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Evaluation of Acute Aflatoxicosis on Humoral Immune Response to Foot-and-Mouth Disease Vaccination in Guinea Pigs

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Summary

The immunosuppression of aflatoxinB_1 (AFB_1) on humoral immune response to foot-and-mouth disease (FMD) vaccination was evaluated. Fifty-two male guinea pigs were assigned to two treatment groups of 20 (A, B) and three control groups of 4. Groups A and B were vaccinated with an inactivated FMD vaccine after they were dosed 0.63mg of AFB_1/Kg once, and on days 1, 3, 14 and 21 respectively. Booster doses were injected 28 days after primary vaccination. Antibody titers against FMD virus were higher in the vaccinated, non-aflatoxin exposed group than in those exposed to aflatoxin. Significantly difference ($P<0.05$) was detected only in group B that was shown an average weight loss of 25g and persisted through second vaccination. The result indicates that aflatoxin can depress the humoral immune responses of guinea pigs to FMD vaccination and the immunotoxicity was found to be dose related.

Key words: aflatoxin, foot-and-mouth disease, vaccination, immunosuppression

Introduction

Foot-and-mouth disease (FMD) is a highly contagious virus infection of sheep, goats, cattle and other, causes both clinical and subclinical infection according to the natural or acquired immunity of the host. Within vaccinated herds FMD may appear

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as acute, mild or subclinical infection, dependent upon to the immune status of the herd, the level of challenge and the efficacy of the vaccine used (Hutber *et al* 1999). Clearly, susceptibility of various infectious diseases may be altered if the immune system is compromised. Immunosuppression may lead to decreased resistance to infectious processes and effective development of immunity in response to prophylactic vaccination. Mycotoxins as immunotoxic agents may suppress, enhance, or change the temporal development of immune responses (Muneer *et al* 1988). Among them aflatoxinB_1 (AFB_1) is widespread naturally occurring mycotoxins in raw-feed materials and animal feed, represent a group of toxic metabolites produced by the fungal species *Aspergillus flavus* and *A.parasiticus*, pose serious threat to human and animal health. The immunosuppressive effect of AFB_1 in farm animals and decrease resistance to some infectious disease in the field were reported (Osweiler & Trampel 1985, Colvin *et al* 1984, Richard *et al* 1983). Direct evidance for aflatoxin-induced immunosuppression in the bovine is lacking.

In parallel with the previous study on evaluation of immune response to FMD virus (Shahsavandi *et al* 2001), this study was undertaken in guinea pigs receiving a booster immunization with a commercially available inactivated FMD vaccine to assess the interaction of aflatoxin with immunization against the disease.

Materials and Methods

Aflatoxin. The sunflower seed, which contained 400ppb aflatoxins was used. Analysis of the seed demonstrated that aflatoxins B_1, B_2, G_1 and G_2 were present in quantities of 66.15%, 2.95%, 29.8% and 1.1%, respectively (Central Veterinary Laboratory, Karaj). The aflatoxin was dissolved in chloroform and was distributed in gelatin capsules to provide a dose equal to 0.63mg of AFB_1 toxic equivalents/kg of body weight or slightly less than a half of the LD_{50} of AFB_1 (1.4mg/kg of body weight). Solvent was allowed to evaporate in the dark at room temperature before capsules were capped and stored in opaque conditions.

Guinea pigs. Fifty-two male guinea pigs, all lacking FMD antibodies, weighing between 450 and 510g were randomly assigned to two groups of 20 and three control groups of 4. The animals had free access to water and pelleted guinea pig ration. They were weighed at the start of the experiment and then at specific intervals.

Vaccine. Inactivated trivalent FMD vaccine including types O_1, A-mardabad and $Asia_1$ strains with aluminium hydroxide and saponin adjuvants (Razi Institute, Karaj) was used for vaccination. The 50% protective does of the vaccine in guinea pig potency test was estimated at 0.5ml.

Experimental design. Forty guinea pigs were divided to two treatment groups of 20 each. Treated guinea pigs in group A were given aflatoxin once on day 1 and other treated group (group B) were given on days 1, 3, 14 and 21. They were vaccinated with the FMD vaccine on day 7. Booster doses were injected 28 days after primary vaccination. Groups C and D were considered as vaccine and aflatoxin controls, respectively. Group E was unvaccinated control, which fed on aflatoxin-free ration. Guinea pigs in all groups were divided into two equal subgroups, white and color. Blood samples were taken from a different group of 5 treated guinea pigs and from controls on postvaccination days 0, 7, 14, 21, 28 and 48. The data from the 12 control guinea pigs were used in analysis of both treatment groups. Collected sera were stored at–20°C until used.

Serological assay. Serum samples were tested for antibodies to FMD virus using the micro serum neutralizing (SN) test as described by Rweyemamu *et al* (1978).

Results

Data on the effect of aflatoxin on antibody titers against FMD virus type O_1 are shown in table 1. Similar results were observed for other types of the virus. The titers were markedly higher in the vaccinated, non-aflatoxin exposed animals (group C) than in those exposed to multi doses of aflatoxin (group B). The statistical

analysis of SN data showed that the effect of aflatoxin on the titers was highly significant ($P<0.05$). Significant differences were observed between the treated groups in relation to whether the aflatoxin was feed singly or in multi doses. Following the second vaccination, the aflatoxin resulted in a dramatic decrease of the antibody titer, indicatind a lack of vaccinal protection.

Table 1. *Mean of serum neutralizing antibody titers (log_{10}) against FMD type O_1 at different days postvaccination*

Group	Status of guinea pigs	Days postvaccination					
		0	7	14	21	28	48
A	Single dose AFB_1 vaccinated	0.60	0.90	1.53	2.54	2.74	2.93
B	Multi doses AFB_1 vaccinated	0.60	0.82	0.93	0.95	1.00	1.07
C	Non-AFB_1 exposed vaccinated	0.70	0.94	1.75	2.77	2.96	3.10
D	AFB_1 exposed unvaccinated	0.75	0.75	0.84	0.80	0.80	0.76
E	Non-AFB_1 exposed unvaccinated	0.55	0.70	0.70	0.80	0.80	0.82

The clinical signs of aflatoxicosis, including depression, loss of weight, weakness and oedema in subcutaneous region, were observed to varying degrees in treated groups and were most severe in group B. White guinea pigs in treated groups reduced more weigh gains in comparison with color guinea pigs (here it is difficult to difining such behavior). The difference in FMD antibody titers between the white and color groups was not significant.

Disscusion

The findings in this study show that exposure of guinea pigs to subclinical level of aflatoxin significantly lowered antibody titers in the animals immunized against FMD compared to nonaflatoxin treated group. As shown in our study when aflatoxin was withdrawn from treated group A, the antibody titers reached just near to the normal values in control group after 3 weeks of its withdrawal. The results revealed that the toxin could not affect the immune response to FMD vaccination except in

severe aflatoxicosis, and possibility changes in the antibody titer were reversible when feeding of AFB_1 was discontinued.

The adverse effects of aflatoxin are thought to be due to both a direct effect on the animal and indirect by suppression of the immune system, and thus increase the susceptibility of poultry and mammals to infectious diseases. It acts as an immunosupperssant primarily by inhibiting cell-mediated immunity (Muneer *et al* 1988). The immunosuppresse effect of aflatoxin has been related to its direct inhibition of protein synthesis (Thurston *et al* 1980) including changes in serum protein, alternation of B and T cell functions which resulted to impairment of IgG and IgA production, and depression of C4 and complement activity (hence depressed opsonization and phagocytic activity). Serum protein changes occure in aflatoxicosis especially from 3th day after feeding of the toxin. By the time necrotic patch on liver is appeare and the organ seem pale and fraible (Chauhan *et al* 1997). Electrophoretic analysis of serum from affected guinea pigs indicated that aflatoxin produced an increase in γ-gloublin, a decrease in α_2-gloublin, and frequency decreased total protein concentration. The extent of these effets is, however, dose dependent (Reddy *et al* 1987, Richared *et al* 1974, Thurston *et al* 1974). Long term of low levels of aflatoxin ingestion also vary. The prime chronic effect of the toxin is the induction of cancer, especially of the liver. The toxin affects DNA replication and hence can produce mutagenic or teratogenic effects. A proportion of aflatoxin ingested by lactating animals can be hydroxylated and excreted in the milk as AFM_1 and AFM_2. It is important for widly consumption of milk and dairy products (Samson 1992).

Although immunization and effective management practices have controlled FMD, and prophylactic immunization against the major infectious disease in cattle is vital to safeguard against the disease outbreaks, at times with heavy economic losses, do occure in developing countries (Hutber *et al* 1999). Lack of adequte protection and interference with immunity of cattle seem to have important roles in

such cases. Because of the large volume of feedstuffs that must be stored and distributed on farms, considerable opportunity exists for mold growth to appear and produce a mycotoxin. Presence of aflatoxin in feed and feed raw materials may also contribute to immunosuppression when ingested either in large single doses or in small quantities over a long period of time. Althought the role of aflatoxin on effectivless of FMD vaccine in field situations is not obviously clear, outbreaks of fowl cholera and infectious boronchities disease have been reported in vaccinated flocks associated with ingestion of aflatoxin contaminated feed (Hagazi *et al* 1991, Anjum 1994). On the other hand, aflatoxin and other mycotoxins are natural contaminators of cereal grains particularly corn (Bryden *et al* 1975, Osweiler 1985) in the field or storage and because of their stability, they may also be found in processed food products. It suggests that mycotoxin contamination must be seriously considered in feed of cattle. Constant monitoring and good on-farm feed management, including good mixing and inclusion of mold inhibitors and binders, will reduce the economic impact of mycotoxins (Quilline 2002). Now, application of HACCP (Hazard Analysis Critical Control Point) method is in fact a logical plan for control of mycotoxins and food safty problems.

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