Influence of Xylazine on Hematology Values in Farmed Red Deer

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Abstract

Reductions in circulating basophils and lymphocytes following xylazine administration to farmed red deer (*Cervus elaphus*) previously observed using automated technology have been confirmed using standard manual procedures. The kinetics of the changes were followed and observed to be maximal at 2–5 min after intramuscular injection, whereas red cell and platelet changes follow a different time course, commencing and peaking later. There is clearly a need to be aware of such changes when interpreting hematological results. The changes seen in basophils and lymphocytes were not associated with splenic sequestration, unlike red cells and, to some extent, platelets. Manual and automated techniques indicated that disruption of basophils appeared to take place on in vitro exposure of blood to xylazine. Although all cell types were affected in vitro, the basophil was much more sensitive, with disruption occurring in under 30 s, compared with loss of other cell types taking place between 5 and 10 min. If basophil disruption occurs in vivo this may play a part in occasional stag deaths due to a hypersensitivity-like reaction.

Key words: Basophil, hematology, red deer, spleen, xylazine

Introduction

Our experience suggests that about 12% of blood specimens from farmed red deer in New Zealand are collected with the aid of sedation. It is well-established that the red cell mass falls during xylazine sedation in several species (Hawkey et al. 1980). Biochemical and some hematological changes take place when sedation is used in white-tailed deer (*Odocoileus virginianus*) and antelope (*Antilocapra americana*) (Seal and Bush 1987). The data for sedated red deer are much more sparse, involving few animals and limited information with regard to formed elements of the blood other than red cells (Drescher-Kaden and Hoppe 1972, 1973; McAllum 1978). Chapman (1977) concludes from the Drescher-Kaden and Hoppe papers that red cell count, hemoglobin, and packed cell volume (PCV) showed small decreases following sedation, with no change in mean red cell volume, mean cell hemoglobin concentration, mean cell hemoglobin, and white cell count.

We have reported on 24 farmed red deer in which the animals showed a substantial fall (22%–30%) in circulating red cell mass in all cases, with reductions in circulating platelets (25%) and lymphocytes (36%) (Cross et al. 1988). Preliminary reports have described a fall in basophils following xylazine administration, and a possible link between the loss of basophils from the circulation and occasional deaths of stags following velvet removal conducted under xylazine sedation (Cross et al. 1989; Mackintosh and Cross 1989). The aim of this study was to assess the short-term kinetics of leukocyte changes during xylazine sedation in vivo, and the effect of the drug on blood cells in vitro.

Materials and Methods

Physical restraint was achieved using a pneumatic crush designed for use with deer. Short-term kinetic studies were carried out on four 18-month-old female red deer. All animals were farmed at the Invermay Ministry of Agriculture and Fisheries (MAF) research station. All four were sampled using physical restraint, then two were given xylazine (2-(2,6-xylidino)-5,6-dihydro-4H-1,3-thiazine, "Rompun," Bayer) intramuscularly at a dose of 1 mg/kg, and all four had blood samples taken at 1, 2, 5, 10, 15, 30, and 60 min. Manual differential counts were performed on ten yearling male red deer. In vitro studies were carried out on samples from four mixed-age adult hinds.

Dipotassium ethylenediaminetetraacetic acid (EDTA) was used as the anticoagulant except where stated otherwise. In the in vitro studies where the cell morphology changes were assessed visually, specimens were obtained from five deer, and xylazine was used at a final concentration of 2, 5, and 10 mg/ml, the reaction being stopped by the addition of 10 volumes of ice-cold phosphate buffered saline. Cytocentrifuge smears were then stained using the Jenner-Giemsa method. This work was done on three occasions, two using EDTA anticoagulated blood (three deer) and one using heparinized blood (two deer). Hematological changes were also assessed by means of the Technicon H6000/C, and the final concentrations of xylazine were 0, 0.25, 0.5, 1, and 2 mg/ml. In this latter case specimens from eight animals were presented to the machine in a sequence to ensure each had approximately 6 min contact with the drug before counting was completed. The Technicon H6000/C was also used to obtain counts on blood specimens used for short-term kinetic studies in vivo.

Manual differential counts on cytocentrifuge smears were carried out by selecting an area where cell morphology was clear, differentiating all the cells in the field with the aid of a 10 × 10 eyepiece micrometer grid, and traversing in a straight line to a new field and again identifying all the cells present. This was repeated until 500 cells had been identified. The 95% probability errors were obtained using a computer program (Cross 1985) adapted for 500-cell counts.

Manual differential counts on whole blood were carried out on well-made blood smears prepared using the spreader-slide technique from freshly collected blood in di-potassium EDTA within 10 min of venepuncture. After staining with Jenner-Giemsa, the smears were scanned using a search pattern commencing near the tail of the film and traversing in a straight line towards the origin until white cell morphology began to appear indistinct. Immediately, two microscope fields were traversed at right angles before tracking back towards the tail. This process was repeated until 200 consecutive leukocytes had been identified.

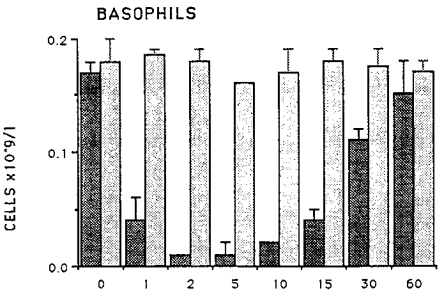
Time for red cell lysis was found by adding 150 µl of 20 mg/ml xylazine to 150 µl of whole deer blood and stopping the reaction by adding 2.7 ml of saline to each tube after 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 min. After centrifuging the tubes, the optical density of the supernatant was measured at a wavelength of 540 nm, within 30 min of commencing the experiment.

Scanning electron microscopy (SEM) was carried out after treating freshly collected blood with xylazine at a final concentration of 10 mg/ml and adding 1 drop to 1% buffered glutaraldehyde after 1, 5, and 10 min. The control consisted of an aliquot of the same cells treated with saline for 10 min.

Results

Intramuscular injection of xylazine was associated with reductions in platelet, lymphocyte, and basophil numbers, and red cell mass. The reductions in red cell mass and platelet count were evident at 5 min and peaked around 30 min, whereas the basophils and lymphocyte reductions were evident at 1 min and peaked around 5 min. The basophil count had returned to initial levels at 60 min, whereas the lymphocyte count was still substantially reduced after 60 min (Fig. 33.1).

The results of manual differentials (Table 33.1) confirmed those found using the automated system, with a moderate increase in neutrophils, significant decreases in lymphocytes and basophils, and no change in eosinophils. No change was seen in the monocytes.



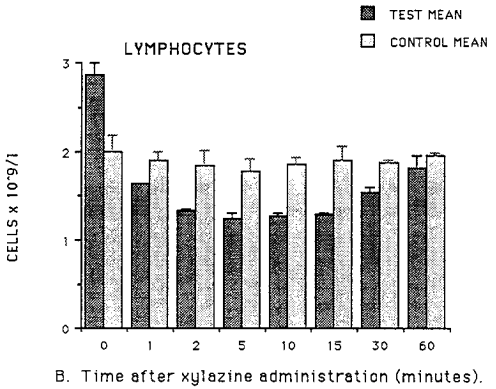


FIGURE 33.1. In vivo effects of xylazine on circulating red deer leukocytes measured using an automated technique (Technicon H6000/C hematology analyser).

In vitro exposure to xylazine brought about a very rapid reduction in basophils, within 25 s, whereas the reduction in lymphocytes was not seen until 5 to 10 min had elapsed (Fig. 33.2). Neutrophils, eosinophils, and monocytes showed similar patterns to lymphocytes. At the same time, an increase in "smear cells" was seen, and occasionally one could see disrupted basophils. Morphological changes were also noted in the nuclear chromatin of the lymphocytes, which developed a more open appearance between 5 and 10 min, suggestive of imminent disruption.

TABLE 33.1. Circulating leukocyte levels (10^9/l) before and 30 min after intramuscular injection of xylazine in farmed red deer using standard manual techniques ($n = 10$)

Cell type	Mean post-xylazine	Mean post-xylazine	Wilcoxon *P* value
Neutrophils	2.05	2.78	0.0125
Lymphocytes	2.45	1.44	0.0051
Monocytes	0.26	0.23	0.5073
Eosinophils	0.35	0.34	0.8785
Basophils	0.16	0.06	0.0217

Release of hemoglobin from red cells exposed in vitro to xylazine showed a similar time scale to the loss of all leukocytes except basophils, with lysis commencing at 8 to 9 min, and complete between 10 and 15 min. The lytic process for red cells was shown by SEM studies to involve a spherocyte transformation prior to lysis. The drug preparation used for these experiments is isotonic according to the suppliers (Ippen, personal communication), and freezing point depression measurements confirmed this, giving an osmolality of 270 mosmol.

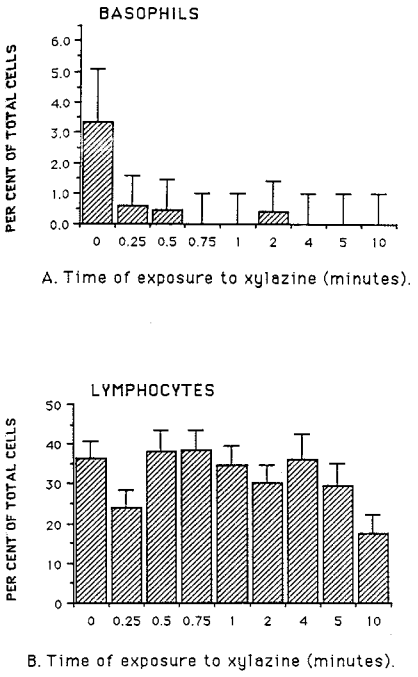


FIGURE 33.2. In vitro effects of xylazine on red deer blood cells measured using manual techniques.

In vitro effects detected using the H6000/C indicated that basophils and lymphocytes were significantly reduced in number at doses of xylazine as low as 0.5 mg/ml, whereas there was no evidence that neutrophils or platelets were reduced under these conditions.

Conclusions

The use of precise technology for leukocyte differential counts (Technicon H6000/C) has been an important aid to the study of leukocyte changes with xylazine. We considered it important that these results be confirmed by standard manual techniques, and the results shown in Table 33.1 clearly show this to be the case.

There are two generally stated hypotheses to account for the red cell mass changes. The first is that splenic contraction prior to sedation leads to a high circulating red cell mass which decreases as red cells return to the spleen once sedation occurs. The second hypothesis is that tissue fluid enters the blood stream due to the blood pressure fall that occurs during sedation with xylazine (Presidente et al. 1973). We have established that the red cell mass changes are greatly reduced in splenectomized red deer, but that the lymphocyte and basophil changes are unaffected (Cross et al. 1989). Therefore, the main reason for the change in circulating red cell mass is return of red cells to splenic stores, and the observed change in platelets can be partly explained in this way.

Since the basophil and lymphocyte falls in vivo precede the red cell and platelet falls, this is a further indication that the mechanisms may differ. The in vitro observations suggest that the basophils are particularly prone to disruption when xylazine is present, and that this is not an IgE-mediated phenomenon. A further indication that it is not due to specific immune hypersensitivity is that the effect is regularly seen in deer not previously exposed to xylazine. It is also evident that a moderate amount of dilution of the drug can take place before the in vitro disruption of basophils is nullified. Experiments are now under way to discover whether in vivo basophil disruption occurs. It is possible that the effects noted in vitro are due to different mechanisms to those seen in vivo. For example, the in vitro loss of lymphocytes could be the result of the action of enzymes released from the basophils.

A wide range of factors can bring about basophil and mast cell degranulation directly, including drugs and chemicals (Fadal 1985). We have drawn attention to the possible role this could play in the occurrence of occasional deaths following xylazine administration (Mackintosh and Cross 1989). Several authors have pointed out that reference values obtained using physical restraint may not be applicable to those obtained using sedation. It has been suggested that "anesthetised animals yield more nearly normal values" (Seal and Bush 1987). While this seems likely to be true in the case of red cell parameters, there must be some doubt that it is always the case for xylazine-treated ones as far as lymphocytes and basophils are concerned.

Acknowledgements

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References

Chapman DI (1977) Haematology of the deer. In Archer RK, Jeffcott LB (eds) Comparative Clinical Haematology. Blackwell Scientific Publications, Oxford, pp 345–364

Cross JP (1985) Program for confidence limits for leukocyte differentials. Lab Med 17:296

Cross JP, Mackintosh CG, Griffin JFT (1988) Effect of physical restraint and xylazine sedation on haematological values in red deer (*Cervus elaphus*). Res Vet Sci 45:281–286

Cross JP, Mackintosh CG, Griffin JFT (1989) Further observations on xylazine and haematological parameters in red deer (*Cervus elaphus*): the effect on reference values, and on splenectomised animals. Proc N Z Vet Assoc Deer Branch 6:146–150

Dacie J, Lewis SM (1975) Practical Haematology, 5th ed. Churchill Livingstone, London

Drescher-Kaden UV, Hoppe P (1972) Vergleichende hämatologische Untersuchungen an wildlebenden Wiederkauern (Rehe, Rotwild, Gemsen, Rentiere) 2 Mitteilung: Erythrozentahl, Erythrozytendurchmesser, Hämoglobingehälte und Hämatokrit. Zeit Jadgdwiss 18:121–132

Drescher-Kaden UV, Hoppe P (1973) Vergleichende hämatologische Untersuchungen an wildkebenden Wiederskauern (Rehe, Rotwild, Gemsen, Rentiere). 2. Mitteilung: Das weibe Blutbidle (Zahl der gesamten und der eosinophilen Leucozyten, Differentialiblutbild). Zeit Jadgdwiss 19:65–75

Fadal RJ (1985) The immunobiology and immunopharmacology of the allergic response. Otolaryngol Clin N Am 18:649–676

Hawkey C, Frankel T, Jones D, Jones A, Nevill G, Hart M, Alderson C (1980) Preliminary report of a study of changes in red blood cells of zoo animals during sedation. In Montali RJ, Migaki G (eds) The Comparative Pathology of Zoo Animals Smithsonian Institution, Washington D.C., pp 625–632

Mackintosh CG, Cross JP (1989) Xylazine study report. Proc N Z Vet Assoc Deer Branch 6:136–143

McAllum HJF (1978) Post capture myopathy syndrome in red deer (*Cervus elaphus*): M.V.Sc. thesis, Massey University, New Zealand, pp 36–39

Presidente PJA, Lumsden JH, Presnell KR, Rapley WA, McCraw BM (1973) Combination of etorphine and xylazine in captive white-tailed deer: II. Effects on haematologic, serum biochemical and blood gas values. J Wildl Dis 9:342–348

Seal US, Bush M (1987) Capture and chemical immobilization of cervids. In Wemmer CM (Ed) Biology and Management of the Cervidae. Smithsonian Institution, Washington D.C., pp 480–504