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Clinical Study of Experimentally Induced Anaphylactic Shock in Goats

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Abstract: This study was carried out to study the characteristics of Caprine anaphylaxis with emphasis on clinical features observed during pre and post Sensitization. Six goats of mixed breed were sensitized to the repeated injections of horse serum. During sensitization common clinical signs observed after second and third antigenic injections were frequent urination, defecation, cough, nasal secretion, irregular respiration and heart beat, dullness and depression confirms the success of sensitization. After a latent period of three weeks, all animals were challenged to horse plasma intravenously. Post challenge clinical signs included respiratory distress characterized by a multifold increase in frequency and thoracolumbar irregular and jerky respiration, nasal secretion, severe coughs, shivering, paddling and kicking. Auscultation revealed moist rales over the lung area. Animals were dull, depressed and unwilling to move. The clinical signs observed during anaphylaxis indicate that the lung may be the major shock organ of anaphylaxis in goats. The results of this study will help in the establishment of appropriate and specific therapy for Type-I hypersensitivity reactions (Anaphylactic-shock) in goat specie.

Key words: Experimental, anaphylaxis, clinical, sensitization, systemic anaphylaxis

INTRODUCTION

Immediate type hypersensitive reactions are inflammatory reactions mediated by certain immunoglobulins isotypes especially IgE, bound to mast cells and basophils; the reactions results from the release of pharmacologically active factors by these cells (Tizard, 1987). The incidence of anaphylaxis and anaphylactic 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 (Acero *et al.*, 2003; Winbery and Liberman, 1995).

Anaphylaxis has been extensively investigated in the guinea pig, rabbit, rat, mouse, dog, calf and horse. Guinea pig dies within 3-5 min due to broncho constriction with labored and gasping breathing (Popa *et al.*, 1973). Rabbit shows respiratory distress and heart failure (Habit *et al.*, 1988; Winbery and Liberman, 1995). In dog profound prostration associated with vomiting, bloody diarrhea and pooling of blood in the liver (Booth *et al.*, 1970; Kitoh *et al.*, 1994). Rat shows dyspnea and intestinal lesions (Church, 1975). In cat, the respiratory distress is a prominent sign followed by vomition and in coordination (Aitken and McCusker, 1969).

In calf, lung has been suggested as the primary target organ of anaphylaxis. I/V antigenic injection lead to respiratory distress with small periods of apnea, coughing and arterial hypotension (Aitken and Sanford, 1969; Berrier, 2003; Eyre *et al.*, 1973; Holroyde and Eyre, 1975; Jean and Bak 1993; Singh *et al.*, 1996). However some have noted several gastro intestinal lesions (Wray and Thomlinson, 1972). In horse, the clinical signs include dyspnea followed by conjunctival congestion, fluid diarrhea, general sweating and pilo erection (Mc Gavin *et al.*, 1972; Eyre and Lewis, 1973). The sensitized sheep responds with respiratory embarrassment and significant changes in lung function (Alexander *et al.*, 1967; Wanner *et al.*, 1979; Mirbahar and Eyre, 1982).

For the characterization of anaphylaxis animals are experimentally sensitized by introducing antigen from a single I/V injection to multiple I/V injections (Aitken and Sanford, 1969; Aitken *et al.*, 1975) or supplemented by sub cutaneous injections (Eyre and Deline, 1976). Some have used intra tracheal injections (Mirbahar, 1985).

Goat is one of the important species of live stock which play a vital role in the economy of Pakistan. In view of the lack or incomplete information (Ladiges and

Garlinghouse, 1981), it seemed of immediate importance to investigate the physio-pathological characteristics of caprine anaphylaxis. This study was therefore carried out to induce the experimental anaphylaxis in goat by using injections of horse plasma as an antigen. The characterization of clinical response of this model will help for the establishment of appropriate treatment of anaphylaxis.

MATERIALS AND METHODS

The study was conducted in 2002 at the Department of Veterinary Pharmacology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam. Six visibly normal goats of mixed breed under one year of age and weighing 18 kg (average) were used. Animals were assigned the identification numbers from G: 1 to G: 6. Animals were allowed to acclimatize for 7 days during which temperature, heartbeat and respiration rates were recorded daily to establish base line values. Animals kept on hay, green and fresh grasses and concentrates. 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 then sensitized by injecting horse serum I/V at the dose of 0.2 mL kg⁻¹ body weight. One hour after the intra venous injection an additional sub cutaneous injection of the same dose of antigen was given in the neck region. Two more sub cutaneous injections of the same dose (0.2 mL kg⁻¹ body weight) of horse serum obtained from freshly collected blood were injected at weekly intervals i.e., 7 and 14th day after the first injection (Aitken and Sanford, 1969; Aitken *et al.*, 1975; Eyre and Deline, 1976). All the animals were observed carefully through the day and the abnormal signs if any were recorded. Heart rate, respiration rate and rectal temperature were recorded after each injection of antigen during the process of sensitization. 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 of antigen (0.2 mL kg⁻¹ body weight) administered intravenously (Eyre and Burka, 1978). Animals were carefully observed for any abnormal reactions such as urination, defecation, respiration, heart beat, temperature coughing, nasal secretion, lacrimation sweating etc. Parameters such as heart beat and respiration were recorded at 10, 20, 30, 40, 50 60 and 240 min post

antigenic challenge. Where as rectal temperature was recorded at 10, 30, 60 and 240 min post antigenic challenge.

Statistical significance was determined using student's t-test to specify differences between means.

RESULTS

Sensitization: After the injection of antigen, the animals were observed continuously for 6 h. The clinically apparent signs noted after the three injections given during the course of sensitization are described in Table 1, 2 and 3, respectively.

As shown, the signs such as urination, defecation, cough, nasal secretion, irregular respiration and heart beat, dullness, depression and unwillingness of animals to stand were more frequent and were observed in almost all animals, while shivering, dyspnoea, tachycardia were least common.

Table 1: Summary of the clinical signs observed after injection of antigen used for experimental sensitization of Goats: Post first dose of antigen: I/V followed by sub cut injection

Symptoms shown	Animals ID No.
Frequent urination (for about 2 h)	All except G: 4
Frequent defecation (for about 2 h)	All except G: 4
Shivering for approximately about 10 min	G: 1, G: 3 and G: 4
Erection of hairs	G: 1, G: 3 and G: 4
Cough (mild for approximately 30 min	G: 1, G: 3 and G: 4
Nasal secretion	G: 1, G: 3 and G: 4
Shallow respiration (approximately for 30 min)	G: 2 and G: 4
Dull and depressed	All except G: 6

Table 2: Summary of the clinical signs observed after the second dose of antigen (one week after the first dose) administered subcutaneously

Symptoms shown	Animals ID No.
Frequent urination (for about 2-3 h)	All animals
Frequent defecation (for about 2-3 h)	All animals
Slightly increased and deep respiration with prominent abdominal movements (for about 2 h)	All except G: 2
Tachycardia (for about 2 h)	G: 2, G: 5 and G: 6
Dull and depressed. All except	G: 3 and G: 4

Table 3: Summary of the clinical signs observed after the dose of antigen one week after the second injection administered subcutaneously

Symptoms shown	Animals ID No.
Frequent urination (at least for 2 h)	All except G: 2 and G: 4
Frequent defecation (at least for 2 h)	All except G: 2 and G: 4
Cough (approximately for 2 days)	All animals.
Severe cough immediately after injection (approximately for 3 h)	G: 2 and G: 3 and G: 6
Nasal secretion (for about 3 days)	Observed in all. Increased in G: 2, G: 3 and G: 4
Irregular respiration (deep and slow, approximately for 2 h)	All except G: 1 and G: 5
Grunting type respiratory sounds (2-3 h)	G: 3
Difficult breathing (Approximately 3 h)	G: 6
Irregular heart beat (at least for 1 h)	All
Weak sounds	
Dull and depressed and unwilling to stand (for about 1-2 h)	All

Systemic anaphylaxis: After a latent period of three weeks, all animals were challenged with horse serum injected intravenously at a dose of 0.2 mL⁻¹ kg body weight. After the antigenic injections, all animals responded with respiratory distress primarily characterized by multifold increase in respiration rate. The change in the character of respiration was manifested as a jerk with rapid thoracic and abdominal movement, irregular respiration with periods of apnea, in coordination, circling, staggering and back walking, unable to stand and falling down. After 60 min of injection the animals were able to stand on their feet with a characteristics posture indicated as neck extended forward and downward and bended towards one side with slightly opened mouth and semi closed eyes, wide opened nostrils and double expiratory movements. Clinically animals improved after one and half hour of injection. All animals looked dull, depressed, lethargic and drowsy in appearance, preferred to sit on the group and were unwilling to move. When forced to move, they exhibited the signs of in coordination. Rubbing and pressing themselves and their head against the sides of the pen was also common. Paddling and kicking was also observed in all animals. Increased nasal secretion was also observed in all animals while only two animals coughed after antigenic challenge. The auscultation of the chest revealed irregular and loud heard sounds, while moist rales were heard over the lung area in all animals. A rise in temperature and Shivering was observed.

Physiological recordings

Heart beat: Heart beat exhibited was significantly (p<0.05) higher after third injection of sensitization (Table 5).

Respiration: Respiration appeared to increase during sensitization. However, the respiration rate was significant (p<0.5) higher at 10 and 20 min post antigenic challenge (Table 6).

Temperature: The rectal temperature exhibited a significant (p<0.5) increase after the third injection of antigen during sensitization (Table-5).

DISCUSSION

Allergic diseases of man and animal were among the diseases of unknown etiology until the experimental observations in animal discovered the methods to establish the state of anaphylaxis or hypersensitivity. Acquired hypersensitivity to agents in our environment might cause a variety of diseases and the list of possible allergic diseases grew rapidly. Delay in recognizing the

Table 4: Summary of the clinical signs observed after I/V injection of challenged antigen in sensitized goats (clinical signs of systemic anaphylaxis)

Symptoms shown	Animals ID No.
Increased respiration (multifold) for 30 min	All
Respiratory distress for about 2 h	All
Jerky respiration with grunting sounds	All
Forced expiratory movements	All
Irregular respiration with short period (10-20 sec) of apnea	All
In coordination, circling, staggering, balk walking, unable to stand and falling down (lasts for 30 min)	All except G: 1
Characteristics posture (for at least 30 min) neck extended, forwarded, downward and bented to one side, wide opened nostrils	All except G: 1
Rubbing, pressing of body and head against walls	All except G: 1 and G: 6
Paddling and kicking	All
Shivering for at least 40 min	All except G: 1 and G: 5
Nasal secretion	All
Cough	G: 1, G: 3, G: 6
Moist rales sounds heard over the lung area	All animals
Irregular and loud heart sounds	All animals
Dull, depressed, lethargic, drowsy, unwilling to stand, in coordination	All animals

signs and symptoms of anaphylaxis can result in mortality. Experimental anaphylaxis has been studied in different species to create an experimental condition similar to natural allergic diseases. These studies will help in differential diagnosis for anaphylaxis and development of appropriate treatments.

Anaphylaxis is a systemic immediate type hypersensitivity reaction resulting from the combination of specific antigen with reaginic antibody, IgE. The reaction ensues after an interval of two to three weeks, by which time the antibodies have, becomes fixed to mast cells and to a lesser extent to basophils along with the smooth muscle cells and capillary endothelium in shock organs. On re exposure to antigen in sufficient quantity, the antigen interacts with the cell fixed antibodies leading to the degranulation of mast cells and basophils causing release of anaphylactic mediators which are responsible for the clinical manifestations (Black, 1979; Eyre and Burka, 1978; Eyre, 1980; Tizzard, 1987; Winbery and Liberman, 1995).

Experimentally, sensitizing animals to foreign proteins and exposing them to the same antigen after 2-3 weeks have induced the anaphylactic reactions (Aitken *et al.*, 1975; Eyre and Burka, 1978).

In this study the success of the sensitization was confirmed by response to 2nd and 3rd antigenic injection suggesting the gradual rise in antibodies. During sensitization re exposure of animals to antigens caused significant increase in heart beat and body temperature (Table 5). This is in agreement with others reporting gradual development of response to repeated

Table 5: Heart rate, respiration rate and body temperature of 6 goats during sensitization to horse serum

Parameter	Control	Post 1st injection	Post 2nd injection	Post 3rd injection
Heart rate (min)	82.00±03.66	80.00±03.87	79.00±03.16	99.00±05.33*
Respiration rate (min)	28.00±00.75	35.00±00.94	38.00±00.94	38.00±00.90
Temperature (°F)	102.4±00.26	102.4±00.60	103.0±00.54	104.6±00.24*

Data is expressed as Mean±SD, * Significantly (p<0.05) different from control

Table 6: Heart rate, respiration rate and body temperature of 6 horse serum sensitized goats recorded at 10, 20, 30, 40, 50, 60 and 240 min post challenge to horse serum

Parameter	Control	After 10 min	After 20 min	After 30 min	After 40 min	After 50 min	After 60 min	After 240 min
Heart rate (min)	90.00±4.31	98.00±4.96	102.00±5.87	102.00±6.08	101.00±6.52	102.00±4.98	100.00±5.80	95.00±3.57
Resp. rate (min)	29.00±2.75	80.00±6.18*	62.00±6.0*	35.00±5.90	31.00±1.63	31.00±3.08	30.00±4.00	30.00±3.08
Temp. (°F)	102.00±0.70	102.8±0.94	-	103.8±1.28	-	-	103.0±1.22	102.4±0.65

Data is expressed as Mean±SD* Significantly (p<0.05) different from control

antigenic administration during the process of sensitization (Aitken and Sanford, 1969; Aitken *et al.*, 1975; Eyre and Deline, 1976; Kitoh *et al.*, 1994; Mirbahar, 1985; Mirbahar *et al.*, 1985; Sharbaugh *et al.*, 1972; Wanner and Reinhart, 1978).

Clinically, the systemic anaphylaxis in goat was characterized predominantly by a multifold increase in respiration rate coupled with wheezing and other respiratory sounds indicating bronchoconstriction (Table 4). Respiratory embarrassment is also a primary feature of anaphylaxis in cattle (Aitken and Sanford, 1969; Berrier 2003; Celly *et al.*, 1989; Dung worth, 1965; Eyre *et al.*, 1973; Eyre and Lewis, 1973; Jean and Bak, 1993; Singh *et al.*, 1996), sheep (Alexander *et al.*, 1970; Wanner and Reinhart, 1978; Wanner *et al.*, 1979) and horse (Mirbahar *et al.*, 1983; Mirbahar *et al.*, 1985). It is reported that immunogenic inflammation in anaphylaxis also causes the neurogenic stimulation leading to the involvement of respiratory system (Meggs, 1995). In these studies lung is believed to be the major target organ of anaphylaxis. It is possible that both immunogenic and neurogenic factors target the lung in the goat.

The results are in contrast to findings of others who have reported tympany, hyper peristalsis, gastrointestinal lesions and accumulation of fluid in the abdominal cavity in sheep and calves and horse (Alexander *et al.*, 1970; Holroyde and Eyre 1975; Wray and Thomlinson, 1972; Sustronck *et al.*, 1993; Singh *et al.*, 1996). However we have observed cumulative numbers of urination and defecation during the process of sensitization but these responses were not observed after challenge in systemic anaphylaxis. Aitken and Sanford (1969) and McGavin *et al.* (1972) reported the same in cattle and horse and suggested the temporarily involvement of Gastrointestinal tract in acute anaphylaxis.

Results of present study are not in agreement with Kitoh *et al.* (1994) who considered liver and portal vessels as important target organs of anaphylaxis in dog. While Wells *et al.* (1973) and Singh *et al.* (1996) reported the involvement of liver in some calves. On the other

hand, Capurro and Levi (1975) reported heart as the target organ of anaphylaxis in the guinea pig. These differences however may reflect species differences.

The clinical and physiological manifestation of acute systemic anaphylaxis is many and varies with each species. Present results indicate that goat can be sensitized to foreign protein. Furthermore the responses suggested the involvement of lung as the major shock organ in caprine specie.

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