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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Evaluation of hyaluronidase in combination with etorphine HCL for immobilizing African elephants (*Loxodanta africana*) in Tanzania

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Abstract

Hyaluronidase (hyalase) has been routinely incorporated with etorphine HCl (M99) during immobilization of herbivores particularly those with thick skin to enhance drug absorption and decrease the induction time. This study evaluated retrospectively the usefulness of hyalase for immobilizing African elephants. Two protocols, one containing M99 (mean total dose 12.8±2.26mg; range 10-15mg) and hyalase (total dose 2500IU) and another M99 alone (mean total dose 12.7±1.84mg; range 10-15mg) were evaluated for first immobilization signs and induction time. Each treatment group consisted of twenty health adult elephants with equal sexes. Results showed the mean induction time were shorter in females than males immobilized using M99 mixed with hyalase or M99 alone but not statistically significant (P>0.05). Similarly, the mean time for the two parameters in the two treatment groups regardless of sex were not statistically significant (Mann-Whitney U test 184.0; p=0.66). Irrespective of the sex, the overall difference in induction time for the two treatment groups did not exceed two minutes. Retrospective analyses of other studies using M99 and varying doses of hyalase (from 1500 to 5000 IU) appear not to shorten the time to first observed signs of immobilization and subsequent induction time. The present study did not observe the clinical significance of incorporating hyalase in M99 for elephant immobilization. However, more data is required to demonstrate any difference in induction time and efficacy of the drug combination of M99 with hyalase.

Keywords: Etorphine-HCL; Hyalase; Induction time; *Loxodanta africana*

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Introduction

Ideal characteristics of a drug for immobilization of free ranging wild animals are short induction time and minimal adverse physiological effects. Other factors which are considered in selecting immobilization protocol include efficacy, animal and human safety, existence of antagonist, stability (under variety of environmental conditions), miscible with a variety of other drugs, availability and cost (Meltzer et al., 2006). Often a drug cocktail is used to shorten the induction time and reduce detrimental effects.

Immobilization protocols for free ranging wild herbivores in Africa particularly giraffe, rhinos and elephants (Meltzer et al., 2006) have recently been documented in which etorphine HCl (M99) in combination with hyalase is recommended. Hyalase is a naturally occurring enzyme obtained from bovine or ovine testes and used widely in human and veterinary medicine. It is an additive in local analgesia and interventional in pain reducing procedures such as adhesiolysis of epidural scar tissue after spinal surgery (Girish and Kemparaju, 2007; Schulze et al., 2008; Cattet and Obbard, 2010). The enzyme works by

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breaking down hyaluronic acid, which makes the ground substance between connective tissues and cells at the injection site thus enhancing drug absorption (Meltzer et al., 2006).

Previous literatures by Haigh (1979), Morkel (1989) and Kock (1992) emphasized the importance of incorporating hyalase in immobilizing drugs to improve drug absorption and decrease the induction time. There is a logistical problem using this enzyme under field conditions due to its storage requirement. The enzyme requires cold chain to retain its viability which is difficult to maintain in remote areas. With scarce scientific evaluation for incorporating hyalase with M99, (Cattet Obbard, 2010), this study was initiated to determine if its use was justified. Retrospective observation was made to compare the average time it took from successful injection to when the animal showed obvious signs of immobilization and full induction time which is the time it took from injection to when it falls down. Two drug protocols were compared, one using M99 in combination with hyalase and the second protocol using M99 alone.

Materials and Methods

Study animals and drugs

Retrospective immobilization records were obtained from healthy adult free ranging elephants captured in Serengeti ecosystem and adjacent protected and unprotected landscapes in Tanzania during placement or removal of radio-collars or other management interventions. A total of 40 elephant records were used in this study where half of them were immobilized using M99 in combination with 2500IU hyalase and the remaining 20 were immobilized using M99 alone. For each immobilization protocol, 10 elephants were females and 10 males. The total dose administered for each category ranged from 10 to 15 mg and the dosage rate was determined based on visual assessment of the age, sex and body condition score as described by Albl (1971). Sub adult females and males were administered with 10-12 mg of M99, the doses for adult females were 12-13 mg of M99 and adult males were given 14-15 mg of M99. All immobilized elephants were relatively in good body conditions.

Data analysis

The mean duration from injection time to first observed (ITFO) signs of immobilization and induction time (IT) was calculated (×±SD) to establish the average time elapsed for observation of first signs and induction time. Mann-Whitney U test was used to compare for the difference in time taken for ITFO and IT for the two immobilization protocols and 95% CI was the criterion for assessing the statistical differences (Systat 10 software package). Comparison was made separately between females and males from the two immobilization protocols (n=10), and later on the comparison was made between two immobilization protocols regardless of sex (n=20).

Results

The mean time elapsed for induction (IT) were shorter in females than males immobilized using M99 in combination with hyalase or M99 alone but this was not statistically significant at P>0.05. Similarly, the mean time for the two parameters in the two treatment groups regardless of sex showed longer mean induction times (2 minutes difference), without hyalase but this was not statistically significant using the Mann-Whitney U test (184.0; P=0.66) (Table 1). The range for the time elapsed in both observations is indicated in bracket after the standard deviation of each respective parameter (Table 1).

Discussion

Observation of first signs of narcotisation is subjective and depends on the experience of the operators but is worth recording in order to have an idea whether the drug has been delivered or not. Thus the operator can prepare for the animal to go down or prepare for a second dose if deemed appropriate. The mean difference in induction time between the two immobilization protocols used in the present study was about 2 minutes. Other studies employing different doses of hyalase in different classes of elephants also show a fairly longer induction time (Table 2). In addition to that the time difference of 2 minutes is insignificant because normally elephants do not move

Table 1: Mean values for first signs of drug effect and induction time for two immobilization protocols in free ranging African elephants

S/N	Drugs	Sex	First signs	Induction time	P-value
1	M99+ Hyalase	F (n=10)	5.4±1.96 (3-8)	9±2.67 (6-13)	
	M99+ Hyalase	M (n=10)	7.3±4.81 (4-20)	13.1±3.09 (7-30)	p>0.05
2	M99 alone	F (n=10)	6.1±2.18 (3-10)	10.9±2.73 (7-15)	
	M99 alone	M (n=10)	5.9±1.29 (4-8)	13.3±3.5 (7-19)	p>0.05
3	M99+Hyalase	F+M (n=20)	5.55±1.88 (3-10)	9.85±2.94 (6-30)	
	M99 alone	F+M (n=20)	6±1.75 (3-10)	12.1±3.29 (7-19)	p>0.05
			Mann-Whitney=184.0		P=0.66

Table 2: Different Immobilization protocols in elephants with or without hyalase, age category of elephants immobilized and induction time

Published Literature	Total dose given in mg (Except when pecified)	Amount of Hyalase (i.u) or else specified	Total number of animal used	Age Category	Induction time (Minutes)
Kock, Martin and Kock. (1993)[1]	11.6±0.3	4500	16	Adult Female	8.9±0.81(range 4.5-23)
	15	4500	16	Adult Female	6.2±0.9 (range 3-24.3)
Schumacher et al (1995)	3.2±0.5µg/kg	None	8	Juvenile	30±21
Osofsky, S.A (1997)	9.5±0.5	2000	20	Adults	8.7±2.4 (range 4.9-14.3)
Hoare, R (1999)	≤ 14	2000	20	Adult	Range 6-40
	≥ 14	2000	28	Adult	Range 2-12
Mpanduji et al., (2003)	15.7±2.0	3-4 drops of TSTA[2]	6	Adults	11.2 (range 5-22)
	18	5000	26	Adults	8.9±2.34 (range 5-25)
Gakuya et al., (2003)	15	3500	14	Sub Adults	9.0±1.41 (range 8-10)
	5	1500	16	Juvenile	9.5±6.36 (range 5-14)
This study	12.8±2.26 (range 10-15)	2500	20	Adult	9.85±2.94 (range 6-30)
	12.7±1.84 (range 10-15)	None	20	Adults	12.1±3.29 (range 7-19)

[1]Same animals used twice for captures in different seasons; [2]Experimental Thermo Stable Tissue Accelerant substance [TSTA] provided by Prof. Henning Wiesner, Munich Germany

very much when immobilized with minimum disturbance. However, when a helicopter is used the elephants are normally guarded to remain at the same area until signs of drug taking effect are observed and the immobilized animal is reluctant to move.

Recommended elephant immobilization procedures incorporate hyalase in their anaesthetic protocols to enhance drug absorption and minimize drug induction time. There is no experimental data to back up this approach and no standardised formula for the amount of hyalase to be used. Many studies (Table 2) reported the amount of hyalase to range from 1500 IU to as much as 5000 IU. However, there is no information on dose effect. The prolonged induction time reported by Schumacher et al. (1995) of up to 30 minutes when etorphine was used alone to immobilize juvenile female elephants from private collection in Florida USA is exceptional. Another study by Mpanduji et al. (2003) incorporated a thermo stable tissue accelerant. This substance offered no added advantage in terms of improvement in induction time (Table 2).

The present study suggests more data be collected on more elephants and with removal of confounding variables, where possible, to demonstrate any difference in induction time and efficacy of the drug combination, using hyalase. Statistical observations obtained from this and other referenced studies show that there is a small difference in induction time when M99 is used alone or in combination with hyalase. Total sample size has a strong bearing on statistical results which might explain the observations in this study. These data might simply reflect the variable influences associated with the whole procedure, affecting drug absorption and narcotic effect and which cannot be standardised under field conditions e.g. dart injection site and depth, animal body weight and metabolism, chase time to darting etc.

In general, the authors support the following principles for immobilization of elephants. Use appropriate darting technique, involving relatively higher doses from published ranges, coupled with selection of a correct gauge and length of dart needles (60mm) and appropriate injection site (rump, shoulder, back on either side of the spine) that will ensure deep intramuscular injection to increase the likelihood of complete discharge of drugs into highly vascularized muscle mass for capturing of elephants. The authors also consider the efficacy of use of hyalase to be equivocal but recognise the statistical weakness in the study design. Potential advantages must also be set against the difficulties of storing the agent due to its temperature sensitivity (Schulze et al., 2008) and added risks of contamination from a second drug being used, especially one which is a protein and therefore, potentially a site for bacterial growth.

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