Hyaluronidase, with xylazine and ketamine, reducing immobilization time in wild cattle (*Bos taurus*)

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SUMMARY

Aim - To evaluate the effects of the addition of hyaluronidase to xylazine and ketamine drug combination on chemical immobilization times in wild cattle.

Study design - Trial study, "blinded" randomized.

Animals - Forty free wild cattle (*Bos taurus*).

Methods - Cattle were divided into two groups and immobilized using chemical immobilization, in group A has been administered an association of 0.4 mg/kg xylazine and ketamine hydrochloride 1 mg/kg, in the group B the same dosage combination of ketamine and xylazine is added 300 UI of hyaluronidase. The time spent between first pharmacological effects and the return at the quadrupedal station (T^1-T^6), heart rate, respiratory rate and body temperature were monitored.

Results - In group B, all monitored times were significantly shorter than in group A, exception made for time T^5.

The total time (T^6) between inoculation of the drug at the return of quadrupedal station was shorter in group B [53 (35/70)] than in group A [72 (7/79)], $P = 0.000$.

Conclusion and clinical relevance - The use of hyaluronidase, in association with xylazine/ketamine, shortens the chemical immobilization time in wild cattle.

KEY WORDS

Cattle; hyaluronidase; xylazine; ketamine; anesthesia.

INTRODUCTION

A growing number of beef cattle farms in southern Italy are specialized in free range or semi-free range breeding. Free animals are often subject to injuries and stress when handled, and sedation is necessary for medical examinations, sampling, diagnostic examinations, tests, vaccinations, antiparasitic treatments, surgical operations, and dental care. Chemical immobilization is proven to be the most effective method for sedating free bovines and other ruminants.[5-6]

Once the animal is hit by the dart however, could escape and reach places far and hard to reach before there are the effects of sedation. We tested the use of immediate-action molecules to shorten the chemical immobilization time, adding hyaluronidase to the anesthesia blend.

Hyaluronidase is a hydrolyzing enzyme with effects on hyaluronic acid, which is a mucopolysaccharide that constitutes the essential element of the connective tissue. Hyaluronidase depolymerizes the complex molecule of hyaluronic acid, reducing its natural viscosity and, consequently, increases the permeability of the tissues for a period of 24-48 hours, necessary for the synthesis of new hyaluronic acid.[1,8,13]

The enzyme is considered a "spreading factor" because enhances the diffusion capacity and bioavailability of injected drugs. Therefore, the enzyme has been used as a local adjuvant to increase the diffusion capacity of local anesthetics, indeed hyaluronidase may increase the tissue penetrating area of the local anesthetic by up to 40%.[2,12,13] The duration of local anesthesia is usually reduced by concomitant administration of hyaluronidase; the association with adrenaline however counteracts the action of hyaluronidase.[1,2,12]

According some authors hyaluronidase enhances the effect of procaine, but not that of lidocaine or mepivacaine. Hyaluronidase is a useful local anesthestic for the retrobulbar ocular area, it reduces the intraorbital pressure, improving the speed and the quality of the peribulbar block.[12]

The use of hyaluronidase has been described as facilitating chemical immobilization in the African buffalo, the gnu, the moose, the polar bear, the giraffe, the rhinoceros and in the African elephant.[9,11] In all these animals the use of hyaluronidase in combination with analgesics or sedatives reduces the time before onset of immobilization. Other authors obtained different results in the horses (Equus caballus) because there not found difference between the group sedated with anesthetics alone compared to the group sedated with anesthetics and hyaluronidase.[18]

The purpose of this study is to evaluate the effect of hyaluronidase, administered together with ketamine and xylazine, on the induction time in wild cattle using chemical immobilization. Our hypothesis is that hyaluronidase may facilitate the absorption of the anesthetic from the injection site, making induction time shorter.[19]

MATERIAL AND METHODS

The study was approved by the University of Messina Review Board for Animals Care (protocol number 032/2019). The

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trials were performed according to the Italian law (DM 116192), the European laws (OJ of L L 358/1, 12/18/1986), and US Animal Welfare Assurance No A5594-01, (Department of Health and Human Services, USA). All owners of the tested animals gave their informed consent.

The experiment was conducted on a group of 40 free wild cattle. Mean age of the subjects ranged between 9 and 36 months, and their weight between 200 and 500 kg. The animals were divided into two groups: group A included 20 subjects, mean age 17.72 ± 7.9 months and estimated weight 332.96 ± 58 kg, group B included 20 subjects, mean age 18.10 ± 7.8 months and average weight 336.20 ± 86 kg. All cattle were nervous and did not allow any approach. Subjects were medium and small cross-breed individuals.

The anesthetics protocols were randomized by lottery: in group A were administered 0.4 mg/kg xylazine 20% (Rompun® Dry Substance, Bayer S.p.a.) and ketamine hydrochloride 10% (Ketavet® MSD Animal Health S.r.l.) 1 mg/kg. In group B the same drugs and dosages were used but with the addition of 300 IU of hyaluronidase. (Jaluran®, Pfizer Italy S.r.l.).

Chemical immobilization was performed from a distance of between 12 and 20 meters. We used syringes (1.5 ml) armed with hypodermic needles 2.0 x 30 mm and syringes (5 ml) armed with hypodermic needles 2.0 x 40 mm.

The manifestation of the first pharmacological effects were hypersalivation, slowness of movement and lowering of the head of the animals. When sedation was obtained and the cows reach recumbency, were left quiet for about 15 minutes. Their eyes were protected with a moistened bandage to prevent corneal dehydration following the anesthesia, all animals were monitored for two hours for possible signs of meteorism (as it had been impossible to establish a fasting period before the procedure, ruminal atonia was a risk), none drug were used like reversal agent.

Cattle were subjected to blood sampling, marking and antiparasitical treatment.

We have not see cutaneous dart reactions, from chemical immobilization, in the subjects.

The monitorated times are: Time 1 (T^1), time between the shot and the first signs of pharmacological effect; Time 2 (T^2), time from the first signs of the pharmacological effect to the recumbency; Time 3 (T^3), the time from recumbency to a plane of deep anesthesia; Time 4 (T^4), time between anesthesia and the first signs of revival; Time 5 (T^5), time from first signs of revival to the quadrupedal station; Time 6 (T^6), total time: from chemical immobilization to quadrupedal station.

Statistical analysis of the data was performed using SPSS 15.0 IBM software for Windows. Shapiro-Wilk normality test was performed, the data are expressed with median and range, differences between groups were compared using the Mann-Whitney U test, $p<0.05$.

RESULTS

Shapiro-Wilk normality Test, highlighted that the data were not normally distributed.

In group A 5(4/6), T^1 time between inoculation of the anesthetic mixture and the first signs of the pharmacological effect were double than in group B 2(1/4) (P = 0.000).

T^2 time, between the first signs of the pharmacological effect and the loss of the quadrupedal station, were very higher in group A 7.32 (4/10) than in B 3 (1/5), P = 0.000.

The time from recumbency to a plane of deep anesthesia (T^3) was of 38.50 (28/58) in group A and 32 (24/54) in group B. P = 0.026.

Also, T^4, time between anesthesia and the first signs of revival are greater than group A 38.50 (28/58) with respect to B 32 (24/54). P = 0.026.

Only, in group T^5, the times from first signs of revival to the quadrupedal station no showed differences statistically significant P = 0.685. Total time (T^6) from chemical immobilization to quadrupedal station was shorter in group B 53 (35/70) than in group A 72 (7/79). P = 0.000.

DISCUSSION

The mechanism of action of hyaluronidase is not fully known, this enzyme is believed to enhance the interstitial permeability through the disruption of the β-1,4-glycosidic bond between the 2-acetamido-2-deoxy-D-glucose and the D-glucuronic acid. The action of this enzyme is quickly reversible.[16]

To try to standardize the subjects we tried to divide them in to two groups, evaluating approximately weight and age, this in order to obtain reliable data.

Obtained data show like, in group B respect group A, there are significant difference in all the monitoring moments.

Our results validate our hypothesis that the addition of hyaluronidase to the anesthetic blend accelerates the diffusion of the anesthetic compound and therefore the effects of the drugs.

This results are similar to those reported in the literature, however other authors used etorphine in association with azaperone, xylazine or alone.[7,11,14]

Table 1 - Time after ketamine/xylazine (A group) and ketamine/xylazine/ialuronidase (B group) administered intramuscularly.
Legend: T1 Time between inoculation of the anesthetic mixture and the first signs of the pharmacological effect; T2 time between the first signs of the pharmacological effect and the loss of the quadrupedal station; T3 time between the achievement of recumbency and deep anesthetic state; T4 time between anaesthesia and the first signs of revival; T5 Time between the first signs of revival and quadrupedalism resumption; T6 Total time: by inoculation of the drug at quadrupedalism resumption. Data were expressed with median and range.
Bold: significant difference between Groups p < 0.05.

Groups	Time (minutes)					
	T1	T2	T3	T4	T5	T6
A	**5 (4/6)**	**7.32 (4/10)**	**9.75 (9/12)**	**38.50 (28/58)**	7 (5/15)	**72 (7/79)**
B	**2 (1/4)**	**3 (1/5)**	**6 (4/8)**	**32 (24/54)**	7.5 (5/15)	**53 (35/70)**
pVALUE	**0.000**	**0.000**	**0.000**	**0,026**	0,685	**0.000**

The anesthetized subjects reached a good degree of muscular relaxation, favouring all mauality.

Decreasing the time of decubitus reduces the possibility of complications such as ruminal atonia, circulatory disorders or respiratory problems.

The use of hyaluronidase is advisable in the chemical immobilization of free cattle and ruminants in general. This enzyme reduces the induction time, accelerates the achievement of recumbency and therefore facilitates patient management.[5-7,11,14]

This protocol could be particularly useful when used in wild animals caught in stressful conditions.

In effect several authors have noted how the stress, in cattle and other ruminants as the fallow deer, determined a prolonged induction time and a greater demand for alpha2-adrenoceptors.[5,6,17]

Based on our findings in other species the results of our study could change due to climatic differences, then, given same conditions of drugs protocols and species, there could be different responses in winter or summer.[1] Moreover, there could be some difficulties in estimating the real weight of the animals, given the huge dimensions of the individuals.

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