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Hematological Study of Experimental Anaphylaxis in Goats

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ABSTRACT

This study was carried out to measure the hematological changes in systemic anaphylaxis in goats. Goats were sensitized to horse serum and were subjected to systemic anaphylaxis shock. Analysis of blood samples collected at 24 h after each injection of antigen during sensitization, as well as at 10, 60 and 240 min after antigenic challenge in sensitized animals revealed a non significant increase in packed cell volume (PCV), hemoglobin (Hb) concentration and total red blood cell (RBC) count. Total leukocyte count (TLC) did not alter significantly during sensitization, while it decreased significantly ($p<0.05$) immediately after antigenic challenge. Study appears to suggest leucopenia may be the primary anaphylactic response and hemoconcentration may be a secondary response in goat specie.

Key Words: Hematological; Experimental; Anaphylaxis

INTRODUCTION

Anaphylaxis or immediate hypersensitivity describes a complex group of immunological reactions, which are characterized by an undesirable enhanced responsiveness to an antigen to which the animal has previously been exposed (Tizard, 1977). The antigens are high molecular weight substances which are foreign to the body and are capable of stimulating an immune response (Bowman & Rand, 1980). The hypersensitivity responses are inflammatory reactions mediated by certain immunoglobulins (especially IgE in mammals), bound to mast cells and basophils; the reactions result from the pharmacologically active mediators by these cells (Winbrey & Liederman, 1995). The incidence of anaphylaxis reactions seems to be increasing and perhaps this rise is due to increased environmental and medical exposure to agents such as foods, drugs, other biologicals and insect venoms (Winbrey & Liederman, 1995). The hematological signs observed in experimentally induced anaphylaxis shock reveal that the involvement of leucocytes is a prominent feature in this type of reaction (Wells *et al*., 1973). Initial leucopenia is almost a consistent finding in anaphylaxis and its relationship to release of chemical mediators has been investigated. This has been observed in calf (Eyre *et al.,* 1973; Wells *et al.,* 1973; Gerros *et al.,* 1995; Nakanishi *et al.,* 1993; Nagaraja *et al.,* 1979), sheep (Aiumlamai *et al.,* 1993), horse (Morris *et al.,* 1992; Cargile *et al.,* 1995; Eaton *et al.,* 1995), dog (Kitoh *et al.,* 1994) and in goat (Takeuchi *et al.,* 1997). They all reported that leucocytes particularly granulocyte count, fall markedly immediately after induction of anaphylaxis and returns to normal after one hour. One of the most profound changes was hemoconcentration accompanied by comparable increase in red cell count and hemoglobin concentration. Wells *et al.* (1973) observed an increase of about 23% in packed cell volume during anaphylactic shock in calves. This increase occurred over a period of 10-60 min from the start of antigen infusion. An increase in hemoglobin concentration and increase in total erythrocyte count were also observed over a similar period. Similar increase in PCV, Hb and RBC count has been reported in cattle (Aitken & Sanford, 1969; Sandhu & Brar, 1989), sheep (Alexander *et al.,* 1970; Aiumlamai *et al.,* 1993), and horse (Eyre & Lewis, 1973; Eaton *et al.,* 1995) dog (Kitoch *et al.,* 1994).

Attempts to create and study anaphylaxis in goats have been few (Takeuchi *et al.,* 1997) and have raised the interest centered on caprine anaphylaxis reaction. In view of the incomplete information, this study has been designed to investigate the influence of anaphylaxis on blood parameters.

MATERIALS AND METHODS

Six visibly normal goats of mixed breed under one year of age and weighing 18 kg (average) were used. Animals were allowed to acclimatize for 7 days during which they were kept on hay, green and fresh grasses and concentrates. Blood was collected from the goats and hematological parameters were recorded to establish base line values. After obtaining control values all animals were used for experiments.

Sensitization. Horse serum was used as antigen. Serum was obtained from blood of the healthy horse. All animals were sensitized by injecting horse serum I/V at the dose of 0.2 mL/kg body weight. One hour after I/V injection an additional subcutaneous injection of the same dose of antigen was given in the neck region. Two more subcutaneous injections of the same dose (0.2 mL/kg body weight) of horse serum obtained from freshly collected blood were injected at weekly intervals i.e. 7^{th} and 14^{th} day after the first injection. Blood samples were collected from goats after 24 h. of each injection and analyzed for possible hematological values. After the last injection, three more weeks were allowed (latent period) before the animals were

challenged to induce anaphylaxis.

Systemic anaphylaxis. After three weeks of sensitization, all the animals were challenged with the same dose (0.2 mL/kg) of horse serum administered intravenously. Blood samples were taken at 10, 60 and 240 min post antigenic challenge and analyzed for alteration in hematological values.

Hematological tests. Packed cell volume (PCV) was determined by means of a Microhematocar method (Bush, 1975).

Hemoglobin concentration (Hb) was measured by Sahlis method (Tharp, 1980). Red blood cell counts and Total leucocytes counts were carried out with a Hemocytometer method (Kolmer *et al.,* 1959).

A two way analysis of variance was used to compare the differences between control and horse serum responses during sensitization and post challenge.

RESULTS

Hematological changes recorded after the three antigenic injections during the course of sensitization are described in Table I, where as changes recorded after 10, 60 and 240 min of antigenic challenge are depicted in Table II.

Hemoglobin. There was a non significant increase in Hb concentration in response to 3rd antigenic injection during the sensitization process (Table I). The increased Hb concentration was also observed over 10 to 60 minutes of post antigenic challenge, however the increase in hemoglobin concentration was non significant (Table II).

Packed cell volume. Analysis of data revealed a non significant increase during sensitization (Table I). Similarly non significant increased PCV values were obtained after challenge when compared to controls (Table II).

Total red blood cell count. As shown in Table I and II, the antigenic administration during the process of sensitization or when used to induce systemic anaphylaxis increase the erythrocyte counts, however these changes were non significant.

Total leukocyte count. The TLC appeared to increase after the first and second dose of antigen and decreases at third dose of antigen during the process of sensitization; however the differences were not statically significant (Table I). On the other hand, the systemic anaphylaxis was characterized by significant ($p<0.05$) decrease in TLC immediately after antigenic challenge. As shown in Table II, after an immediate drop, the TLC returned to normal and exhibited a non significant increase at 240 min post antigenic challenge.

Table I. Hematological values of 6 goats during sensitization to horse serum. Samples were taken at post first injection, post second injection and post third injection

Parameter	Control	Post 1st injection	Post 2nd injection	Post 3rd injection
Hemoglobin G%	09.32 ± 00.42	09.10 ± 03.87	09.00 ± 00.49	10.20 ± 00.25
Packed cell volume %	18.33 ± 01.03	19.21 ± 00.80	19.50 ± 00.84	20.00 ± 01.26
Red blood cell count m/cum	07. 09 ±00.97	06.92 ± 01.02	07.087 ± 00.72	07.99 ± 00.64
Total leukocyte count 10^3/ cumm	08.03 ±01.12	08.71 ±01.18	08.21 ±01.25	07.78 ±00.85

Data is expressed as Mean ± S.D.
* Significantly ($P < 0.05$) different from control.

Table II. Hematological values of 6 goats after challenge to horse serum. Samples were taken at 10, 60 and 240 minutes post challenge.

Parameter	Control	After 10 minutes	After 60 minutes	After 240 minutes
Hemoglobin G%	09.30 ± 00.48	09.85 ± 00.30	09.95 ± 00.495	09.13 ± 00.25
Packed cell volume %	19.50 ± 01.03	20.50 ± 01.22	20.83 ± 00.89	18.83 ± 01.06
Red blood cell count m/cum	07. 55 ±00.63	08.97 ± 00.61	08.26 ± 00.72	07.79 ± 00.64
Total leukocyte count 10^3/ cumm	09.16 ±01.12	04.26* ±00.71	08.21 ±00.52	10.28 ±01.05

Data is expressed as Mean ± S.D.
Significantly ($P < 0.05$) different from control

DISCUSSION

Principle aim of present study was to investigate the hematological changes accompanied during experimental anaphylaxis in goats. Change in leucocytes is a prominent feature of anaphylaxis and has been reported in dog (Kitoh *et al.,* 1994), cattle (Wells *et al.,* 1973; Geroos *et al.,* 1995, Nakanishi, *et al.,* 1993), horse (Cargile *et al.,* 1995; Morris *et al.,* 1992; Eaton *et al.,* 1995), and sheep (Aiumlamai *et al.,* 1993). It seems likely, therefore, that leucocytes may play an integral part during the anaphylaxis in goat. In this study, leucocytes count significantly decreased ($p<0.05$) immediately after antigenic challenge (Table II). Initial leucopenia which may result from the sequestration of the cells in vascular bed is almost a consistent finding in anaphylaxis and has been reported in calf (Wells *et al.,* 1973; Geroos *et al.,* 1995; Nakanishi, *et al.,* 1993), sheep (Aiumlamai *et al.,* 1993), and horse (Cargile *et al.,* 1995; Eyre & Lewis, 1973). The initial leucopenia was returned to normal followed by a marked rise in leucocytes at 240 min post challenge. Wells *et al.* (1973), Eyre and Lewis (1973), Morris *et al.* (1992), Singh and Sodhi (1992) and Aiumlamai *et al.* (1993) reported the similar finding in cattle, horse and sheep. On the other hand Nagaraja *et al.* (1979) reported the persisted leucopenia in anaphylactic like reaction in calves, which returned to normal in 12 h post endotoxin administration. It has been reported that in cow and horse whole blood histamine concentration fall significantly during initial leucopenia between 10 and 20 min after the start of anaphylaxis (Wells *et al.,* 1973; Eyre & Lewis, 1973). This suggests the important correlation between decreased histamine concentration and leucopenia.

The decrease in leucocytes count observed in this study may be reflection of the above process there by implicating histamine as the primary mediator. This however needs further study in goat.

Hemoconcentration increased during anaphylaxis. The blood analysis revealed an increase in RBC count, Hb concentration and PCV volume. The increased hemoconcentration may have been due to loss of circulatory fluid by vascular permeability changes. In addition the systemic hypotension during anaphylaxis leads to an increase in systemic sympathetic activity with resultant splenic contraction raising the circulatory erythrocytes (Eyre & Lewis, 1973). Significant increases in PCV, Hb concentration and RBC count during anaphylaxis have been observed in different species including cattle (Aitken & Sanford 1969; Wells *et al.,* 1973; Sandhu & Brar, 1989), sheep (Alexander *et al.,* 1970), horse (Eyre & Lewis, 1973) and dog (Kitoh *et al.,* 1994). The findings of this investigation in goats with respect to RBC, Hb and PCV after antigenic challenge are not in complete agreement with other studies. As shown in Table I and II, there was a non significant increase in RBC count, Hb concentration and PCV volume. Similar findings were also reported during endotoxin induced anaphylactic reactions in calves (Nakanishi *et al.,* 1993) and in horse (Cargile *et al*., 1995). One possible explanation for the non significant rise in RBC may be that, among the domestic animals, the erythrocytes in goats are the smallest in diameter and the roulex formation is also absent (Sastri, 1989), thus the number ob RBC may have increased but due to smaller size may not have reached the statistical significance.

This study confirms the successful sensitization and induction of experimental anaphylaxis in goat. The hematological changes indicate that leucopenia is prominent feature during systemic anaphylaxis in goat. However further studies are required to explore the specificity of mediator of anaphylaxis in this specie.

REFERENCES

Aitken, M.M. and J. Sanford, 1969. Experimental anaphylaxis in cattle. *J. Comparative Path*., 79: 131–9

Alexander, F., P. Eyre and K.W. Head, 1970. Effects of anaphylaxis and chemical histamine liberators in sheep. *J. Comparative Path.,* 80: 19–30

Aiumlamai, S, H. Kindhai, P. Ingkaniun and P. Poomvises, 1993. Low doses of endotoxin in ewes. *Proceed., 11th Int. Symp. of the World Association of Veterinary Food Hygienists*, pp. 322–6. Oct. 1993, Thailand

Bowman, W.C. and M.J. Rand, 1980. *Text Book of Pharmacology.* 2nd Ed. Black well Science publication. London. 13, 1–35

Bush, B.M., 1976. *Veterinary Laboratory Manual.* Unwin Brs. Ltd.., The Gresham Press, Old working, Surry. p. 125

Cargile, J.L., R.J. Mackay; J.R. Dankert and L. Skelley, 1995. Effect of treatment with a monoclonal antibody against equine tumor necrosis factor (TNF) on clinical, hematologic and circulating TNF responses of miniature horses given endotoxin. *American J. Vet. Res.*, 56: 1451–9

Eaton, S.A, D. Allen, S.C. Eades and D.A. Schneider, 1995. Digital starling forces and hemodynamics during early laminitis induced by an aquous extract of black walnut in horses. *American J. Vet. Res.,* 56: 1338–44

Eyre, P. and A.J. Lewis, 1973. Acute systemic anaphylaxis in the horse. *British J. Pharmacol.,* 48: 426–73

Eyre, P., A.J. Lewis and P.W. Wells, 1973. Acute systemic anaphylaxis in the calf. *British J. Pharmacol.,* 47: 504–16

Geroos, T., S.D. Semrad and R.A. Proctor, 1995. Alterations in clinical, hematological and metabolic variables in bovine bovine neonatal endotoximia. *Canadian J. Vet. Res.*, 59: 34–9

Kitoh, K., K. Watoh, K. Chaya, H. Kitagawa and Y. Sasaki, 1994. Clinical, hematological and biochemical findings in dogs after induction of shock by injection of heart worm extract. *American J. Vet. Res.,* 55: 1535–41

Kolmer, J.A, E.H. Paulding and H.W. Robinson, 1959. *Approved Laboratory Techniques.* 5th Ed. Appleton, NewYork

Morris, D.D, J.N. Moore, N. Crowe and L.L. Moldawer, 1992. Effeect of experimentally induced endotoximia on serum interleukin–6 activity in horses. *American J. Vet. Res.*, 53: 753–6

Nagaraja, T.G, E.E. Bartley, H.D. Anthony, H.W. Leipold and L.R. Fina, 1979. Endotoxin shock in calves from intravenous injection of rumen bacterial endotoxin. *J. Anim. Sci.,* 49: 567–82

Nakanishi, N, Y. Nakamur; S. Ura, N. Tisuji, N. Taira, N. Tanimura and M. Kubo, 1993. Sudden death of calves by experimental infection with strongyloids papillosus. III. Hematological,. Biochemical and histological examinations. *Vet. Parasitol.,* 47: 67–76

Sandhu, H.S. and R.S. Brar, 1989. Hematological alterations induced by acute toxicity of monocrotophos in buffalo calves. *Indian J. Anim. Sci.,* 59: 1263–4

Sastri, G.A., 1989. Hematology and blood biochemistry. *Veterinary Clinical Pathology.* 3rd Ed. Philadelphia

Singh, D.V. and S. Sodhi, 1992. Effect of shock on certain biochemical profiles of blood in calves. *Indian J. Anim. Sci.,* 62: 1031–6

Takeuchi, Y., T. Kikusui, D. Kizmui, H. Ohnishi and Y. Mori, 1997. Pathophysiological changes evoked by lipopolysaccharide administration in goats. *J. Vet. Medical Sci.,* 59: 125–7

Tharp, G.D., 1980. *Experiments in Physiology.* 4th Ed. University of Nobraska, Lincolin, Burgess Pub. Company

Tizzard, J.R., 1977. An introduction to veterinary immunology. 1st ed. W.B. Saunders Co., Philadelphia. 1: 15–8

Wells, P.W, P. Eyre and J.H. Lumsden, 1973. Hematological and pathological changes in acute systemic anaphylaxis in calves; Effects of pharmacological agents. *Canadian J. Comparative Medicine.,* 37: 119–29

Winbery, S.L. and P.L. Lieberman, 1995. Anaphylaxis: Systemic Reaction. *Immunology and Allergy Clinics of North America*., 15: 447–75

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