Study of Cell-Mediated Immune Response in Experimental Aflatoxicosis of Buffalo Calves

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Abstract

Humoral immune response due to the B-lymphocytes and cell-mediated immune response (CMIR) due to T-lymphocytes are the two arms of immune response. This study was undertaken to investigate CMIR in buffalo calves with experimental aflatoxicosis. Six buffalo calves were randomly divided into two groups of three animals each. One group was fed aflatoxin B1 (AFB1) at the rate of 10 µg/kg body weight daily for 30 days (treatment group) while the other group was left as control. CMIR in both treated and control calves was studied by erythrocyte (E) rosette technique as well as the delayed type hypersensitivity (DTH) skin test. T-lymphocytes status in these animals before and at 10 days intervals after experimental aflatoxicosis was studied by using E-rosette technique. The mean percent E-rosette in control calves remained unchanged throughout the experiment (i.e. day 0, 10, 20 and 30) whereas it increased in AFB1 intoxicated animals on day 0 and 10 and decreased thereafter. DTH skin test, carried out by initial sensitisation at day 20 followed by challenge on day 30 post-intoxication by BCG vaccine antigen. It revealed an increase in mean skin thickness from 3.43±0.12 mm pre-challenge to 10.50±0.75 mm 48 hr post challenge in control calves as against 3.57±0.37 mm to 4.57±0.32 mm in treated calves. There was a highly significant (P<0.01) reduction in skin thickness in AFB1 intoxicated calves when compared with the controls. These findings provided evidence of CMIR suppression in AFB1 intoxicated buffalo calves.

Keywords: Aflatoxin, Immune response

Introduction

Buffalo is an important producer of milk in Nepal and most part of Central Asia. In Nepal, she buffalo is reared for milk and he buffaloes for meat and draft purposes. So, the importance of the buffalo in the Nepalese meat industry is now widely recognised. There are 3.4 million buffaloes in Nepal producing a total of 729.4 thousand metric ton milk and 117.4 thousand metric ton meat per year which is 70% of total milk and 65% of total meat produced in the country (CBS 1998).

The aflatoxin producing fungi i.e. Aspergillus flavus and Aspergillus parasiticus are commonly found as toxic contaminants of many food commodities in the tropics (WHO 1979). These may pose threat to animal and human health when present in feed and food sources. Great economic losses occur from aflatoxin contamination of animal feeds which causes reduced growth rate, low productivity, infertility and may cause livestock mortality and rejection of animal products in the international market. Among all the aflatoxins, aflatoxin B1 (AFB1) is the most abundant and potent toxin (Kang 1970). Aflatoxin causes a variety of biological effects on animal system, including teratogenicity (Di Paola et al. 1967), mutagenecity (Ong 1975), carcinogenecity (Pier & McLoughlin 1985) and immunosuppression (Richard 1978). This immunosuppressive action appears to be antigen-specific. Aflatoxins are linked with liver cancer in humans and animals (Enomoto & Saito 1972). Aflatoxin also decreases resistance to bacterial, viral, fungal and parasitic diseases by interfering with both humoral immunity (Virdi et al. 1989) and cell-mediated immunity (Ghosh et al. 1991). The nature and specific mechanism by which aflatoxins impair the immune
As the melting point of aflatoxins ranges from 237°C to 299°C (Castegnaro et al 1980), these toxins are not readily degraded under normal cooking conditions (Goldblatt 1969). Metabolites of aflatoxin are present as residues in milk, muscle, liver and kidneys of farm animals including buffalo. It is therefore, a high risk of aflatoxin intoxication in human beings through meat, milk, eggs and other livestock products. As no specific symptoms are observed when consumed in lower dose levels, it is often considered as a silent killer.

In recent times study of aflatoxin has gained a great significance. Buffalo calves are highly susceptible to aflatoxicosis with a high mortality rate. However, comparatively less information is available about the effect of aflatoxin on the immune system of buffaloes. The present investigation was therefore designed to study the cell-mediated immune response (CMIR) in experimentally induced aflatoxicosis in buffalo calves. Enumeration of T-cells was done using E-rosette technique which is being increasingly used as a method for evaluating cell-mediated immune response (Kersey & Gaji-PecZalska 1975). Furthermore, CMIR was also studied by using the delayed type hypersensitivity (DTH) skin test using Mycobacterium sp. antigen (Dimitri & Gobal 1996).

Materials and Methods

Six apparently healthy Murrah male buffalo calves aged one year and weighing about 35 to 40 Kg purchased and used for the study. They were screened by coprological examination for the presence of any protozoal cysts and helminth ova, and for any other clinical disease. Only disease free animals were used for the experiment. All the experimental animals were given the same feed and kept under the same management condition. The buffalo calves were randomly divided into two groups i.e. control and treatment, comprising of three buffalo calves in each group. Aspergillus flavus (Link ex Fries) was obtained from Aflatoxin Laboratory for post - graduate studies, Department of Botany, Bhagalpur University of India. The production of aflatoxin in laboratory was done by culturing spores of toxigenic strain of Aspergillus flavus in SMKY liquid medium (Diener & Davis 1966). Aflatoxin was extracted in the laboratory, characterised by thin layer chromatography and quantified spectrophotometrically (Nabney & Nesbitt 1965) before use. Treatment group was fed AFB1 at the rate of 10 µg / Kg body weight starting from day 0 daily for 30 days orally in propylene glycol. The control group received only the same amount of propylene glycol. E-rosette technique and DTH skin test determined CMIR in treated and control groups. All chemicals used in the experiments were of analytical reagent grade.

E-rosette technique

Peripheral venous blood was collected from all calves on day 0, 10, 20 and 30. Ten ml of blood was collected from the Jugular vein of each animal in heparinised tube (Heparin 10 i.u./ml of blood as anticoagulant). Enumeration of T-cells using E-rosette technique was done by the method of Kaura et al. (1979) with slight modification. Briefly, lymphocytes were separated by density gradient centrifugation by over-layering the blood over histopaque (Sigma Co., USA). The viability of separated lymphocytes was determined by trypan blue dye exclusion technique (Jain & Jesper 1967). The concentration of lymphocytes was adjusted to $10^5$ cells/ml of medium (RPMI-1640) containing 10 ml HEPES [N-12-hydroxyethyl piperazine N'-2-ethane sulphuric acid], buffer and 10% heat inactivated foetal calf serum. Sheep red blood cells (SRBC) was collected in Alsever’s solution and was washed thrice in 0.05 M Phosphate Buffer Solution (PBS), pH 7.3 and finally suspended in PBS to a concentration of 5%.

The lymphocyte suspension of 0.25 ml was mixed with 0.25 ml of SRBC and incubated at 37°C for five minutes and then centrifuged at 1000 rpm for five minutes at 4°C in thermostatically controlled centrifuge machine. The cell pellets were re-suspended gently and equal volume of 0.2% cold glutaraldehyde in PBS and 50µl of foetal calf serum was added. This was mixed gently and one drop was placed on a counting chamber of haemocytometer, and rosette-forming cells (RFC) were counted. For calculation of percentage in each case at least 200 lymphocytes were counted. The lymphocytes surrounded by three or more than three erythrocytes attached firmly around them were only considered as RFC.

DTH skin test

The role of aflatoxin on CMIR was also judged by measuring the DTH skin test using Mycobacterium sp. antigen (Dimitri & Gobal 1996). Freeze dried BCG vaccine prepared by BCG Vaccine Laboratory, Guindy, Madras-32, India was used for this study. The flank region of the control and treated buffalo calves were cleanly shaved (3 cm² area) taking all sort of precautions to avoid scratches on skin. To study the DTH skin test a dose of 0.5ml BCG vaccine was inoculated intradermally on day 20AFB1 intoxication. This injection served as a sensitising dose. After 10 days i.e. on day 30, a challenge dose of 0.5ml BCG
vaccine was inoculated intra-dermally in the centre of shaved region of all buffalo calves. A Vernier calliper was used to measure the skin thickness of flank site before application of challenge dose of BCG vaccine. The skin thickness was again measured after 48 hours.

**Statistical analysis**

The significant effect of aflatoxin on CMIR was calculated using the t-test for DTH skin test and analysis of variance for E-rosette technique (Snedecor & Cochran 1967).

**Results**

**T-lymphocyte count by E-rosette technique:**

The mean percent E-rosette values were 17.83±2.33 and 18.20±3.88 respectively in control and treatment groups of calves on day 0. The difference between two groups was not significant (P=0.94). This value remained at the same level throughout the experiment in control calves (Figure 1). However, the percent E-rosette in treated calves increased to 23.73±3.41 on day 10, and started to decline continuously thereafter reaching 15.20±1.76 on day 20 and 10.63±1.10 on day 30 (Figure 1). However, the difference in E-rosette value between two groups was significant only on day 30 (P<0.05).

When the mean percent E-rosette values for two groups of calves for the whole period of experiment was compared with each other (17.64±0.68 and 16.94±1.86 for control and treated calves respectively), the mean T-cells in the treated group decreased by 0.7 percent than that of control. No significant effect of AFB1 between groups, periods and the interaction between groups and periods could be observed.

**DTH skin test**

Results of the delayed type hypersensitivity reaction as measured by skin thickness (mm) are depicted in Figure 2. It is evident that the mean value of initial skin thickness was not different between the groups. The mean skin thickness in AFB1 intoxicated buffalo calves increased from 3.57±0.32 mm pre-challenge to 4.57±0.32 mm 48 hrs after the challenge, whereas in the control group it increased from 3.43±0.12 mm pre-challenge to 10.50±0.75 mm post-challenge. On statistical analysis a highly significant reduction (P<0.01) in skin thickness was observed after 48 hours in treated group when compared with the control group (Figure 2).

**Discussion**

Present study demonstrated that, as in many other livestock species, aflatoxicosis suppresses the cell-mediated immune response in buffaloes. It was demonstrated both by enumerating the T-cells (Figure 1) and by delayed type hypersensitivity skin reaction (Figure 2).
aflatoxin on CMIR. Though the experimental animals received a fixed dose of aflatoxin in the present study earlier workers have reported that in different species of animals the effect of aflatoxin on T-cell count is dose dependent (Vishalkshan et al. 1984).

The DTH skin test conducted after one month of experimental intoxication with AFB1 in treated calves demonstrated conclusively the diminished response of intoxicated buffaloes to cutaneous hypersensitivity reaction (Figure 2). The mean skin thickness of 4.57±0.32 and 10.50±0.75 mm was observed in treated and control calves, respectively 48 hours after challenge with tuberculin antigen as against the group mean skin thickness of 3.57±0.37 and 3.43±0.12 mm respectively before challenge. Similar reductions in lymphocyte indices and skin reactions had previously been reported in rabbits (Dimitri & Gobal 1996), pigs (Miller et al.1979) and chickens (Kadian et al.1988) intoxicated with aflatoxin. Furthermore, aflatoxin had also been shown to affect cellular immunity in human beings (Savel et al.1970). Aflatoxin concentration of >10 µg/ml significantly suppressed lymphocyte response of normal animals to phytotherenes, lymphocyte response of Mycobacterium bovis infected animals to specific antigen PPD (Paul et al.1977). Other workers had shown that the chemotactic ability of leucocytes and phagocytic ability of heterophil was inhibited in chicken receiving dietary aflatoxin (Chang et al.1976). The effect of aflatoxin on tuberculin skin reaction may be due to its direct effect on the T-lymphocytes which play a significant role in cell mediated immunity and consequently the delayed hypersensitivity (Dannalberg 1991). It seems that aflatoxins have affected transfer of cutaneous reactions through impairing lymphokine production by circulating T-cells and inhibiting migration of macrophages (Pier et al.1980, Thurston et al.1972).

**Conclusion and Recommendation**

The mechanism by which aflatoxin suppress CMIR is not clearly known. However, aflatoxin has been reported to cause thymic involution, impaired lymphocyte function, moderate lymphocytopenia and reduced phagocytosis as assessed by in-vivo and in-vitro tests (Bodine et al.1984, Thurston and Richard 1980). In addition to the direct effects of aflatoxins, a serious consequence of impaired CMIR may result to the increased susceptibility of the animals towards various microbial infections. It becomes therefore important to take steps to prevent aflatoxin contamination in animal feed. The above CMIR findings serve as and adjunct for diagnosis of aflatoxicosis in buffalo calves. As there is heavy contamination of animal and poultry feed by aflatoxin in the Indian sub-continent (Sinha et al.1999), effective education and extension programmes are required to reduce the prevalence and severity of aflatoxicosis in livestock and human in this part of the world.

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**References**


