2.3. Duration of immobilisation

The total time for detailed monitoring and manipulations was approximately 35 minutes for each baboon. All baboons injected with BAM-Ket reached full immobilisation after a single injection. The baboons were stably immobilised for a minimum of 40 minutes, with most of the baboons remaining immobilised until receiving reversals approximately 50 minutes after induction. However, two baboons showed signs of awakening before receiving any reversals. The first signs of awakening were seen in these baboons 42 minutes and 48 minutes after induction, respectively.
4.2.4. Anaesthetic depth

All the animals in study II obtained immobilisation scores of either 3 or 4. The scoring in the different categories was given as $n_3 / n_4$ ( $n_3 =$ number of animals with score 3, $n_4 =$ number of animals with score 4, $n_3 + n_4 =$ total animals in the category). The distribution of immobilisation scores in the different dose categories was: D1: 2/3, D2: 1/5, D3: 2/2. The immobilisation scores in different genders were as following: females: 5/1, and males: 4/5. Most baboons showed good muscle relaxation (jaw tone score: 1) throughout the immobilisation and four baboons exhibited occasionally mild jaw tone (jaw tone score: 2). Palpebral reflexes were either absent or weak in all baboons (mean palpebral reflex score: 1.4 ± 0.4). None of the baboons reacted notably to the manipulations, as no changes could be seen during micro-chipping or intubation, except for one animal that slightly coughed during removal of intra-tracheal tube.

4.2.5. Monitoring

The main physiological values recorded during the chemical restraint are presented in table 4. All animals suffered from some degree of adverse effects, in the form of hypoxia and hypercapnia. Other physiological values stayed within acceptable limits throughout the immobilisation.
Table 4. Physiological response of baboons during a 40-minute period, whilst being immobilised with BAM (butorphanol-azaperone-medetomidine) in combination with a low dose of ketamine. The table represents recorded measurements from 15 animals during three different measurement periods. Values are given as mean ± standard deviations and minimum – maximum range.

<table>
<thead>
<tr>
<th>Variable</th>
<th>10–20 min</th>
<th>20–30 min</th>
<th>30–40 min</th>
<th>Overall (min–max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR [bpm]</td>
<td>96.8 ± 18.8</td>
<td>90.7 ± 20.2</td>
<td>86.5 ± 18.5</td>
<td>54.0–133.0</td>
</tr>
<tr>
<td>RR [brpm]</td>
<td>28.8 ± 9.7</td>
<td>27.3 ± 8.2</td>
<td>27.2 ± 8.6</td>
<td>11.0–47.0</td>
</tr>
<tr>
<td>RT [°C]</td>
<td>39.0 ± 0.7</td>
<td>38.6 ± 0.7</td>
<td>38.6 ± 0.8</td>
<td>37.1–40.7</td>
</tr>
<tr>
<td>SBP [mmHg]</td>
<td>103.8 ± 7.6</td>
<td>101.9 ± 7.4</td>
<td>98.5 ± 6.3</td>
<td>86.0–118.0</td>
</tr>
<tr>
<td>DBP [mmHg]</td>
<td>58.1 ± 11.0</td>
<td>59.7 ± 9.4</td>
<td>56.6 ± 8.3</td>
<td>34.0–78.0</td>
</tr>
<tr>
<td>MAP [mmHg]</td>
<td>73.6 ± 8.6</td>
<td>74.2 ± 8.6</td>
<td>69.9 ± 8.8</td>
<td>51.0–91.0</td>
</tr>
<tr>
<td>SpO₂ [%]</td>
<td>56.9 ± 12.7</td>
<td>60.8 ± 13.1</td>
<td>68.3 ± 12.9</td>
<td>33.0–94.0</td>
</tr>
<tr>
<td>EtCO₂ [mmHg]</td>
<td>60.0 ± 9.2</td>
<td>63.7 ± 8.5</td>
<td>63.2 ± 8.7</td>
<td>44.0–82.0</td>
</tr>
<tr>
<td>PaO₂ [mmHg]</td>
<td>35.1 ± 7.3</td>
<td>37.2 ± 10.5</td>
<td>41.3 ± 10.9</td>
<td>20.0–64.0</td>
</tr>
<tr>
<td>PaCO₂ [mmHg]</td>
<td>60.2 ± 8.5</td>
<td>62.4 ± 8.7</td>
<td>65.3 ± 9.1</td>
<td>46.3–80.1</td>
</tr>
<tr>
<td>pH</td>
<td>7.3 ± 0.04</td>
<td>7.3 ± 0.04</td>
<td>7.3 ± 0.04</td>
<td>7.2–7.37</td>
</tr>
<tr>
<td>Lac [mmol/L]</td>
<td>2.1 ± 1.9</td>
<td>1.3 ± 1.0</td>
<td>1.1 ± 0.7</td>
<td>0.5–8.1</td>
</tr>
</tbody>
</table>

Notes: HR, heart rate; RR, respiratory rate; RT, rectal temperature; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; SpO₂, haemoglobin oxygen saturation; EtCO₂, end-tidal carbon dioxide; PaO₂, partial pressure of arterial oxygen; PaCO₂, partial pressure of arterial carbon dioxide.

4.2.6. Thermoregulation

The ambient temperature during immobilisation of different baboons varied from 20 to 32°C (25.2 ± 3.2°C). The rectal temperature at the start of the immobilisation was also higher in association with higher ambient temperatures \( p = 0.002 \). However, there is no influence of the ambient temperature on the decline trend of rectal temperature over time (figure 3). There was a steady decline over time in both mean rectal temperature \( p < 0.001 \) and mean lactate levels \( p < 0.001 \) in all baboons (Figure 4a & 4b). The mean rectal temperature declined from 39.1°C at the beginning to 38.5°C at the end of the monitoring period. The mean lactate levels declined from 1.88 mmol/l to 0.64 mmol/l. Furthermore, a positive association was seen between lactate levels and body temperature \( p = 0.001 \). The level of lactate being on an average 0.96 ± 0.2 mmol/l in temperatures below 25°C and 2.0 ± 1.3 mmol/l in temperatures over 25°C.
Figure 3. Illustration of rectal temperatures of baboons immobilised with BAM (butorphanol-azaperone-medetomidine) and a low dose of ketamine, in relation to the ambient temperature. The figure shows higher rectal temperatures at higher ambient temperatures (≥25°C) as opposed to lower ambient temperatures (<25°C). The time trends are similar in both groups.

4.2.7. Cardiovascular effects

During the immobilisation, no significant changes in arterial blood pH could be observed in any of the baboons. HR declined throughout the immobilisation (Figure 4c). HR AUC was positively associated with the ambient temperature (p = 0.003). However, the ambient temperature had no influence on the HR change over time. The capillary refill times were less than 2 seconds in all animals and SBP AUC / minute (101 ± 7.3) stayed within normal range throughout the immobilisation.
Figure 4. Changes in arterial lactate (a), rectal temperature (b), heart rate (c) and respiratory rate (d) (mean ± standard deviation) during immobilisation of chacma baboons with BAM (butorphanol-azaperone-medetomidine) together with a low dose of ketamine.

4.2.8. Respiration

All animals showed signs of severe hypoxia (PaO₂ < 60): some animals throughout and some animals occasionally during the immobilisation. Apnoea was not observed in any of the baboons and RR remained stable (Figure 4d), without noticeable response to increased carbon dioxide levels or low blood oxygenation. The RR AUC (p = 0.009) and SpO₂ AUC (p = 0.007) were mostly influenced by the weight of the animal – both RR and SpO₂ being lower in heavier individuals. Furthermore, SpO₂ was influenced by the ambient temperature, with SpO₂ AUC values significantly decreased as ambient temperatures increased (p = 0.003). Neither the oxygenation nor RR were significantly influenced by the dose. However, a dose-effect was
observed on the expired carbon dioxide levels (Figure 5), with the EtCO$_2$ AUC / minute values being significantly lower in the lowest dose group (D1: 59.7 ± 3.7 mmHg min$^{-1}$) in comparison to the highest (D3: 72.3 ± 7.4 mmHg) ($p = 0.005$). The dose showed no significant effect on any of the other measured parameters, including PaCO$_2$. Throughout the immobilisation, male baboons showed significantly higher values of both EtCO$_2$ ($p < 0.001$) and pCO$_2$ ($p = 0.001$) compared to females. The EtCO$_2$ AUC / minute in females was 55.4 ± 4.0 mmHg and in males 68.3 ± 6.2 mmHg and the pCO$_2$ was in females 55.6 ± 5.1 mmHg versus 67.8 ± 5.2 mmHg in males.

![Figure 5](image-url)

**Figure 5.** The influence of dose on the exhaled carbon dioxide (EtCO$_2$) during immobilisation of chacma baboons with BAM (butorphanol-azaperone-medetomidine) and a low dose of ketamine. The concentrations of BAM in each dose group are: low (D1; n = 5): 0.0067–0.0079 ml/kg, medium (D2; n = 6): 0.0080–0.0099 ml/kg, high (D3; n = 4): ≥0.01 ml/kg. The values are given as mean ± standard deviation for each measurement point.
4.2.9. Recovery

The recovery was smooth and calm for all animals. Time from injection of antidotes to first signs of recovery was $3.1 \pm 1.3$ minutes, and time to full recovery was $4.8 \pm 2.8$ minutes. Two baboons showed signs of awakening before receiving any reversals. These baboons were initially immobilised with the following doses: $0.008 \text{ ml/kg of BAM + 2.353 ml/kg of ketamine}$ and $0.009 \text{ ml/kg of BAM + 1.789 ml/kg of ketamine}$. For safety reasons these baboons were re-immobilised by administering an additional dose of ketamine (100 mg) and were thereby excluded from the recovery analysis. There was no association found between recovery time and actual BAM-Ket dose category. All baboons recovered well from the immobilisation.
5. DISCUSSION

5.1. Dose titration

As demonstrated in the dose titration study, a low dose of ketamine is needed as an addition to the protocol, since BAM on its own is unable to produce complete immobilisation. An explanation for this could be the unique pharmacodynamics of butorphanol in baboons. Butorphanol is used in veterinary medicine primarily for its sedative effect, which is mainly derived from κ-receptor activation. In addition to being a κ-receptor agonist, butorphanol is known to act as an antagonist on μ-receptors in different ungulate and carnivore species, in which BAM is commonly used. However, the mode of action of butorphanol has proven to be different in primates, in both the affinity as well as the type of activation of both κ- and μ-receptors. Butorphanol has a stronger agonistic affinity and efficacy on μ-receptors than on κ-receptors in primates, resulting in a lesser sedative effect, but better analgesic properties (Butelman et al., 1995; Liguori et al., 1996). Since the sedative effect of butorphanol is weaker, also BAM’s sedative effect is compromised and thus is unable to produce full immobilisation on its own. However, in a study by Malinowski et al. (2019) done on rhesus macaques, BAM was able to produce complete immobilisation on its own. The doses of BAM used in macaques are similar to the doses used in the present study. However, while BAM failed to produce immobilisation in the baboons at doses up to 0.025 ml/kg, the macaques were fully immobilised already at concentration of 0.016 ml/kg. It is possible that the differences are due to species specific reactions, and that the baboons potentially would have reached full immobilisation, had the doses in the titration studies been higher. But considering the low oxygenation values obtained already at the doses that were used (<90%), it was considered unethical to increase the dose. Further it may be speculated that the differences in capture techniques between the two studies played a significant role in the induction of the immobilisation. The macaques were pre-captured and kept in controlled environments prior to the immobilisation, which would have
resulted in reduced stress-levels at the time of the actual immobilisation. The semi-wild baboons, by contrast, were immobilised in direct connection with the capturing procedure. After seeing troop-members being captured, immobilised and taken away, it is reasonable to assume that the baboons would have been much more agitated and more stressed at the time of injection compared to the macaques. As the efficacy of the anaesthetic is decreased by higher excitement levels, it could explain why the baboons, in contrast to the macaques, did not reach full recumbency on BAM alone. The excitement level may especially have influenced the efficacy of medetomidine, as adrenaline is a direct competitive antagonist to medetomidine (Sinclair, 2003).

5.2. Physiological effects of BAM-Ket

5.2.1. Duration of immobilisation

The earliest signs of awakening were seen in one baboon after 42 minutes of immobilisation. At this time, it is reasonable to assume that the effect of ketamine would have been negligible, considering the low dose and the relatively fast elimination time of the drug (Mion & Villevieille, 2013). When comparing BAM-Ket to other anaesthetics used in primates, the duration of action can be considered reasonable. For example, ketamine used alone in macaques at a dose of 10 mg/kg, has a significantly shorter anaesthetic duration of 28 minutes (Lee et al., 2010). In a study on olive baboons, a protocol combining ketamine and xylazine (10 mg/kg; 0.5 mg/kg), produced an immobilisation lasting for approximately 1 hour before spontaneous recovery, with some physiologic effects seen several hours later when no reversals were used (Langoi et al., 2009). By contrast, a ketamine-medetomidine combination (3.0 mg/kg; 0.15 mg/kg) has a reported duration of up to 70 minutes in the macaques (Sun et al., 2003), and a slightly shorter duration of 45 minutes in golden headed tamarins when a dose of 10 mg/kg of ketamine and 0.02 mg/kg of medetomidine is used (Selmi et al., 2004).
5.2.2. Cardiovascular signs

Considering the stability of cardiovascular parameters within different dose groups, the therapeutic dose range of BAM-Ket seems to be fairly wide, as cardiovascular parameters (HR, BP, CRT) stayed within acceptable limits and a constant decrease of the HR was seen in all animals throughout the immobilisation. Both HR (91 ± 19 bpm) and SBP (101± 7 mmHg) were lower in chacma baboons compared to base line values documented in for example olive baboons (HR = 145 bpm, SBP = 150 mmHg) (Langoi et al., 2009). However, when the olive baboons were immobilised with ketamine-xylazine, the cardiovascular parameters (HR <100 bpm and SBP < 90 mmHg) were more similar to those seen in the chacma baboons in this study. The ambient temperature significantly influenced the HR in the beginning of immobilisation, resulting in higher HR at the start of immobilisation. This occurrence may be explained by the need for increased cardiac output as the body temperature and metabolic rate is increased due to the high ambient temperature.

5.2.3. Respiration

All animals treated with BAM-Ket suffered from hypoxia (PaO$_2$ < 60 mmHg) (Ehrenfeld et al., 2011). Occasionally, when medetomidine is administered, low SpO$_2$ values, if obtained from pulse-oximeter readings, may be false. Low oxygenation values can be a secondary finding as a result of peripheral vasoconstriction commonly associated with the use of α2-agonists. This occurrence was for example described in a study by Malinowski et al. (2019) in macaques immobilised with BAM. Findings of hypoxia also correlate with studies in which butorphanol containing protocols have been used in primates (e.g. Butelman et al., 1995; Liguori et al., 1996; Zucker et al., 1987). As some of the PaO$_2$ values obtained in present study were critically low, ranging from 20.0–64.0 mmHg, the trustworthiness of these values may unfortunately be questionable. Especially since no noticeable adverse effects could be seen in the baboons after
immobilisation, which would be expected after prolonged severe hypoxia. The values may have been exaggeratedly low due to a potential mix up of arterial and venous blood or a problem in the calibration of the instruments. However, even though the level of hypoxia leaves room for doubt, it is reasonable to believe that some degree of hypoxia was present, as both SpO₂ and PaO₂ values were well outside of normal range.

In addition to hypoxia the animals suffered from slight hypercapnia. The lack in physiological response to counteract hypercapnia and severe hypoxia was evident as respiration failed to be stimulated. Although a significant decrease in RR was not seen during the immobilisation, it is possible that the tidal volume may have been influenced by the anaesthetics. Measurements of the tidal volume were not taken, but one baboon exhibiting low oxygen saturation was shortly ventilated with an Ambu bag, which resulted in a noticeable increase in the SpO₂ value. This occurrence would support the assumption that hypoxia is mainly a result of a decrease in tidal volume, or at least decreased ability to correct hypercapnia and hypoxia by increasing the tidal volume.

Both medetomidine and butorphanol are known to have some inhibitory effects on the pulmonary system. Medetomidine may cause a decrease in RR, especially in combination with other sedatives (Sinclair, 2003). There are several studies in which the respiratory effects of medetomidine has been evaluated in primates. In macaques anaesthetised with a combination of medetomidine and ketamine, minimal negative cardiopulmonary effects were observed (Lee et al., 2010). Selmi et al. (2004) conducted a study in which tamarins were immobilised with a combination of ketamine and medetomidine. No significant decrease in oxygen saturation was seen in the tamarins, and SpO₂ values stayed above 94% throughout the immobilisation, although a continuous decrease in RR was observed. On the other hand, butorphanol has shown to produce significant decrease in oxygen saturation in a variety of primate species. In macaques, respiratory depression has been seen in doses ranging from 0.001–0.32 mg/kg without a ceiling effect, and in humans doses equivalent to 0.03–0.06 mg/kg reported to produce significant respiratory depression (Butelman et al., 1995; Zucker et al., 1987). A study by Liguori et al. (1996) systematically shows how different opioids affect macaques’ response to hypercapnia. Butorphanol decreased the minute volume by more than 60% from the control values, when given at a dose of 0.3 mg/kg (Liguori et al., 1996). The decreased sensitivity to respond to
increasing CO₂ values may be explained by activation of μ-opioid receptors in different locations within the central nervous system, including areas in pons (pre-Bötzinger complex) responsible for regulating respiratory rhythm (Boom et al., 2012). These occurrences support the assumption that even though both medetomidine and butorphanol are known to influence respiration, the hypoxia observed in this study would mainly be due to the action of butorphanol.

The dose showed no influence on the level of oxygenation or the respiration and no association was found between the dose and PaCO₂, however, the dose had an influence on the EtCO₂. As PaCO₂ and EtCO₂ values are expected to correlate with each other and the fact that only EtCO₂ values are significantly influenced by the dose is paradoxical and may be due to some errors in measurement of the arterial blood gases. An association was seen between EtCO₂ and gender, as it was higher in male individuals. High EtCO₂ may be an indication of deeper anaesthetic depth, which would mean that BAM-Ket has a higher potency in males. A higher potency could be connected to bioavailability. Bioavailability is influenced by factors such as body composition, which is known to differ between males and females (Ranasinghe et al., 2013). However, since body composition analysis was not systematically performed in the study, this hypothesis cannot be confirmed. Furthermore, if the potency of BAM-Ket was in fact higher in males compared to females, other parameters such as induction time and anaesthetic depth (immobilisation score) should also differ between them. Since neither one of these variables differed significantly between the genders, the exact reasons behind higher EtCO₂ in males would need more research to determine.
6. CONCLUSION

There is a need for safe and efficient ways of handling baboons, both in wild populations as well as semi-wild settings. When sole physical restraint is considered insufficient, chemical restraint may be used. None of the anaesthetics in use today fulfils all the criteria for an ideal immobilisation protocol in baboons, which makes investigations into novel agents imperative. In this study it was demonstrated how BAM-Ket can be used as an alternative protocol for short term immobilisation in baboons. The protocol supplies sufficient analgesia that allows for mildly painful procedures. Advantages of the BAM-Ket protocol include rapid recovery due to the complete reversal of butorphanol and medetomidine with atipamezole and naltrexone. Furthermore, the synergism of the combined drugs allows for a reduction of the dose of each substance and thereby the dose-dependent adverse effects of these compounds can be minimized. However, the use of BAM-Ket in baboons proved to expose the animals to hypoxia, and thus supplemental oxygen is recommended when using this protocol. Due to this, BAM-Ket is not considered to have any superiority to previously used protocols including medetomidine-ketamine combinations. However, further investigations are needed to determine the precise mechanisms that lie behind the negative effects on respiration, and if these are related to decreased tidal volumes. In addition, it may be worth investigating different dose combinations in order to gain maximal effect of BAM-Ket and reduce adverse effects.
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ÜLDKOKKUVÕTE

Uudne protokoll karupaavianide anesteesiaks

Nii loomaedades elavate kui metsikute paavianide käsitlemiseks on vaja ohutuid ja tõhusaid anesteetikume. Tänasel päeval paavianidel kasutatavatest anesteetikumidest ei taga kahjuks ükski ideaalse immobiliseerimise. Butorfanooli, asaperooni ja medetomidiini (BAM) kombinatsioon on osutunud erinevatel loomaliikidel heaks vahendiks saavutamaks piisav anesteesia loomade käsitlemiseks siis vöib BAM olla sobiv vahend ka paavianide käsitlemisel. BAM-ile on olemas ka antidoot (naltreksooni-atipamezooli kombinatsioon) mis on tema kasutamise juures suur eelis. Samuti võimaldab kasutavate ravimite sünergism kasutada väiksemaid doose mis vähendab võimalike kahjulike kõrval mõjude esinemist. Opioididel ja a2-agonistidel on valuvaigistavad omadused, mis võivad osutuda kasulikuks eriti kergelt valulike protseduuride korral. BAM-i sedatiivsed omadused tulenevad butorfanooli ja medetomidiini sünergismist. Primaatidel on butorfanooli farmakodünaamika siiski mõned sõraliste ja lihasõjade liikidega võrreldes erinev. Kuna butorfanooli aktiivsus u- opioidiretseptoritele on antagonistliku asemel agonistlik ja afiinsus u-opiooidiretseptorite suhtes on suurem kui primaatide κ-opiooidiretseptorite suhtes, võib butorpanooli rahustav toime paavianidel olla väiksem. See nõrgendaks ka BAM-i võimet paaviane immobiliseerida ja seetõttu lisati paavianide täielikuks immobiliseerimiseks BAM-le väike annus ketamiini (BAM-Ket). Esialgsetes doosikatsetes kasutati kuut isast karu paaviani (Papio ursinus) ja nendes selgas, et ketamiini lisamine tagas parema immobilisatsiooni. Ravimi efektiivsuse ja ohutuse hindamiseks jälgiti BAM-Ket anesteesia ajal 15 paaviani füsioloogilisi parameetreid, samuti induktsooni, anesteesia ja taastumise kvaliteeti. Kasutati järgmisi annuseid: BAM 0,01 ± 0,005 ml/kg (butorfanool 0,31 ± 0,15 mg/kg, asaperooni 0,12 ± 0,06 mg/kg, medetomidiini 0,12 ± 0,06 mg/kg) ja ketamiin 2,04 ± 0,22 mg/kg. BAM-Ket anesteesia tagas minimaalselt 40-minutilise immobilisatsiooni, induktsooni oli 3,46 ± 1,36 minutit ja täielik taastumine toimus 4,8 ± 2,8

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minutit peale antidoodi (atipamezool, naltreksooni) manustamist. Põhjustatud kõrvaltoimed olid hüpoksia (SpO2: 62 ± 13%; PaO2: 37 ± 10 mmHg), samuti pisut kõrgenenud EtCO2 (63 ± 9 mmHg) ja PaCO2 (63 ± 9 mmHg). Kokkuvõtteks võib öelda, et väikese ketamiinianiusega BAM tekitab paavianides lühiajalise üldanesteesia, mis võimaldab väiksemaid veterinääprotseduure, nagu vere kogumine ja mikrokiibistamine. Esinenud hüpoksia tõttu on paavianidel siiski soovitav BAM-Ket-i anesteesia korral kasutada lisahapniku. Samuti on vaja täiendavaid uuringuid, selgitamaks mehhanisme mis mõjuvad pärssivalt hingamisele. Lisaks tuleks BAM-Ket-i maksimaalse efekti saavutamiseks ja kahjulike mõjude vähendamiseks paavianidel uurida erinevaid annuste kombinatsioone.
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APPENDIXES

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